Molecular Medicine

Leukocyte Activity in Patients with ST-Segment Elevation Acute Myocardial Infarction Treated with Anakinra

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Anakinra, the recombinant form of the human interleukin (IL)-1 receptor antagonist, blunts the acute systemic inflammatory response in patients with ST-segment elevation myocardial infarction (STEMI), by determining a fall in peripheral blood leukocyte and plasma C-reactive protein levels. The aim of the present study was to determine the effects of anakinra on the activity of leukocytes measured *ex vivo*. Blood was collected 72 h after admission in 17 patients enrolled in the Virginia Commonwealth University - Anakirna Remodeling Trial (2) (VCU-ART2) and randomly treated with anakinra (N = 7) or placebo (N = 10). Whole blood was cultured at 37°C for 24 h to measure spontaneous production of IL-6 or stimulated with *Escherichia coli* lipopolysaccharide (LPS) for toll-like receptor (TLR)-4 or heat-killed *Staphylococcus epidermidis* (SE) for TLR-2 activation. The cultures of anakinra-treated patients produced significantly less IL-6 spontaneously (71 pg/mL (27–114)) compared with placebo-treated patients (290 pg/mL (211–617), $p = 0.005$). LPS- or SE-induced IL-6 production, on the other hand, was not statistically different between anakinra- versus placebo-treated patients (344 pg/mL (94–560) versus 370 pg/mL (306–991), $p = 0.32$ for LPS, and 484 pg/mL (77–612) versus 615 pg/mL (413–871), $p = 0.31$ for SE, respectively). IL-1 blockade with anakinra in STEMI patients results in reduced spontaneous leukocyte activity *ex vivo* without impairing the responsiveness to bacterial stimuli.

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INTRODUCTION

Post-ischemic heart failure is one of the leading causes of death worldwide (1). Adverse cardiac remodeling after an acute myocardial infarction (AMI) remains one of the most important determinants of survival, despite the striking improvements made in the diagnosis and treatment of AMI in recent years (2). Over recent years, a large amount of evidence stressed the importance of the inflammatory response in adverse cardiac remodeling after AMI (3,4). More specifically, specific inflammation mediators are emerging as novel therapeutic targets (3,4).

Interleukin (IL)-1 is a key mediator of infectious and sterile inflammatory responses (5). IL-1–targeted therapy is commonly used to treat a broad spectrum of autoinflammatory diseases (5,6). However, only recently has IL-1 emerged as a novel target for intervention in heart disease (7). The initial experience with anakinra, the recombinant form of the naturally occurring human IL-1 receptor antagonist, in patients with ST-segment elevation myocardial infarction (STEMI) suggests that IL-1 blockade blunts the acute systemic inflammatory response, since leukocyte counts and plasma C-reactive protein (CRP) levels were reduced, as well as the incidence of heart failure (8,9). Yet, the effects of anakinra on leukocyte activity in patients with STEMI remain unknown. The aim of this study was to determine the effects of anakinra on the activity of leukocytes measured *ex vivo*. Therefore, we measured the spontaneous and inducible production *ex vivo* of IL-6, a key secondary proinflammatory cytokine, in leukocytes obtained from 17 patients with STEMI enrolled in a clinical trial of anakinra or placebo (9).

MATERIALS AND METHODS

Demographic and clinical data were obtained from 17 patients enrolled for

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the Virginia Commonwealth University - Anakirna Remodeling Trial (2) (VCU-ART2), representing 57% of all patients (9). The VCU-ART2 study enrolled 30 patients with ST-segment elevation acute myocardial infarction, randomized, double-blinded, to anakinra 100 mg daily or placebo for 14 d. The primary endpoint of differences in interval change in left ventricular end-systolic volume index between anakinra and placebo at 3 months was measured with cardiac magnetic resonance, as an expression of adverse remodeling (www.clinicaltrials.gov, NCT01175018) (9).

Seven (41%) patients had been randomly assigned to blinded treatment with subcutaneous injections of 100 mg anakinra daily for 14 d (Kineret; Swedish Orphan Biovitrum, Stockholm, Sweden) and 10 (58%) to matching placebo injections. The whole blood assay was performed at 72 h after admission when the peak inflammatory response during STEMI is seen (10). Blood was collected in EDTA (ethylenediaminetetraacetic acid) tubes. A total of 250 μL whole blood was placed in 1.5-mL screw-top cryotubes containing 750 μL RPMI (Roswell Park Memorial Institute) tissue culture medium without any stimulants for spontaneous IL-6 production. Spontaneous IL-6 production reflects cytokine production as affected by the disease severity and the presence of concurrent medications. Additional tubes contained stimulants *Escherichia coli* LPS (1,000 ng/mL) to activate toll-like receptor (TLR)-4 and heat-killed *Staphylococcus epidermidis* (5 million bacteria per million white blood cells [WBCs]) for TLR-2 activation. All tubes were closed tightly and incubated at 37°C. After 24 h, cultures were lysed with Triton-X (0.5% final concentration) and frozen at –20°C.

After thawing, the lysates were assayed by using multiplex immunoassay (Human Cytokine Mosaic; R&D Systems, Minneapolis, MN, USA) in a Quansys Imager System (Quansys Biosciences, Logan, UT, USA). Complete WBC count and high-sensitivity CRP levels were also measured at the same time of whole blood cultures, as described (9). Data are expressed as pg/mL but normalized to cytokine per leukocytes. The data are presented as median and interquartile range, and the nonparametric Mann-Whitney test was used for the potential deviation from the Gaussian distribution associated with the small sample size. The analyses were completed by using SPSS, version 19.0 (IBM, Armonk, NY, USA).

Table 1. Characteristics of the patients.

	Placebo	Anakinra	*p* Value
Number of patients	10	7	
Age (years)	57 (48–80)	60 (52–65)	0.81
Gender (males)	10 (100%)	3 (43%)	0.015
Race (Caucasians)	6 (60%)	3 (43%)	0.64
Hyperlipidemia	5 (50%)	5 (71%)	0.62
Hypertension	7 (70%)	3 (43%)	0.35
Diabetes mellitus	3 (30%)	2 (28%)	1.0
Tobacco use	7 (70%)	3 (43%)	0.35
Infarct size (%)	24 (14–33)	22 (19–23)	0.43
LVEF (%)	59 (49–68)	64 (51–67)	0.88
WBCs (×1,000/mm^3)			
Admission	10.9 (7.2–13.9)	11.3 (10.4–13.5)	0.96
72 h	7.8 (7.2–10.3)	6.4 (4.9–10.7)	0.41
CRP (mg/L)			
Admission	3.8 (1.9–6.1)	4.2 (0.7–9.7)	0.96
72 h	30.9 (15.9–41.6)	2.8 (1.9–42.6)	0.088

Discrete variables are presented as number and percentage; continuous variables are presented as median and interquartile range. LVEF, left ventricular ejection fraction.

RESULTS

The clinical characteristics of the patients are reported in Table 1. The two groups had similar clinical characteristics with the exception of a significant imbalance in gender (3 [43%] males in the anakinra group compared with 10 [100%]; $p = 0.015$). WBC count and CRP levels were not statistically different between the two groups at admission (Table 1). The increase in CRP between admission and 72 h was significantly reduced in anakinra-treated patients compared with placebo-treated patients (+1 [–4/+19] versus +27 [+12/+37], $p = 0.022$, respectively).

Spontaneous IL-6 production in the whole blood cultures was significantly lower in anakinra-treated compared with placebo-treated patients, as expressed as absolute IL-6 amount ($p = 0.005$; Figure 1) or corrected per 1 million WBCs ($p = 0.010$; Figure 1). There was no correlation between the clinical variables listed in Table 1 and the IL-6 production (data not shown).

IL-6 production in response to LPS or to SE, on the other hand, was not statistically different between anakinra- versus placebo-treated patients expressed as absolute protein amount or corrected per million WBCs (all $p > 0.3$; Figure 1).

DISCUSSION

This study shows for the first time that IL-1 blockade with anakinra in patients with STEMI inhibits spontaneous leukocyte activity *ex vivo*, as revealed by spontaneous IL-6 production but without impairing the responsiveness to bacterial stimuli. We have recently reported that, in patients with STEMI, anakinra significantly blunted the acute systemic response measured as leukocyte count and CRP levels (8,9). Here we describe the effects of anakinra on leukocyte activity. IL-1 blockade with gevokizumab, a human monoclonal antibody that specifically neutralizes IL-1β but not IL-1α in patients with type 2 diabetes, revealed a reduction in the spontaneous production of IL-6 from leukocytes *ex vivo* (11). Accordingly, we report a significant inhibi-

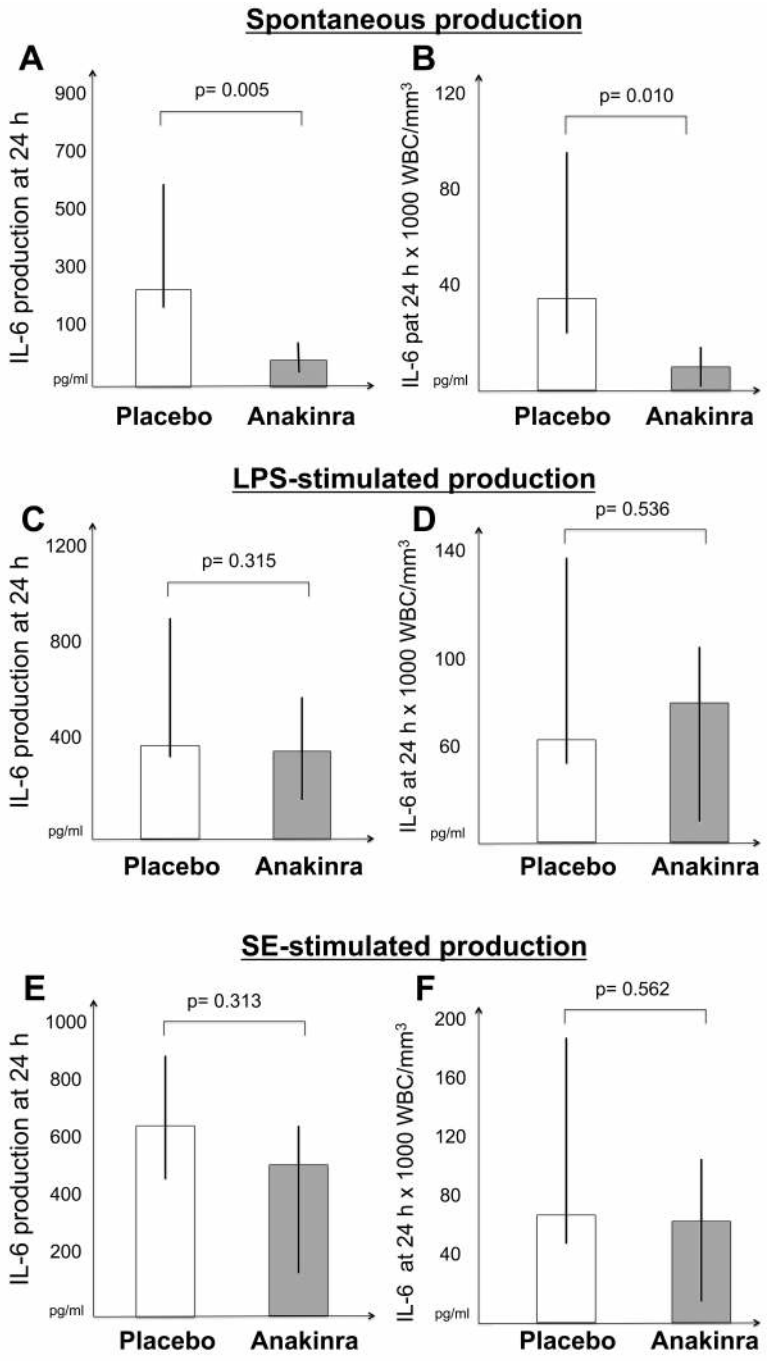

Figure 1. Leukocyte activity *ex vivo*. (A, B) IL-6 production from leukocytes in unstimulated conditions expressed as absolute IL-6 amounts or normalized per 1,000 leukocytes per mm^3, respectively. (C, D) IL-6 production in response to *Escherichia coli* lipopolysaccharide (LPS) 1,000 ng/mL expressed as absolute IL-6 amounts or normalized per 1,000 leukocytes per mm^3, respectively. (E, F) IL-6 production in response to heat-killed SE 1:1,000 dilution expressed as absolute IL-6 amounts or normalized per 1,000 leukocytes per mm^3, respectively.

tion of leukocyte activity in the anakinra-treated patients 72 h after admission for STEMI.

Despite the blunting of the systemic inflammatory response and inhibition of the spontaneous IL-6 production, the leukocyte reactivity to bacterial-derived stimuli was not impaired by anakinra treatment. Thus, leukocytes retain their ability to respond to bacterial products. IL-6 is an established biomarker of disease severity and, in this context, unimpaired IL-6 production implies unimpaired responses to bacterial infections from gram-negative and gram-positive organisms. An appropriate IL-6 response in leukocyte after LPS or SE stimulation requires an intact sensing and processing innate immune response comprising viable cells and the expression of functional toll-like receptors, signaling molecules like myeloid differentiation factor-88 or interleukin-1 receptor–associated kinase-4, and nuclear transcription factors (primarily the κB isoform) (12). As such the lack of impairment in such response with anakinra highlights how these mechanisms that are essential in the defense toward infections are not impaired. If the leukocyte response to exogenous stimuli is intact, yet the spontaneous production of IL-6 is reduced, in line with a reduced increase in CRP levels, this suggests that in STEMI anakinra blunts the acute inflammatory response secondary to the sterile myocardial injury (13).

Indeed, IL-1 induces cardiac dysfunction and cardiomyocyte death, and as such, anakinra may limit the effects of the ischemic injury and the ensuing acute sterile inflammatory response on the heart (7,14). This result is consistent with the finding of reduced incidence of heart failure with anakinra-treated patients with STEMI (8,9) and the reported improvements in cardiac function, even in patients with advanced heart failure and established injury (15,16).

The unimpaired response to bacterial stimuli in leukocytes *ex vivo* is also consistent with the lack of opportunistic infections in patients treated with anakinra (17–19). This result is in contrast to the use of tumor necrosis factor (TNF)-α blockers, which resulted in a significant and worrisome increase in opportunistic infections, death due to overwhelming sepsis and worsening heart failure (20–22). A direct comparison between

anakinra and TNF-α blockers in this *ex vivo* model is however lacking, and therefore conclusions should be drawn cautiously.

Similar to many pilot studies (23), this report has several inherent limitations. First, the small number of cases represents the major limitation, in particular considering that this is a subgroup of 17 cases from a randomized pilot study of 30 patients, and result in imbalances in the randomization. Second, we only explored one *ex vivo* time point (72 h) on the basis of the expected peak inflammation, but have no baseline or follow-up information on the leukocyte activity in these subjects. Third, we used IL-6 as a surrogate for leukocyte activity, because it is an easily and reliably assessable secondary cytokine often used for this purpose, but we cannot exclude imbalances in other more specific cytokine responses.

CONCLUSION

IL-1 blockade with anakinra is emerging as a safe antiinflammatory treatment in heart disease. In patients with STEMI, anakinra inhibits the spontaneous leukocyte activity *ex vivo* without impairing the leukocyte response to bacterial-derived stimuli.

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DISCLOSURE

CA Dinarello received consulting fees from Swedish Orphan Biovitrum. BW Van Tassell received research funds or served on advisory boards for Gilead and Novartis. A Abbate received research funds or served on advisory boards for Swedish Orphan Biovitrum, Gilead, Janssen, Novartis and XOMA.

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