Selective inhibition of TNF-α or IL-1β does not affect E. coli-induced inflammation in human whole blood

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ARTICLE INFO

Article history:
Received 18 February 2010
Accepted 24 February 2010
Available online 23 March 2010

Keywords:
Innate immunity
Sepsis
E. coli
CD14
Complement
DAMP (Danger Associated Molecular Pattern)

ABSTRACT

Inhibition of the inappropriate and excessive inflammatory response has been a main issue in sepsis-related research. Historically, TNF-α and IL-1β have been postulated as key mediators in sepsis, but selective inhibition of these cytokines has failed in clinical trials. Recently it was found that inhibition of upstream recognition by complement and CD14 could efficiently reduce Escherichia coli (E. coli)-induced inflammation. An ex vivo model with lepirudin-anticoagulated human whole blood was used to explore the significance of selective inhibition of TNF-α and IL-1β in E. coli-induced inflammation. The effect of TNF-α, IL-1β, complement and CD14 on the inflammatory response was assessed by adding highly specific neutralizing agents to these mediators. Proinflammatory cytokines, expression of CD11b and oxidative burst were measured. The controls included relevant isotype-matched immunoglobulins and peptides. Selective inhibition of TNF-α or IL-1β had no impact on E. coli-induced release of proinflammatory cytokines, CD11b-upregulation or oxidative burst. In contrast, the combined inhibition of complement and CD14 virtually abolished these responses. These data suggest that both TNF-α and IL-1β are downstream mediators and as single mediators play a limited role within the complex inflammatory reactions induced by E. coli.

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1. Introduction

Severe sepsis and septic shock are major challenges in intensive care medicine. The incidence and mortality are high and does not seem to decrease (Karlsson et al., 2007; Finfer et al., 2004; Martin et al., 2003). Antimicrobial therapy is usually sufficient to treat the underlying infection, but is not capable of reversing the systemic inflammatory response and companion organ failure. Hence, one of the main challenges in sepsis-related research has been to intervene and inhibit the detrimental effects of the excessive inflammatory response.

During the eighties much attention was directed towards cytokines, their inflammatory properties and their potential ability to cause endogenous harmful systemic effects. Tumor necrosis factor-alpha (TNF-α) was regarded as the most important proinflammatory cytokine, and different observations and experimental results connected the role of TNF-α directly to the pathogenesis of sepsis. The level of TNF-α seemed to correlate with the outcome of sepsis (Waage et al., 1987; Debets et al., 1989). Administration of TNF-α to animals created both cardiovascular alterations and inflammatory responses similar to what was seen in septic patients (Tracey et al., 1986; Remick et al., 1987; Natanson et al., 1989), and inhibition of TNF-α protected animals against lethal effects induced by experimental sepsis (Beutler et al., 1985; Tracey et al., 1987; Emerson et al., 1992). However, several clinical trials using TNF-α inhibitors have shown moderate or no effect on the outcome of sepsis in humans (Abraham et al., 1998, 2001; Panacek et al., 2004; Rice et al., 2006). IL-1β is another central proinflammatory cytokine postulated to be a key mediator in sepsis. As with TNF-α inhibition, targeting IL-1β alone or in combination with TNF-α inhibitors has not been successful in clinical trials (Fisher et al., 1994; Opal et al., 1997; Remick et al., 2001). It is therefore reasonable to question the role of TNF-α and IL-1β as truly upstream mediators in the Gram-negative bacteria-induced inflammatory reaction in humans. A unique whole blood ex vivo model for investigation of crosstalk of the human inflammatory network has made it possible to investigate these matters (Mollnes et al., 2002).

Complement and the Toll-like receptors (TLRs) constitute two important upstream sensor and effector-systems of innate immunity. Both complement and TLRs possess germline-encoded
pattern-recognition receptors (PRRs), mediating “danger signaling” from highly conserved structures or patterns on exogenous invading microbes or endogenous danger motifs (Medzhitov and Janeway, 2000; Matzinger, 2002; Kohl, 2006). The fluid-phase complement cascade system plays an essential role in protection against infectious diseases (Sjoberg et al., 2009). Under normal conditions, complement acts locally, but uncontrolled systemic activation may induce an overwhelming inflammatory response. Systemic activation as seen in sepsis causes cardiovascular deterioration, impaired oxygenation and metabolic acidosis (Younger et al., 2001). Excessive generation of the complement split product C5a may lead to a vast cytokine production, upregulation of adhesion molecules, paralysis of neutrophils and disseminated intravascular coagulation (Ward, 2004). The Toll-like receptor family (TLRs) is the main cellular arm of innate immunity, and individual TLRs have different properties (Cook et al., 2004). TLR4 acts as the PRR for the exogenous lipopolysaccharide (LPS) on Gram-negative bacteria. However, TLR4 is dependent on interaction with the co-receptors MD-2 and CD14, the latter being a key molecule for LPS recognition (Shimazu et al., 1999; Latz et al., 2002; kashi-Takamura and Miyake, 2008). Once activated, the CD14/MD2/TLR4 complex induces NF-κB to be responsible for TLR4 function, since it has been shown to be important not only for TLR4 function, but also for TLR2 (Muta and Takeshige, 2001; Nilsen et al., 2008), CD14 may be regarded as an upstream molecule in the TLR system when it has been shown to be important not only for TLR4 function, but also for TLR2 (Muta and Takeshige, 2001; Nilsen et al., 2008), and TLR3 (Lee et al., 2006).

The present study examines the significance of the traditionally regarded early cytokines TNF-α and IL-1β, compared to the central upstream PRRs of innate immunity, complement and CD14, as mediators of and candidates for inhibition of E. coli-induced inflammation in human whole blood.

2. Materials and methods

2.1. Reagents and equipment

Sterile phosphate buffered saline (PBS) and recombinant human tumor necrosis factor-α (rTNF-α) 10 μg/mL, was purchased from Sigma–Aldrich (St. Louis, MO). Recombinant lyophilized human IL-1β (rHL-1β) was purchased from R&D Systems (Minneapolis, MN). CryoTubes™ (polypropylene) was purchased from Nunc AS (Roskilde, Denmark). Lepirudine (Refludan®) was purchased from Pharmion (Copenhagen, Denmark).

2.2. Bacterial preparations

Briefly, E. coli strain LE392 (ATCC 33572) was obtained from American Type Culture Collection (Manassas, VA). E. coli was grown overnight in LB-medium (trypton 1%, yeast extract 0.5% and sodium chloride 0.9%), then centrifuged and washed in PBS. After resuspension in PBS, E. coli was heat-inactivated 1 h at 60 °C. Growth control confirmed that all bacteria were killed. Thereafter, the bacteria were washed nine times to remove extra-bacterial LPS, counted by flow cytometry and stored at 4 °C in batches of 1 × 10⁷ E. coli/mL PBS.

2.3. Antibodies and inhibitors

Infliximab (Remicade®), a chimeric IgG1κ monoclonal antibody, with a molecular weight (m.w.) of 149 kDa, composed of human constant and murine variable regions, was purchased from Schering-Plough (Leiden, the Netherlands). Infliximab was reconstituted in sterile water to a concentration of 10 mg/mL. Further dilution was done in sodium chloride, 9 mg/mL. Etanercept (Enbrel®), a dimeric fusion protein with a m.w. of 150 kDa, consisting of the extracellular ligand-binding portion of the human tumor necrosis factor receptor II (TNFRII) linked to the Fc portion of human IgG1, was purchased from Wyeth Pharmaceuticals (Hampshire, UK) as single-use prefilled SureClickTM, 50 mg/mL. Further dilution was done in PBS. Both infliximab and etanercept bind specifically to soluble and membrane bound TNF-α. Rituximab (MabThera®), a genetically engineered chimeric murine/human IgG1κ mAb with a m.w. of 145 kDa, directed against CD20, was purchased from Roche (Grenzach-Wyhlen, Germany) as a preservative-free liquid, 10 mg/mL. Further dilution was done in PBS. Rituximab was used as an isotype-matched control antibody to infliximab and etanercept. Anakinra (Kineret®), a non-glycosylated form of human interleukine-1 receptor antagonist (IL-1ra), with a m.w. of 17.3 kDa, was purchased from Amgen B.V. (Breda, the Netherlands) in prefilled glass syringes, 150 μg/mL. Further dilution was done in PBS. The mouse anti-human CD14 (clone 18D11) F(ab′)2 and a control F(ab′)2 (clone BHI1) was purchased from Diatec AS (Oslo, Norway). The potent Compstatin analog Ac–[CV(1MeWQ)DWAHRC]7, which binds to and inhibits cleavage of C3, was produced as previously described (Morikis et al., 1998). The inactive peptide Ac–IAVQQDWHHRAT was used as control. All inhibitors were stored in aliquots at −70°C.

2.4. Whole blood model

The ex vivo whole blood model has been described in detail previously (Mollnes et al., 2002). Briefly, fresh human whole blood was obtained from healthy donors and anticoagulated with lepirudin (Refludan). Whole blood was preincubated with inhibitor, control or PBS for 5 min at 37 °C. PBS or E. coli, in a final concentration of 10⁶ E. coli bacteria/mL whole blood, was then added and incubated for 2 h at 37 °C. EDTA (20 mM) was added to stop the complement activation and the tubes were centrifuged for 15 min at 1400 × g at 4 °C. The plasma was stored at −70°C until analysed. Informed written consent was obtained from each donor, and the study was approved by the Local Ethical Committee.

2.5. Multiplex analysis

Plasma samples were analysed using multiplex technology—an immunoassay which simultaneously measures a range of human biomarkers. Both human 27-plex kits and mixed single-kits were used. The kits were purchased from Bio-Rad Laboratories (Hercules, CA). The following analytes were measured: IL-1ra, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-α, IFN-γ, eotaxin, FGF-basic, G-CSF, GM-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES and VEGF. The multiplex 27-plex assay is based on 27 colored beads, each with a unique cytokine detection antibody. The beads are incubated with the samples, a secondary biotin-conjugated antibody is added and then a reporter molecule, streptavidin-PE, which binds to biotin. The samples are run in a modified flow cytometer, with two lasers, one which excites the beads and one which excites the reporter molecule. In this way, the beads are “separated” and the amount of each of the 27 cytokines can be quantified.

The analyses were performed according to the instructions from the manufacturer. Six single observations were excluded due to extreme out-of-range values.
2.6. Flow cytometry

2.6.1. Leukocyte CD11b expression

Whole blood was preincubated with inhibitors or controls for 5 min at 37 °C. *E. coli*, 10^8 bacteria/mL, was then added and the incubation continued for 10 min at 37 °C. The cells were fixed with 0.5% (vol/vol) paraformaldehyde for 4 min at 37 °C, stained with anti-CD14 FITC/anti-CD11b PE (Becton Dickinson, San José, CA) and the nuclear dye LDS-751 (Sigma–Aldrich, St. Louis, MO) for 15 min at room temperature in the dark. Samples were analysed with a LSRII flow cytometer (Becton Dickinson) with threshold at LDS-751 to exclude red cells, platelets and debris. Granulocytes and monocytes were gated in a side scatter (SSC)/CD14-dotplot and CD11b expression was given as median fluorescence intensity (MFI).

2.6.2. Oxidative burst

Whole blood was preincubated with inhibitors or controls for 5 min at 37 °C. *E. coli*, 10^8 bacteria/mL, was then added and incubated for 10 min at 37 °C. Oxidative burst was measured with the commercial kit, Bursttest (Orpegen Pharma, Heidelberg, Germany), using dihydrorhodamine 1,2,3 as a substrate. Gates were set on granulocytes and monocytes in a forward/side scatter (FSC/SSC) dotplot, in order to analyse each population with regard to MFI.

2.7. Statistics

GraphPad Prism version 5 (GraphPad Software, San Diego, CA) was used for the statistical analyses. The data were analysed by one-way ANOVA followed by Bonferroni’s multiple comparison test. A p-value of 0.05 was considered statistically significant.

3. Results

3.1. Addition and neutralization of recombinant TNF-α and IL-1β in human whole blood

Whole blood was incubated with incremental doses of rhTNF-α and rhIL-1β, and the amount of the cytokines was measured in plasma (Fig. 1, upper panels). The TNF-α inhibitors infliximab and etanercept, which bind to TNF-α, dose-dependently neutralized 10 ng/mL of TNF-α added to whole blood (Fig. 1, lower left panel). Complete neutralization of TNF-α to the baseline level was obtained using etanercept at a dose of 200 μg/mL and infliximab at a dose of 0.5 μg/mL whole blood. The reduction in measured TNF-α is consistent with binding of the inhibitors to epitopes needed for detection in the assay. The IL-1 receptor antagonist, anakinra, does not bind IL-1β and IL-1β can therefore not be used as readout to measure the effect of neutralization. Functional inhibition of IL-1β was therefore examined using IL-8 as readout. IL-8 concentration was dose-dependently decreased by anakinra and completely

![Fig. 1. Addition and neutralization of recombinant TNF-α and IL-1β in human whole blood.](image-url)
inhibited at a dose of 1 μg/mL whole blood when blood was incubated with rhIL-1β at 1 ng/mL whole blood (Fig. 1, lower right panel).

3.2. Effect of E. coli and pure recombinant TNF-α and IL-1β on cytokine production

E. coli, 10^3 to 10^7 bacteria/mL, dose-dependently induced expression of TNF-α, IL-1β, IL-6, IL-8, MIP-1α and IL-1ra (Fig. 2). Incremental doses of rhTNF-α, 0.04–25 ng/mL, dose-dependently induced production and release of IL-1β, IL-6, IL-8, MIP-1α and IL-1ra (Fig. 3). Incremental doses of rhIL-1β, 0.1–10 ng/mL, dose-dependently induced production and release of TNF-α and IL-8, whereas the amount of MIP-1α was hardly affected and IL-6 and IL-1ra were not affected (Fig. 3).

3.3. Comparison of E. coli versus rhTNF-α and rhIL-1β on cytokine production

The impact of rhTNF-α and rhIL-1β on cytokine production in whole blood was compared with the cytokine response induced by E. coli. In order to do this, we determined “E. coli-equivalent” concentrations of both rhTNF-α and rhIL-1β.

E. coli at 10^6 bacteria/mL whole blood, induced approximately the same TNF-α concentration as was measured after addition of 5 ng/mL of rhTNF-α, and approximately the same IL-1β concentration as was measured after addition 1 ng/mL of rhIL-1β (Fig. 4, hatched bars). Thus, the individual effects of “E. coli-equivalent” doses of recombinant cytokines were compared to the effect of whole bacteria when incubated in fresh human whole blood for 120 min at 37 °C. The average levels of IL-1β, IL-6, IL-8 and MIP-1α were significantly higher when incubated with E. coli compared to rhTNF-α in equivalent doses (Fig. 4). Similarly, the average levels of TNF-α, IL-6, IL-8 and MIP-1α were significantly higher when incubated with E. coli compared to rhIL-1β in equivalent doses. IL-1ra, however, was significantly increased when incubated with rhTNF-α compared to E. coli, whereas rhIL-1β induced similar amount as E. coli (Fig. 4).

3.4. Effect of specific inhibitors on E. coli-induced cytokine release

The effect of selective TNF-α- and IL-1β-inhibition on E. coli-induced cytokine production using a final concentration of 10^6 bacteria/mL whole blood was then examined (Fig. 5). Based on the titration experiments, the TNF-α inhibitors etanercept and infliximab were used at a concentration of 50 μg/mL whole blood and the IL-1 inhibitor anakinra at 10 μg/mL whole blood. For comparison, the following CD14- and complement-inhibitors known to attenuate this reaction were included: anti-CD14 (10 μg/mL) and compstatin (25 μM). Infliximab and etanercept efficiently neutralized TNF-α by 100% and 85%, respectively (p < 0.001), as expected. A non-significant change by 20% for IL-6 by infliximab was observed, which was not seen for etanercept (Fig. 5). The expression of the other cytokines was not influenced by the TNF-α inhibitors (non-significant change was 0–9%). Anakinra had no effect on any of the measured cytokines. Anti-CD14 alone significantly inhibited...
Fig. 3. Effect of pure recombinant TNF-α and IL-1β on cytokine production. Whole blood was incubated for 120 min at 37 °C with incremental doses of rhTNF-α, from 0.4 to 25 ng/mL, or incremental doses of rhIL-1β from 0.1 to 10 ng/mL. TNF-α, IL-1β, IL-6, IL-8, MIP-1α and IL-1ra were measured and data given as means and SD (n = 5). T0 represents the baseline sample after 0 min and T120 the spontaneous activation after 120 min incubation.

all the measured cytokines (p < 0.001) (Fig. 5). Comstatin significantly inhibited the release of TNF-α (p < 0.05) and IL-1β (p < 0.001), insignificantly reduced the release of IL-6, but did not influence IL-6 or MIP-1α. The combined inhibition of compstatin and anti-CD14 significantly reduced the expression of all cytokines by 88–96% (p < 0.001) (Fig. 5).

3.5. Effect of specific inhibitors on E. coli-induced oxidative burst and expression of CD11b

Infliximab, etanercept and anakinra had no effect on the level of granulocyte- and monocyte-oxidative burst or on the level of CD11b-upregulation on granulocytes and monocytes (Fig. 6). Inhibition of complement by compstatin almost abolished oxidative burst in granulocytes and markedly reduced it in monocytes. Anti-CD14 markedly reduced oxidative burst in granulocytes but less pronounced in monocytes. The combination of compstatin and anti-CD14 reduced the level of oxidative burst in both cell types by 93–97%, below complement inhibition alone. Compstatin only and anti-CD14 only reduced the expression of CD11b in both cell types. The combined inhibition of the two further reduced the expression by 70% in both cell types (Fig. 6).

4. Discussion

Mediator-directed therapy attenuating the detrimental immune response in sepsis has been a major task and goal in sepsis-related research. Many studies have been conducted, targeting mediators like the proinflammatory cytokines TNF-α and IL-1β. Although these are traditionally regarded as early inflammatory mediators, they indeed belong to the downstream actors as compared to the real upstream molecules being the sensors of danger. There has been a gap between promising results in experimental studies and results from clinical trials when using inhibitors of TNF-α and IL-1β. Accordingly, in the present study we demonstrate that selective inhibition of TNF-α or IL-1β has no impact on cytokine production, oxidative burst or expression of the cell-surface marker CD11b in E. coli-induced inflammation. This was in contrast to the convincing inflammation attenuating effect of the combined inhibition of two important upstream PRRs of innate immunity, CD14 and complement. To our knowledge, it is the first time the effect of selective inhibition of downstream cytokines compared to upstream inhibition of PRRs in E. coli-induced inflammation in whole blood has been explored. The effect of pure rhTNF-α and rhIL-1β as inflammatory inducers in fresh human whole blood was explored in pilot experiments. Multiplex analysis revealed modest production and release of the inflammatory mediators TNF-α, IL-1β, IL-6, IL-8, MIP-1α, and IL-1ra. All of these selected mediators dose-dependently increased when whole blood was incubated with rhTNF-α. The cytokine response induced by rhIL-1β was less pronounced and only TNF-α and IL-8 responded.

In order to explore the impact of pure rhTNF-α and rhIL-1β as inducers of inflammation, their effect was compared to the proin-
Fig. 4. Comparison of *E. coli* versus rhTNF-α and rhIL-1β on cytokine production. The effect of *E. coli* versus rhTNF-α and rhIL-1β on cytokine production was compared. Whole blood was incubated for 120 min at 37 °C with either 10⁶ *E. coli* bacteria/mL, or with "*E. coli*-equivalent" doses of recombinant TNF-α and IL-1β (hatched bars). TNF-α, IL-1β, IL-6, IL-8, MIP-1α and IL-1ra were measured. Data are presented as means and 95% confidence intervals (*n* = 5). *p < 0.05 versus *E. coli*.

Inflammatory effect of the Gram-negative bacterium *E. coli*. The *E. coli* concentration in whole blood was similar to what has previously been used as a sublethal dose in experimental sepsis on baboons (Creasey et al., 1991). rhTNF-α and rhIL-1β were added at concentrations similar to those induced by *E. coli*. *E. coli*-induced a substantially broader and more potent proinflammatory response compared to pure recombinant TNF-α or IL-1β. Notably, and in contrast to the effect on the proinflammatory cytokines, rhTNF-α significantly induced more IL-1ra compared to *E. coli*. IL-1ra is considered as an anti-inflammatory mediator because it blocks the triggering of the IL-1 receptor, thereby preventing the proinflammatory effects of IL-1β (Dinarello, 1991). It has been shown that complement activation inversely affects IL-1ra (Lappegard et al., 2009). In contrast to rhTNF-α, which is inert with respect to complement activation, *E. coli* is a potent complement activator. This may explain the discrepancy in production and release of IL-1ra, as complement activation by *E. coli* would suppress the production.

Of the TNF-α inhibitors, infliximab neutralized TNF-α slightly more efficiently than etanercept, and even high concentrations of etanercept did not completely abolish all TNF-α added. Both inhibitors were present in excess but different biochemical and mechanistic profiles may explain the different efficacy. Infliximab binds both the 17-kDa monomer and the 51-kDa trimer form of TNF-α and can bind two TNF-α molecules simultaneously in contrast to etanercept which only binds the trimer form of TNF-α in a one-to-one ratio (Scallon et al., 2002). On the other hand, etanercept is the only TNF-α antagonist which also binds to lymphotoxins (LT) and evidence suggests a higher binding affinity to LTα3 ligands compared to TNF-α (Tracey et al., 2008; Gudbrandsdottir et al., 2004).

Inhibition of TNF-α or IL-1β did not have any impact on oxidative burst, production and release of other cytokines nor upregulation of CD11b. However, combined inhibition of complement and CD14 virtually abolished all measured inflammatory mediators induced by *E. coli*. This is in accordance with previous reports on the effect in human whole blood (Brekke et al., 2007, 2008). Further, both the endogenous meconium induced inflammation in human whole blood and the exogenous *E. coli*-induced inflammation in porcine whole blood were attenuated similarly by this inhibitory regimen (Salvesen et al., 2008; Thorgersen et al., 2009). Thus, the combined inhibition of these two important upstream PRRs of innate immunity is effective in different species and attenuate exogenous as well as endogenous induced inflammation. Inhibition of complement C3 convertase by compstatin prevents both the bacterial opsonization and the formation of C5a which is crucial for upregulation of the important phagocytosis-receptor CR3 (CD11b/CD18) (Mollnes et al., 2002). Inhibition of CD14 prevents TLR4-induced intracellular signaling and induction of immune-response genes (Akira et al., 2006). According to previous results (Brekke et al., 2007, 2008; Thorgersen et al., 2009), inhibition of complement and CD14 attenuated *E. coli*-induced inflammation differentially. In the present study complement inhibition attenuated *E. coli*-induced IL-1β and IL-8 leaving the other cytokines relatively unchanged, in contrast to anti-CD14 which significantly attenuated all the measured cytokines. On the other hand, oxidative burst was more dependent on complement than CD14. Evidence suggests an existence of crosstalk between these two upstream independent branches of pattern recognition (van Bruggen et al., 2007; Mollnes et al., 2008). An efficient suppression of the *E. coli*-
induced inflammation is therefore dependent on a combined inhibition of complement and CD14, supported by the present findings.

Sepsis is a condition which includes a broad and heterogeneous patient population (Dellinger et al., 2008). Interventional clinical trials including patients with severe sepsis and septic shock have resulted in large variation in baseline risk of death and are therefore associated with uncertainty (Annane, 2009). This may partly explain why previously clinical trials have failed to show beneficial results using TNF-α or IL-1β inhibitors. Anti-TNF-α treatment has been successful in the treatment of rheumatoid arthritis (Scott and Kingsley, 2006). This condition is characterized by high production of TNF-α in synovial joints inducing local detrimental effects (Tracey et al., 2008). A chronic condition with persistent presence of TNF-α is totally different from what is seen in septic patients, and it is in our opinion not surprising that anti-TNF-α treatment is efficient in rheumatoid arthritis but not in sepsis. Furthermore, the concentration of TNF-α and IL-1β in the blood differs between patients with sepsis and may fluctuate individually. In experimental models of sepsis, TNF-α typically reach a peak level 90 min after exposure and thereafter returns to baseline levels (Hesse et al., 1988). IL-1β follows TNF-α in a delayed but similar manner. A treatment regimen focused on specific cytokine inhibition is therefore obviously biased by these fluctuations. The main problem, however, is rather related to the fact that the inflammatory network is broadly activated by upstream molecules, limiting the effect of neutralization of single downstream mediators.

The present study was conducted ex vivo, obviously lacking the complexity which arises in a whole organism. However, the model used in this study applying fresh human whole blood anticoagulated with lepirudin, is a particularly suitable and reliable method for studies of inflammatory crosstalk. Lepirudin is a highly specific thrombin inhibitor, and thus, except for thrombin all inflammatory mediators in whole blood are able to interact mutually. Lepirudin neither interfere with complement activation nor the blood cells, and the blood physiology is preserved close to the condition in vivo (Mollnes et al., 2002).

According to our results, it is reasonable to assume that inhibition of TNF-α or IL-1β would have a limited effect on the E. coli-induced inflammation also in vivo and consequently a limited role in intervention in E. coli-induced inflammation. The data support the notion that TNF-α and IL-1β, although early appearing cytokines, are not key upstream mediators when the inflammatory network is triggered by E. coli. In contrast, combined inhibition of real upstream actors like CD14 and complement effectively inhibited E. coli-induced inflammatory mediators, and represent a potentially treatment regimen in Gram-negative sepsis.
Intensity (MFI) is presented as mean and range of two separate experiments. Spontaneous background activity is subtracted from all results. Presented as grey bars: positive (P), rituximab (R) and F(ab’2) (Ig). All inhibitors and controls were used in the same concentrations as described in Fig. 5. Median fluorescence intensity (MFI) is presented as mean and range of two separate experiments. Spontaneous background activity is subtracted from all results.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

Acknowledgments

This work was supported by The Research Council of Norway, Medinnova SF, The Norwegian Council on Cardiovascular Disease, The Regional Health Organization Helse Sør-Øst, The Family Blix Foundation, The Odd Fellow Foundation and NIH grant AI068730 (JDL).

References


Fig. 6. Effect of specific inhibitors on E. coli-induced oxidative burst and expression of CD11b. Whole blood was preincubated with inhibitors for 5 min, thereafter incubated with E. coli for 10 min at 37 °C. Inhibitors were etanercept (E), infliximab (I), anakinra (A), compstatin (C), anti-CD14 (α) and the latter two in combination (Co). Controls are presented as grey bars: positive (P), rituximab (R) and F(ab’2) (Ig). All inhibitors and controls were used in the same concentrations as described in Fig. 5.


