Increased Production of Inflammatory Cytokines in Patients With Silent Myocardial Ischemia

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OBJECTIVES The aim of the study was to examine the inflammatory cytokines in patients with myocardial ischemia to evaluate whether silent ischemia patients exhibit any particular cytokine pattern.

BACKGROUND Silent myocardial ischemia is frequently observed in patients with coronary artery disease. Various endogenous mechanisms control a patient’s perceived intensity of pain. Among them, the inflammatory process and the related cytokine production are known to modulate the threshold for activating the primary afferent nociceptors.

METHODS Seventy-eight patients with reproducible exercise-induced myocardial ischemia were studied: 34 symptomatic patients, with rest and/or stress angina; 44 asymptomatic patients, with no symptoms during daily life activities or during positive exercise stress test. Venous blood samples were taken from all patients to evaluate the expression of CD11b receptors both on neutrophils and monocytes. Frozen plasma samples (at −80°C) were used to quantify the anti-inflammatory (interleukin-4 and -10, transforming growth factor-β) and the proinflammatory cytokines (tumor necrosis factor-α, interferon-γ, interleukin-1β and -6).

RESULTS In asymptomatic patients, lower CD11b receptor expression and higher concentration of anti-inflammatory cytokines were observed. Proinflammatory cytokine production was similar in the two groups.

CONCLUSIONS The data suggest that an “anti-inflammatory pattern” of cytokine production correlates with silent ischemia and that the immune and inflammatory system activation may be crucial for angina symptoms. (J Am Coll Cardiol 2001;38:1895–901) © 2001 by the American College of Cardiology

Immunocytes and cytokines are known to play a key role in the pathogenesis of the atherosclerotic process and coronary artery disease (CAD) (1,2). Silent myocardial ischemia frequently occurs at rest, during daily life activities or after physical or emotional exertion (3–6); moreover, history of anginal pain might not be reported after acute myocardial infarction (MI) or during an angioplasty-induced coronary occlusion (7,8). The mechanisms responsible for silent ischemia are not well understood, and individual differences in pain threshold may only partially explain the variability in pain perception (3–6). Patients with silent ischemia usually show a generalized hyposensitivity and higher pain threshold as compared with symptomatic patients (5–8); production of the endogenous opiates may be partially responsible for decreased perception of pain (3).

The relationship between inflammatory process and atherosclerosis has been extensively debated, but no data have yet been reported to describe the link between inflammatory system activation and silent myocardial ischemia. All stages of the atherosclerotic process are characterized by systemic endothelial dysfunction with subsequent endothelial damage and inflammation (1,2); these mechanisms are also involved in the atherosclerotic plaque instability and rupture (1,9–12), which may occur in acute ischemic event. Several other studies suggest that inflammatory cytokines may directly damage the endothelial surface, leading to ischemia, underlining the direct link between proinflammatory cytokine blood concentrations and cardiovascular risk (13–15).

Cytokine production also correlates with clinical manifestations of acute coronary syndrome (14,15). Local vascular and perivascular inflammation occurs within the injured tissue. Immune recruitment results from a multistep, sequential engagement of various adhesion molecules (1,9,11,16,17). The activated phagocytes (granulocytes and monocytes) express the CD11b/CD18 adhesion molecule, thus mediating their sticking adhesion to the endothelial cells and leading to phagocyte chemotaxis toward the inflamed tissue.

In addition, the CD11b/CD18 binds to the activated complement factor (C3a), enhancing the inflammatory process, and recognizes fibrinogen-coated surfaces, leading to neutrophil-clot adhesion and fibrin digestion (11,17). Within the inflammatory site, the activated phagocytes release the cytokines interferon-gamma (IFN-γ), interleukin (IL)-4, IL-10 and transforming growth factor-beta (TGF-β), which further enhance cellular migration; other proinflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)-α and IL-6 are known to be released within inflamed tissue (18–20). Peripheral inflammation stimulates peripheral nerve endings causing hyperalgesia, which is due to enhanced localized inflammatory mediators (18,19); the local release of cytokines and endogenous opioids might be able to modulate the threshold for peripheral nerve-ending activation. Pain perception may result from microenviron-

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Abbreviations and Acronyms

CAD = coronary artery disease
CGRP = calcitonin gene-related peptide
ECG = electrocardiogram or electrocardiographic
IFN-γ = interferon-gamma
IL = interleukin
MAb = monoclonal antibodies
MESF = molecules of equivalent soluble fluorescein
MI = myocardial infarction
NF-κB = nuclear factor-κB
PBR = peripheral benzodiazepine receptor
SP = substance P
TGF-β = transforming growth factor-beta
TNF-α = tumor necrosis factor-alpha

In our study we investigated inflammatory cytokines in patients with transient myocardial ischemia to determine whether silent ischemia correlates with any particular pattern of cytokine production. Inflammatory system activation markers were detected in patients with symptomatic angina and in patients with silent myocardial ischemia episodes. The CD11b adhesion molecule expression was detected on phagocytes, and proinflammatory (IL-1β, TNF-α, IL-6, and IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines (19,20).

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METHODS

Patient selection. Seventy-eight male patients were studied: 34 patients with anginal pain during daily life activities (group P) and 44 patients with silent ischemia (group A). Two positive exercise stress tests documenting transient myocardial ischemia were performed in each patient. Patients who always refer anginal symptoms were enrolled in group P; patients who do not complain of anginal pain were enrolled in group A. Only male patients were studied. Patients with intercurrent inflammatory conditions, and patients who were on nonsteroidal anti-inflammatory drugs and steroids, were excluded. Pharmacologic washout was performed using standardized fluorescein microspheres (quantum 26 FITC 7.3-μm diameter hydrophobic microspheres, FACS, Research Triangle Park, North Carolina, obtained by Becton Dickinson, Milan, Italy).

Cytokine assay. Quantitative measurement of IL-1β, IL-4, IL-6, IL-10, TNF-α and IFN-γ was performed using a solid-phase sandwich ELISA test with microtiter plate precoated with the specific MAb (Biosource, Celsbio, Milan, Italy). Quantitative measurement of TGF-β1 was performed using a solid-phase enzyme amplified sensitivity immunoassay with microtiter plate precoated with the specific MAb (Biosource; Celsbio, Milan, Italy). Tests were performed according to the supplier’s instructions. Patient sample and cytokines standard samples were assayed simultaneously, in duplicate. The standard curve for the IL-1β assay ranged from 0.31 to 20 pg/ml; for IL-4 it was 0.39 to

mental balances between proinflammatory (IL-1β, TNF-α, IL-6, and IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines (19,20).

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Seventy-eight patients (mean age 62.5 ± 8.6 years) were included in the study. Group P consisted of 34 anginal patients: 19 patients with effort angina, 4 patients with angina at rest and 11 patients with mixed angina. Silent ischemia was documented during exercise stress test in 44 patients (group A); 27 patients with silent ischemic episodes during daily life activities and documented by Holter recording were also included within group A. Clinical data are reported in Table 1. There were no statistically significant differences in age, ECG-documented severity of angina, hypertension, hyperlipidemia, diabetes and smoking. Coronary angiography results were similar within the two groups. All patients in group P complained of angina during exercise test; moderate to severe dyspnea was experienced during exercise stress test in group A patients. The entity of ST-segment depression at peak exercise was similar in the two groups. No difference was found in the CD11a/CD18 expression on monocytes. The CD11b/CD18 expression was lower in asymptomatic patients both on granulocytes and monocytes (p < 0.01) (Fig. 1). In symptomatic patients, MESF values (× 10⁷/cells) were 68.1 ± 7 for granulocytes and 80.8 ± 6 for monocytes; in asymptomatic patients, MESF values (× 10⁷/cells) were 56.4 ± 7 for granulocytes and 48.4 ± 8 for monocytes. In asymptomatic patients, anti-inflammatory cytokine levels were significantly higher: IL-4, 0.634 ± 0.062 ng/ml vs. 1.790 ± 0.195 ng/ml, p < 0.005; IL-10, 5.804 ± 0.364 ng/ml vs. 15.350 ± 1.129 ng/ml, p < 0.001; TGF-β, 2.511 ± 0.232 ng/ml vs. 9.671 ± 0.533 ng/ml, p < 0.001 (Fig. 2A). Proinflammatory cytokine concentrations were similar in the two groups: IL-1β, 0.376 ± 0.027 ng/ml vs. 0.412 ± 0.025 ng/ml, p = NS; IL-6, 2.581 ± 0.534 ng/ml vs. 2.805 ± 0.426 ng/ml, p = NS; TNF-α, 5.746 ± 0.401 ng/ml vs. 5.733 ± 0.322 ng/ml, p = NS; IFN-γ, 26.362 ± 2.193 pg/ml vs. 24.860 ± 3.406 pg/ml, p = NS (Fig. 2B).

**RESULTS**

Our data show that, patients with asymptomatic ischemia, there is higher production of anti-inflammatory cytokines with lower expression of CD11b/CD18 adhesion molecule on phagocytes. These observations may indicate that the clinical feature of silent myocardial ischemia might correlate with a particular biochemical pattern of inflammatory system activation.

**Inflammation opioid system and peripheral benzodiazepine receptors.** Inflammation is involved in the pathogenesis of atherosclerotic process, in plaque instability and rupture and in tissue injury during MI. In this regard, several studies showed that unstable and complicated atherosclerotic plaque might result from recruitment of neutrophils and monocytes within the plaque owing to local

| Table 1. Clinical, Angiographic and Ergometric Data in Symptomatic (Group P) and Asymptomatic Patients (Group A) With Coronary Artery Disease |
|--------------------------------------------------|-----------------|---------|
| **Clinical features**                             | **Group P** (n = 34) | **Group A** (n = 44) | **p Value** |
| Mean age (yrs ± SD)                               | 60.7 ± 8.6       | 64.9 ± 9.4 | NS        |
| Arterial hypertension                            | 4                | 6        | NS        |
| Hyperlipidemia                                   | 10               | 14       | NS        |
| Diabetes mellitus                                | 3                | 4        | NS        |
| Smoking                                          | 9                | 12       | NS        |
| **Coronary angiography**                         |                  |          |           |
| One- vessel disease                              | 10               | 7        | NS        |
| Multivessel disease                              | 14               | 26       | NS        |
| Ejection fraction (%) ± SD                       | 58.1 ± 10.2      | 60.1 ± 9.4| NS        |
| LVEDV (ml) ± SD                                  | 115 ± 26         | 124 ± 21 | NS        |
| LVEDP (mm Hg) ± SD                               | 16.4 ± 8.1       | 14.6 ± 3.9| NS        |
| **Exercise stress test**                         |                  |          |           |
| Baseline RPP (beats/min × mm Hg) ± SD            | 9.243 ± 2.232    | 9.623 ± 2.056| NS        |
| Peak RPP (beats/min × mm Hg) ± SD                | 21.043 ± 4.585   | 22.347 ± 6.573| NS        |
| Peak ST depression (mm) ± SD                     | 1.92 ± 1.51      | 2.24 ± 1.82| NS        |

LVEDV = left ventricular end-diastolic volume; LVEDP = left ventricular end-diastolic pressure; RPP = rate-pressure product.
production of inflammatory metabolites and cytokines (9–12). The local inflammatory reaction further mediates tissue damage and may be responsible for the modulation of the pain transmission pathways (1,2,10,13,15). Immunocytes locally release inflammatory cytokines as well as beta-endorphins, thus interfering with the endogenous opioid-mediating system (3,4,8,20,21–27). The resulting inflammatory microenvironmental production may alter the threshold for activation of peripheral nerve endings (1,2,10,13,15). Higher threshold of pain transmission pathway activation has been demonstrated during strenuous exercise and after physical training due to increased plasma levels of endogenous opioids (see beta-endorphins), as part of physiological stress response (4,8).

During myocardial ischemia, the endogenous opioids, which derive from local immune cells, interact with specific receptors on sensory nerves, leading to strong and clinically measurable analgesia (5,21,22,24–27). It has also to be stressed that morphine, which is the best pain-relieving drug currently available for cardiac patients, is able to decrease leukocyte activation and to exert anti-inflammatory properties (see beta-endorphins), as part of physiological stress response (4,8).

Several observations suggest that inflammatory cytokines orchestrate nociception and also suggest the role of proinflammatory and anti-inflammatory cytokines in the perception of pain. Particularly, proinflammatory cytokines are known to induce the release of pain mediators, such as bradykinin and calcitonin gene-related peptide (CGRP) and to activate vagal afferents (37). The IL-1β is a proinflammatory cytokine able to induce hyperalgesia. Central administration of IL-1β enhances the nociceptive neuronal response (38–40) and, in monoarthritic rat, dose-dependently enhances the spinal-cord–evoked release of substance P (SP) and CGRP (41). By contrast, the IL-1 receptor antagonist attenuated the IL-1β–induced hyperal-
necrosis factor-alpha. Pentoxifylline was demonstrated to inhibit TNF-alpha. Size prostaglandins (43), In experimental inflammation, pentoxifylline was demonstrated to inhibit TNF-alpha release, with consequent decrease of pain-related behavior (44).

By contrast, anti-inflammatory cytokine IL-10 administration was demonstrated to reverse the dynorphin-induced allodynia, which is a model of neuropathic pain in the mouse, thus suggesting that allodynia might be modulated by the inflammatory cytokine system (45). The IL-10 inhibits the positive inflammatory feed-forward loop by inhibiting the initial induction and the subsequent amplification of proinflammatory cytokines (46). A single peripheral administration of IL-10 was demonstrated to decrease the nerve injury-induced thermal hyperalgesia (46,47) and the intradermal endotoxin-induced hyperalgesia (48). Our data show that IL-10 levels increase significantly in asymptomatic patients. Transforming growth factor-beta stimulates connective tissue growth and collagen formation, and it can virtually and strongly inhibit all the immune and hematopoietic functions, especially if present before cell activation (49,50). It also has a role in mediating inflammation and cytotoxic reactions (49–51): TGF-beta blocks the IFN-gamma-induced cell activation, thus increasing the production of ROS, prostaglandins and nitric oxide; IL-4 has similar effects to that of TGF-beta in limiting the inflammatory hyperalgesia (51,52).

Role of the transcription factor nuclear factor-kappaB. Cytokine production is primarily regulated at the transcriptional level (53,54) by specific transcriptional factors linking the receptor-driven cytoplasmic signaling events with changes in gene expression. The nuclear factor-kappaB (NF-kappaB) activation induces nociceptive protein production such as proinflammatory cytokines, inducible nitric oxide synthase (S), cyclo-oxygenase (COX-2) and pre-prodynorphin. Several stimuli, such as proinflammatory cytokines, oxidative stress, nerve growth factor, and protein kinase C, can activate NF-kappaB (53–56). The relevance of NF-kappaB to nociception is supported by the observation that hyperalgesic doses of nerve growth factor in vivo activate NF-kappaB in dorsal root ganglia (56). Moreover, capsaicin-induced hyperalgesia associates with enhanced expression of NF-kappaB (56). Anti-inflammatory cytokine administration (such as IL-10) correlates with NF-kappaB reduced activity and with decreased intensity of pain perception (47). Our data may suggest that alterations of anti-inflammatory cytokine production might exert an inhibitory effect on NF-kappaB, which is crucial for nociception, leading to an ischemic angina episode.

Study limitations. Our results showed that an anti-inflammatory pattern of cytokine production is activated in patients with silent myocardial ischemia. The data suggest that silent ischemia might result from a particular microenvironmental pattern of inflammatory system activation leading to higher threshold for the stimulation of peripheral nerve endings. The findings should be interpreted cautiously. Cytokines have a very short half-life in the bloodstream, and their biologic effects seem to be more related to the length of cytokine exposure than to their absolute circulating concentrations; in addition, cytokine levels were detected only once in the study, and our observational study did not include any follow-up controls. Sequential measurement of circulating cytokines and clinical correlation with patients’ symptoms and illness stages are necessary to further confirm our data. Moreover, we lack a biologic model describing stimuli able to cause Th1 or Th2 activation in cardiovascular patients, and we did not investigate whether...
a particular pattern of cytokines might correlate with clinical outcome. In addition, we also cannot exclude the idea that inflammatory cell activation and cytokine release are epiphenomena occurring during myocardial ischemia, without any pathogenetic implication with the basic illness. Moreover, pain perception might only depend on individual pain threshold and/or other unknown mechanisms. Nevertheless, the positive association described between silent myocardial ischemia and anti-inflammatory cytokine production cannot be excluded.

Conclusions. The significant increase of levels of anti-inflammatory cytokines together with the decrease of leukocyte adhesion molecule expression might identify one of the mechanisms for silent ischemia. In patients with silent ischemia it might be possible that the Th2 activation will induce higher production of anti-inflammatory cytokines together with endogenous opioid production and higher expression of PBRs. The anti-inflammatory cytokines might be able to block the pain transmission pathway activation by increasing the threshold for nerve activation. In addition, the anti-inflammatory cytokines will exert a prolonged inhibitory effect on transcription factor NF-κB, leading to reduction and/or elimination of pain-mediating substances. Further studies are needed to explain the effects of these cytokines on clinical progression and prognosis and on the atherosclerotic process. These findings might also indicate that immune system cytokine production can modulate nociception, thus providing us with data on widespread clinical implications.

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REFERENCES