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Local Cytokine Concentrations and Oxygen Pressure Are Related to Maturation of the Collateral Circulation in Humans

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Objectives Our aim was to determine cytokine and oxygen gradients over the collateral circulation in humans.

Background The molecular background of the maturation of the collateral circulation in response to coronary narrowing is poorly understood in humans, partly because of difficulties in obtaining local samples from the human collateral circulation.

Methods Coronary collateral blood was sampled in 60 patients with nontotal ($n = 25$) or total coronary occlusions ($n = 35$) using a special wide-lumen catheter. Coronary collateral flow index (CFI) was assessed by intracoronary pressure measurements. Oxygenation and lactate content was measured as well as 30 cytokines potentially involved in collateral artery growth, using a custom-made multiplex assay.

Results No rise in lactate or change in pH was found in collateral blood. Oxygen gradient between coronary and collateral arterial blood correlated inversely with CFI ($r = -0.61$, $p < 0.001$). Locally increased plasma levels were found for basic fibroblast growth factor, eotaxin, macrophage migration inflammatory factor, monocyte chemoattractant protein-1, and transforming growth factor-beta, while stem cell factor and stem cell growth factor-beta were significantly decreased. The highest cytokine gradients were found in patients with the least developed collateral circulation. The majority of cytokines correlated more strongly with the pO_2 gradient across the collateral bed than with CFI.

Conclusions Intravascular ischemia during brief balloon coronary occlusion is absent in human coronary collateral arteries. The oxygen gradient found over the collateral circulation is increased in patients with a less matured collateral circulation and relates to local levels of several known pro-arteriogenic cytokines. In case of a more developed collateral circulation, relatively low levels of cytokines are present, suggesting that growth factor therapy might be beneficial at this stage. (J Am Coll Cardiol 2009;53:2141–7) © 2009 by the American College of Cardiology Foundation

Although mechanisms of arteriogenesis have been well elucidated in experimental models, knowledge of cytokines and growth factors that mediate collateral artery growth in humans is still limited (1). Also, the role of ischemia in human collateral artery growth is presently unknown. Local plasma sampling directly from the human coronary collateral circulation can provide such data. This is cumbersome, however, because of the poor accessibility of the collateral circulation for blood sampling. Therefore, previous studies have investigated systemic cytokine levels in patients with different degrees of coronary collateralization. These studies have provided conflicting results, probably due to the fact that systemic levels of growth factors do not reliably reflect the local process of collateral artery growth (2,3). Direct

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Abbreviations and Acronyms

bFGF = basic fibroblast growth factor
CAD = coronary artery disease
CFI = collateral flow index
CTO = chronic total occlusion
CVP = central venous pressure
ELISA = enzyme-linked immunosorbent assay
GM-CSF = granulocyte-macrophage colony-stimulating factor
HGF = hepatocyte growth factor
IL = interleukin
MCP = monocyte chemoattractant protein
MIF = macrophage migration inflammatory factor
MIP = macrophage inflammatory protein
TGF = transforming growth factor
TNF = tumor necrosis factor

sampling from the collateral circulation, as performed in previous studies, yielded only a small volume of blood in a limited percentage of patients. Such small blood volumes have nevertheless allowed the analysis of a few cytokines showing increased levels of basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-beta in the coronary collateral circulation (4,5).

In the present study, to allow analysis of a wide array of cytokines potentially involved in human arteriogenesis, we have used the Proxis catheter (St. Jude Medical, St. Paul, Minnesota), a wide-lumen suction catheter originally developed as a proximal embolic protection device (6), to obtain larger amounts of blood from the collateral circulation. In addition to plasma levels of cytokines, the use of this catheter facilitated additional blood gas and lactate analyses of locally sampled collateral blood, which have not been performed previously in human coronary collateral arteries. Together with the analysis of plasma cytokines, these metabolic parameters can provide valuable insights into the growth and functional status of the collateral circulation in response to epicardial obstructions.

Methods

Patient selection. This study was approved by the medical ethics committee of the Academic Medical Center, Amsterdam, the Netherlands. Sixty Caucasian patients scheduled for elective percutaneous coronary intervention for stable coronary artery disease (CAD) with symptoms of angina pectoris for ≥4 weeks were included after giving informed consent. Patients with a subtotal stenosis (≥70%) were selected if they had single-vessel CAD; patients with chronic total occlusions (CTOs) had single-vessel or multivessel disease. Exclusion criteria were previous myocardial infarction in the area of collateralization, previous cardiac surgery, severely depressed left ventricular function, diabetes mellitus, neoplastic disease, and signs of inflammatory illness.

Intracoronary instrumentation. After pre-dilation of the stenosis, a 7-F (inner diameter 0.051 inches) proximal embolic protection device (Proxis catheter, St. Jude Medical) was advanced through the guiding catheter and into the treated coronary artery, which received collateral blood. Inflating a low-pressure (0.67 atm) balloon at the end of the catheter temporarily stopped antegrade blood flow in the artery. Complete obstruction of antegrade blood flow was ensured in a pilot study of 5 patients showing no contrast dye entering the epicardial artery after balloon occlusion (data not shown). Subsequently, gentle suction was applied manually to the proximal end of the catheter. The first 3 ml aspirated was discarded to prevent contamination with contrast medium or with blood already in the epicardial vessel before balloon occlusion (and thus not from the collateral circulation). Approximately 5 to 15 ml blood (depending on collateral flow) was then aspirated within 60 s. After deflation of the balloon, the catheter was withdrawn into the guiding catheter to obtain a control sample from the coronary circulation. Blood was transferred into citrate tubes and centrifuged at 1,550 *g* for 30 min. Plasma was taken off carefully, aliquoted, snap frozen in liquid nitrogen, and stored at −80°C. Collateral flow index (CFI) was measured as previously published (7).

Oxygen, pH, and lactate measurements. Immediately after aspiration, partial oxygen (pO_2) and carbon dioxide pressure (pCO_2), pH, and saturation were measured from 1 sample per site per patient on a Rapidlab 865 (Siemens, Germany). Lactate concentrations were measured using a standard clinical chemistry test.

Multiplex assay for the measurement of cytokine concentrations. A custom-made panel of 30 cytokines was measured using the Bio-Plex suspension array system (Bio-Rad Laboratories, Hercules, California) according to the manufacturer's instructions as previously described (8). The panel consisted of interferon-alpha-2, hepatocyte growth factor, macrophage colony-stimulating factor (MCSF), monocyte inflammatory factor (MIF), monokine induced by gamma-interferon, nerve growth factor-beta, stem cell factor, stem-cell growth factor-b, stromal cell-derived factor-1a, tumor necrosis factor (TNF)-alpha, TNF-beta, TNF-related apoptosis inducing ligand, interleukin (IL)-1b, IL-4, IL-6, IL-8, IL-10, IL-16, eotaxin, bFGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma, interferon-gamma-inducible protein-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1α), MIP-1β, platelet-derived growth factor, beta chain, regulated on activation, normal T expressed and secreted (RANTES), and vascular endothelial growth factor. Plasma samples were thawed at room temperature, diluted 1:1 in the manufacturer's sample diluent, and incubated with bead-bound antibodies to previously mentioned cytokines for 60 min on a 96-well filter plate. After repeated washing, biotinylated detection antibody was added, and the plate was incubated for another 60 min. Finally, streptavidin-PE (phycoerythrin) was pipetted onto each well. After a short incubation, the constituents of each well were drawn up into the flow-based Bio-Plex suspension array system, which identifies and quantifies each cytokine concentration based on bead color and fluo-

rescence. Data were analyzed using Bio-Plex Manager software, version 3.0 (Bio-Rad Laboratories).

Enzyme-linked immunosorbent assays (ELISAs). Because human interferon-beta and TGF-beta were not represented on our multiplex assay, we tested concentrations of these cytokines using commercially available ELISA kits (Quantikine ELISA, R&D Systems, Minneapolis, Minnesota, and PBL Interferonsource, Piscataway, New Jersey) according to the manufacturer's instructions. Since the concentrations of bFGF were often found to be too low to be properly detected with the multiplex system, a high sensitivity ELISA assay was performed (HS Quantikine ELISA, R&D Systems) according to the manufacturer's instructions. Briefly, samples were incubated on a 96-well plate coated with the appropriate antibody. After the necessary washing steps, a biotinylated antibody and subsequently streptavidin-conjugated horseradish-peroxidase were linked to the adherent probes. Using tetramethylbenzidine as a substrate, absorption was measured at 450 nm in an EL808 spectrophotometer (BioTek, Winooski, Vermont).

Statistical analysis. Clinical characteristics are presented as mean ± SD or median and interquartile range for quantitative variables and as observed numbers (%) for nominal variables. Fisher exact test was used for testing association in 2 × 2 contingency tables. From pO_2, oxygen gradient was calculated as: $pO_{2\ gradient} = pO_{2\ coronary} - pO_{2\ collateral}$. pCO_2 gradient was calculated as: $pCO_{2\ gradient} = pCO_{2\ coronary} - pCO_{2\ collateral}$. Cytokine gradients were calculated as: $concentration_{collateral} - concentration_{coronary}$. Oxygen and cytokine gradients were calculated on a logarithmic scale to correct for skewed distributions. Oxygen and cytokine concentrations are shown as median (interquartile range). Comparisons among 2 groups were performed by Student *t* test for normally distributed parameters and Wilcoxon test for non-normally distributed parameters. Statistics on cytokine concentrations were corrected for multiple testing using Benjamini-Hochberg correction (9). False discovery rates were calculated and expressed as percentage. Comparisons between 3 groups were performed by analysis of variance testing. Correlations were calculated using a Pearson correlation.

Results

Patient characteristics. Sixty patients were included in the study. Patients were age 61.0 ± 11.4 years, and CFI ranged from 0.09 to 0.65 (mean value 0.31 ± 0.12). Thirty-five patients had a CTO of a coronary artery, while 25 had a subtotal stenosis (ranging from 60.1% to 91.5% diameter stenosis in quantitative coronary angiography). All lesions undergoing intervention were proximal lesions, resulting in arterial diameters of 3.0 to 4.0 mm. All CTO patients had sufficient collateralization to prevent ischemia during balloon occlusion as indicated by absence of ST-segment deviation, and no symptoms of angina pectoris at rest. Patients with a nontotal occlusion were grouped into collateral responders (CFI >0.21, n = 13) and nonresponders (CFI ≤0.21, n = 12). ST-segment elevation during balloon occlusion (shown as median [interquartile range]) was significantly ($p < 0.001$) different between collateral responders (0.0 [1.0] mm) and nonresponders (2.0 [2.0] mm). Clinical baseline characteristics did not correlate with CFI and were not different between the 3 groups (nonresponders, responders, CTOs) (Table 1).

Metabolic parameters in the coronary collateral circulation. Oxygen pressure measurements were performed in 45 patients in total in this study. In the complete patient group, average pO_2 was found significantly lower in the collateral sample than in the coronary sample (10.2 [2.75] kPa vs. 11.3 [2.4] kPa; $p < 0.001$). In contrast, lactate concentrations were equal in coronary and collateral blood (0.94 mmol/l vs. 0.91 mmol/l, p = NS), and also pH did not differ significantly (coronary: 7.42, collateral: 7.41, p = NS). Lactate and pH remained unchanged in collateral versus coronary blood in collateral nonresponders, responders, and patients with CTOs. There was no significant correlation between CFI and lactate ($r = 0.19$, $p = 0.29$) or CFI and pH ($r = 0.12$, $p = 0.44$). Also, pCO_2 was not found different between proximal and distal sampling sites (5.18 vs. 5.17 kP, p = NS).

Comparing the 3 patient groups, nonresponders showed a significantly higher oxygen gradient than responders or CTOs (2.24 [2.0] kPa vs. 1.63 [1.0] and 0.95 [0.77] kPa, $p = 0.003$). In the patient group as a whole, a significant inverse correlation was found between pO_2 gradient and CFI ($r = -0.61$, $p < 0.001$) (Fig. 1). Similarly, an inverse correlation was found between oxygen saturation ratio and CFI ($r = -0.46$, $p = 0.002$). Conversely, the pCO_2 gradient did not correlate with CFI or oxygen gradient ($r = 0.17$, p = NS) (Fig. 2).

Local cytokine concentrations in the collateral circulation. In the complete patient population, eotaxin, MCP-1, MIF, bFGF, and TGF-beta were found in significantly higher plasma concentrations in the collateral specific samples as compared with the coronary samples, while stem cell factor and stem cell growth factor-beta were significantly down-regulated (Table 2). Other cytokines measured did not show significant differences between collateral and coronary samples. Interferon-beta was not detectable in either the coronary or the systemic sample in the majority of patients (35 of 60 patients).

Dividing patients into the 3 groups (nonresponders, responders, CTOs) resulted in the highest collateral-coronary gradients for all of the previously mentioned cytokines in the nonresponders, followed by the responders, while patients with CTOs displayed smallest concentration differences (Fig. 3).

Group-specific cytokine profiles in relation to CFI and oxygen gradient. In the patient group with nontotal occlusions, a positive correlation with oxygen gradient between

Table 1 Baseline Characteristics

	Nonresponders (CFI ≤0.21, n = 12)	Responders (CFI >0.21, n = 13)	CTOs (n = 35)	p Value
Age, yrs	62.8 ± 13.5	62.6 ± 11.4	59.8 ± 10.9	0.63
Male sex	9 (75)	8 (61.5)	24 (68.8)	0.80
Body mass index, kg/m^2	27.2 ± 2.6	27.0 ± 2.8	27.1 ± 4.2	0.99
Body surface area, m^2	2.0 ± 0.2	2.0 ± 0.1	2.0 ± 0.3	0.71
Hypertension	7 (58.3)	8 (61.5)	22 (62.9)	0.92
Hypercholesterolemia	5 (41.7)	8 (61.5)	15 (42.9)	0.54
Family history of CAD	10 (83.3)	8 (61.5)	19 (54.3)	0.24
Current smoker	4 (33.3)	1 (7.7)	5 (14.3)	0.44
Ex-smoker	6 (50)	7 (53.8)	19 (54.3)	0.44
Weeks anginal symptoms*	26 (12, 73)	12 (6, 65)	25 (11, 68)	0.41
Beta-blockers	11 (91.7)	10 (76.9)	27 (77.1)	0.53
Statins	10 (83.3)	12 (92.3)	31 (86.6)	0.78
Aspirin	11 (91.7)	12 (92.3)	32 (91.4)	1.0
Clopidogrel	6 (50)	11 (84.6)	22 (62.9)	0.18
Calcium antagonists	5 (41.7)	3 (23.1)	8 (22.9)	0.42
Nitrates	6 (50)	6 (46.2)	19 (54.3)	0.88
ACE inhibitors/ARBs	4 (33.3)	4 (30.8)	15 (42.9)	0.70
Diameter stenosis (QCA) (%)	72.4 ± 9.4	74.9 ± 9.2	100 ± 0.0	<0.001
C-reactive protein, mg/dl*	1.75 (0.7, 6.3)	1.4 (0.8, 3.5)	1.5 (1.0, 3.3)	0.75
Hemoglobin, mmol/l	8.63 ± 0.61	8.75 ± 0.42	8.75 ± 0.65	0.84
Leukocytes, 10^9/l	6.6 ± 1.9	7.2 ± 1.3	6.9 ± 1.7	0.62
Total cholesterol, mmol/l	4.0 ± 0.9	3.9 ± 0.8	4.2 ± 0.9	0.63

Clinical characteristics were comparable between the 2 groups. Values are presented as n (%) or mean ± SD unless otherwise indicated. *Indicates non-normal distribution; these values are presented as median (first quartile, third quartile).

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CAD = coronary artery disease; CFI = collateral flow index; CTO = chronic total occlusion; QCA = quantitative coronary angiography.

coronary and collateral arterial blood was found for coronary-collateral gradients of MCP-1, eotaxin, MIF, MIP-1β, and hepatocyte growth factor. Conversely, a negative correlation with oxygen gradient was found for gradients of nerve growth factor-beta and TNF-related apoptosis-inducing ligand (TRIAL) (Table 3). No cytokine gradient correlated significantly with CFI (data not shown) in this group. After correction for multiple testing, none of the coronary-collateral cytokine gradients correlated with oxygen gradient (false discovery rate >25%) or CFI in patients with CTO.

Discussion

In this study on the molecular background of the maturation of the coronary collateral circulation in man, no changes in

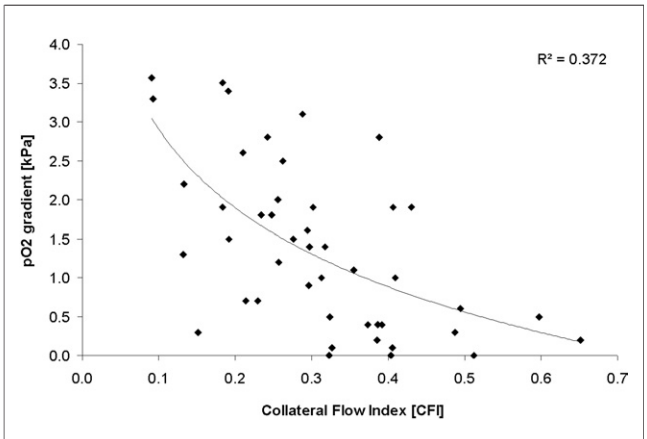

Figure 1 Oxygen Gradient Correlates With CFI

Partial oxygen (pO_2) gradient is plotted against collateral flow index (CFI) in the whole patient group. Patients with adequately developed collateral arteries demonstrated low oxygen gradient, while collateral nonresponders had a high oxygen gradient, resulting in a significant correlation.

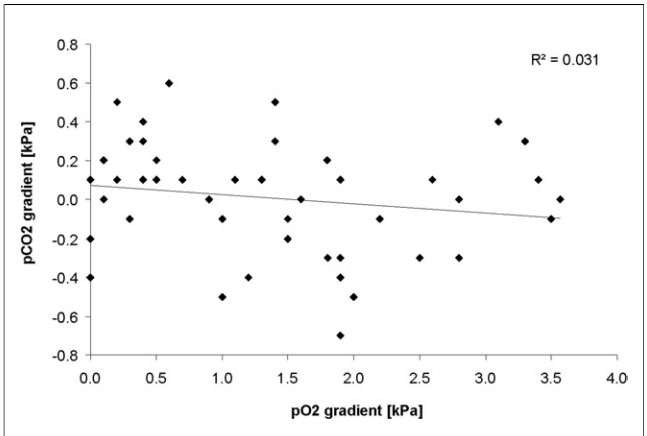

Figure 2 Lack of Correlation Between Oxygen Gradient and Carbon Dioxide Gradient

In contrast to pO_2, partial carbon dioxide (pCO_2) gradient over the collateral circulation does not correlate with CFI. As shown in this figure, pCO_2 gradient also did not correlate with oxygen gradient over the collateral circulation ($p = 0.25$). Abbreviations as in Figure 1.

Table 2 Cytokine Concentrations in the Collateral and Coronary Samples (in pg/ml)

Cytokine	Coronary	Collateral	Gradient	Log-Ratio	FDR (%)
bFGF	3.32 (4.18)	10.49 (9.69)	6.82 (9.86)	2.09 (2.07)	<0.1
MIF	91.75 (57.35)	111.02 (80.88)	22.25 (65.77)	0.37 (0.86)	0.2
Eotaxin	20.89 (32.14)	30.65 (50.78)	3.48 (18.02)	0.20 (0.92)	0.2
MCP-1	14.01 (4.31)	15.85 (9.95)	1.57 (5.14)	0.16 (0.47)	0.2
TGF-beta	3,830.62 (1,892.84)	4,161.25 (2,815.69)	493.25 (1,133.53)	0.19 (0.44)	2
SCF	359.50 (143.75)	283.84 (155.55)	−29.26 (72.83)	−0.14 (0.32)	<0.1
SCGF-beta	2,624.00 (1,646.38)	2,349.57 (1,602.63)	−83.98 (905.27)	−0.07 (0.65)	2

Data are reported as median (interquartile range). Enhanced concentrations of basic fibroblast growth factor (bFGF), macrophage migration inflammatory factor (MIF), eotaxin, monocyte chemoattractant protein (MCP)-1, and transforming growth factor (TGF)-beta were found in collateral plasma, while stem cell factor (SCF) and stem cell growth factor (SCGF)-beta were found to be decreased. The table displays cytokine gradients in the complete patient group (n = 60). Please note that due to calculations of the median values and interquartile range, the gradient is not equal to the difference of the median collateral and the median coronary cytokine concentration. The second column to the right indicates log2-transformed values that have been used for Figure 3.

levels of pH or lactate were found in the collateral circulation, showing that no intravascular ischemia is present in the human collateral circulation. While pCO_2 and pH were unchanged in collateral versus systemic blood, pO_2 was significantly lower in collateral as compared with coronary blood samples, suggesting local oxygen extraction in the collateral circulation. Oxygen gradient between coronary and collateral arterial blood correlated with CFI. Eotaxin, bFGF, MCP-1, MIF, and TGF-beta were found selectively increased in collateral blood plasma, and cytokine levels in the collateral circulation correlated well with oxygen gradient. Collateral cytokine gradients were strongest in patients with insufficient collateral arteries and almost absent in patients with CTOs.

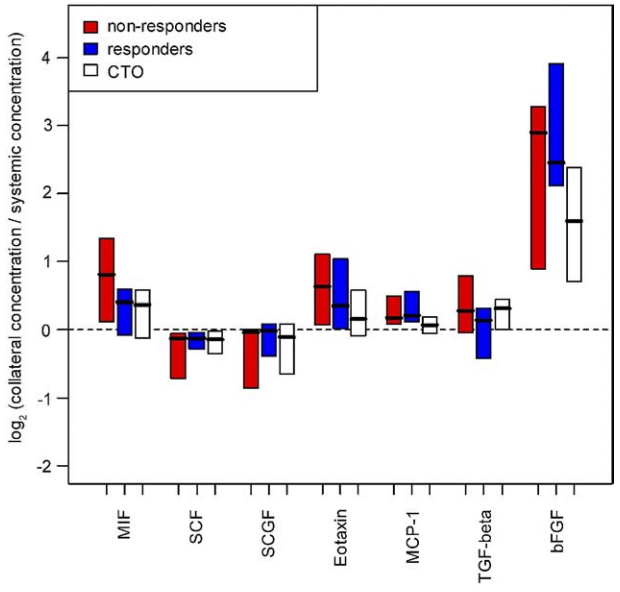

Figure 3 Cytokine Gradients

The **box plot** shows the relative change of collateral as compared with coronary cytokine concentrations (significantly different cytokines for nonresponders, responders, and chronic total occlusions [CTOs]). Concentrations were log-transformed to correct for skewed parameter distributions and improve visualization of median values with large interquartile ranges. Most cytokine gradients were strongest in nonresponders and almost absent in patients with CTOs. bFGF = basic fibroblast growth factor; MCP = monocyte chemoattractant protein; MIF = macrophage migration inflammatory factor; SCF = stem cell factor; SCGF = stem cell growth factor; TGF = transforming growth factor.

Patient selection. For a comprehensive analysis of human arteriogenesis, we selected patients with single-vessel CAD and a severe coronary narrowing (nonresponders and responders) as well as patients with CTOs. This approach allows an analysis of the collateral vascular development in 2 distinct clinical phases (i.e., during an accelerated phase in 1-vessel disease and a more quiescent phase in chronic coronary occlusion). Moreover, the analysis of cytokine concentrations was performed in a uniform fashion allowing, for the first time, an interpretation of the adaptation of collateral vessels in a wide range of coronary narrowing. Patients with previous myocardial infarction, as shown by echocardiography or pathological Q waves on electrocardiogram, were excluded from the study.

Collateral-specific blood sampling. The Proxis embolic protection device has been shown earlier to safely occlude coronary vessels and provide retrograde blood aspiration (10). For the first time, we describe the use of the catheter for the selective collection of coronary collateral blood. Previous studies using an over-the-wire catheter or a multifunctional probing catheter for coronary-collateral-specific blood sampling resulted in limited amounts of blood and possible activation of the sample because of the small lumen

Table 3 Correlations of Collateral-Coronary Cytokine Gradients With Oxygen Gradient in Patients With Subtotal Stenosis

Cytokine	Correlation Coefficient	p Value	FDR (%)
MIF	0.59	0.01	11
Eotaxin	0.56	0.02	11
MCP-1	0.55	0.01	11
MIP-1β	0.54	0.02	11
HGF	0.53	0.02	11
bNGF	−0.57	0.02	11
TRAIL	−0.54	0.02	11

A good correlation was detected between gradients of several cytokines and oxygen gradient. Both p value and percentage FDR are mentioned. Note the negative correlation of the gradients of nerve growth factor-beta (bNGF) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) with oxygen gradient.

HGF = hepatocyte growth factor; MIP = macrophage inflammatory protein; other abbreviations as in Table 2.

of the catheter (4,5). Using pericardial fluid or coronary sinus blood results in sufficient sample volumes that are, however, not selective for the collateral circulation (11,12).

In the clinical setting, catheter-assisted aspiration of collateral blood cannot exclude potential admixture of capillary/venous blood, especially in patients with low CFI. The fact that in the present study the fall in pO_2 is not accompanied by a rise in pCO_2 or a decrease in pH in the retrogradely drawn samples, however, makes significant admixture of venous blood less likely although contamination of a small amount of distal coronary capillary blood cannot be fully excluded.

We postulate that the gradient in arterial oxygen pressure between coronary and collateral arterial blood found in this study rather relates to actual oxygen extraction along the collateral anastomoses. This corroborates experimental studies also reporting significant oxygen extraction along arteriolar systems (13,14). Local production of cytokines by the activated endothelium of the collateral circulation is an energy-consuming process. Indeed, in patients with a high oxygen gradient we also found the highest levels of cytokines.

Cytokines in human arteriogenesis. The collateral-specific up-regulation of MCP-1, bFGF, and TGF-beta confirms earlier data on the involvement of these cytokines in arteriogenesis (4,5). Other previously described pro-arteriogenic cytokines, such as GM-CSF, granulocyte colony-stimulating factor, and TNF-alpha, were not found differential between the 2 sampling sites. Next to CCL2 (MCP-1), we here also first describe enhanced expression of another CC-chemokine, CCL11 (eotaxin) in collateral arteries. Its up-regulation in collateral arterial blood might reflect a role in the fine-tuning of mononuclear cell homing, partly counteracting the effects of MCP-1 (15). MIF has earlier been described to stimulate angiogenesis, but a link to collateral artery growth has not been reported previously (16). MIF acts as a major positive regulator of inflammatory cell recruitment (17) and macrophage response to lipopolysaccharide (18), both of which play an important role in arteriogenesis.

Cytokine expression in different phases of collateral artery growth. Since no intravascular ischemia could be detected in the collateral arteries, collateral-specific expression of cytokines is not regulated by intravascular hypoxemia or tissue ischemia directly adjacent to the collateral circulation. However, ischemia in tissue distant from the major collateral arterioles still occurs, particularly in patients with a nonsufficient collateral circulation, as shown by ST-segment elevation during balloon occlusion. Our findings are in agreement with earlier results from experimental studies showing that arteriogenesis takes place proximal to the site of tissue ischemia (19). Low oxygen gradient between coronary blood and blood in well-developed collateral arteries demonstrated the physiological significance of these bypass vessels, which are capable of supplying myocardial tissue with normal oxygen concentrations.

Interestingly, cytokine concentrations in the collateral circulation could be correlated to the oxygen gradient between coronary and collateral blood. We show that the degree of collateral maturation is reflected by oxygen pressure in the collateral artery. Cytokine gradients between collateral and coronary blood were greatest in nonresponders and almost absent in CTO patients. The correlation of cytokine concentrations with the oxygen extraction shows strong arteriogenic activity where collateral vessels are not yet capable of providing sufficient oxygen levels. Those cytokines that most significantly correlated with oxygen gradients were the same that were most strongly up-regulated in collateral compared with coronary plasma. Interestingly, a number of cytokine gradients were found to inversely correlate with the oxygen gradient, suggesting a potential antiarteriogenic role. In the chronic phase of arteriogenesis (i.e., in CTOs), a reduction of cytokine gradients indicates that collateral artery growth has reached a plateau phase where less cytokines and growth factors are secreted. This is in line with the results of a study by Werner et al. (5) showing higher bFGF concentrations in more recent occlusions, where arteriogenesis was presumably still ongoing, as well as increased concentrations of MCP-1, placental growth factor, and TGF-beta in smaller collateral arteries with higher shear stress. Even if CFI does not reach high levels in all patients, collateral artery growth may have come to a halt. This is consistent with earlier findings showing a genetic predisposition of individual patients to develop an adequate or inadequate collateral circulation (7) and supports strategies in which local delivery of growth factors/cytokines in the coronary circulation is striven for. In that earlier study, increased monocyte inflammatory signaling was found in patients with weak collateralization, which is supported by plasma measurements in the present study showing increased cytokine gradients in insufficient collateral development. Potentially, inhibition of antiarteriogenic signaling selectively at the site of interest is an alternative treatment strategy for the stimulation of collateral artery growth.

Assessment of collateral flow index. Central venous pressure (CVP) was assumed to be 5 mm Hg instead of measuring CVP in every patient individually because of the already extensive study protocol. This assumption, however, reduces accuracy of the CFI measurements especially in patients with low CFI (20,21). To evaluate if differences in CVP would significantly influence our study results, we recalculated correlations of CFI with pO_2 and pCO_2 gradients as well as cytokine gradients using a CVP of 0, 5, and 10 mm Hg. Correlation of CFI with pO_2 gradient was almost unchanged ($R = 0.60$, $p < 0.001$). Similarly, changing the CFI cutoff to 0.25 did not significantly influence the results, still showing significantly different oxygen gradients (1.4 [1.8] vs. 2.1 [1.6]) in collateral responders versus nonresponders. Correlations with cytokine gradients and increased gradients in nonresponders

compared with responders were also unchanged with CVP varying from 0 to 10.

Study limitations. A limitation to pressure-derived CFI measurements is reduced accuracy in low collateral flow, when back pressure increases due to increased wall stress, which may influence CFI measurements (22). No data on left ventricular end-diastolic pressure are available from the present study population as it requires additional intracavitary instrumentation. However, patients with depressed left ventricular function on echocardiography were excluded from the study, which limits the possibility of variation in back pressure as a confounding factor in the present study.

Finally, applying negative pressure during intracoronary blood withdrawal might have affected collateral flow rate, which potentially has led to an actual underestimation of the pO_2 and cytokine gradients in patients with a low CFI.

Conclusions

CFI can directly be related to the gradient of oxygen pressure over the collateral vascular bed. In patients with a nonsufficient collateral circulation, the increased oxygen gradient is accompanied by higher local levels of cytokines, pointing to an active phase of arteriogenic remodeling. Less pronounced cytokine gradients in collateral arteries in CTOs indicate a quiescent phase of arteriogenic activity, which supports strategies of local growth factor therapy in these patients.

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