Galectin-2 expression is dependent on the rs7291467 polymorphism and acts as an inhibitor of arteriogenesis

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Aims

In patients with obstructive coronary artery disease (CAD), the growth of collateral arteries, i.e. arteriogenesis, can preserve myocardial tissue perfusion and function. Monocytes modulate this process, supplying locally the necessary growth factors and degrading enzymes. Knowledge on factors involved in human arteriogenesis is scarce. Thus, the aim of the present study is to identify targets in monocytes that are critical for arteriogenesis in patients with CAD.

Methods and results

A total of 50 patients with a chronic total coronary occlusion were dichotomized according to their collateral flow index. From each patient, RNA was isolated from unstimulated peripheral blood monocytes, monocytes stimulated by lipopolysaccharide (LPS) or interleukin (IL)-4, and from macrophages. Increased mRNA expression of galectin-2 was found in three out of four monocytic cell types of patients with a low capacity of the collateral circulation ($P = 0.03$ for unstimulated monocytes; $P = 0.02$ for LPS-stimulated monocytes; $P = 0.20$ for IL-4-stimulated monocytes; $P = 0.02$ for macrophages). Additionally, galectin-2 mRNA expression was significantly associated with the rs7291467 polymorphism in LGALS2 encoding galectin-2 in all four monocytic cell types. Patient with the rs7291467 CC genotype displayed highest galectin-2 expression, and also tended to have a lower arteriogenic response. To evaluate the effect of galectin-2 on arteriogenesis in vivo, we used a murine hindlimb model. Treatment with galectin-2 markedly impaired the perfusion restoration at Day 7.

Conclusion

Collectively, these results identify galectin-2 as a novel inhibitor of arteriogenesis. Modulation of galectin-2 may constitute a new therapeutic strategy for the stimulation of arteriogenesis in patients with CAD.

Keywords

Collateral circulation • Coronary artery disease • Galectin-2 • Monocytes • Genetic polymorphism

Introduction

In patients with coronary artery disease (CAD), the perfusion of the myocardial tissue is impaired. Remodelling of small arterioles into large collateral arteries that divert blood flow around stenotic lesions, i.e. arteriogenesis, is a powerful natural mechanism that can restore myocardial tissue perfusion in these patients. Well-developed collateral arteries are present in approximately one-third of patients with CAD and are associated with better preservation of myocardial function, reduced risk for adverse
cardiac events, and increased survival. Although the severity of stenosis is one of the most important determinants of the extent of arteriogenesis, Pohl et al. have demonstrated that a large heterogeneity exists in the arteriogenic response in patients, even in case of a fully occlusive coronary stenosis.

Circulating mononuclear cells, in particular monocytes have emerged as important regulators of collateral artery growth. We previously showed that differences in RNA and protein expression of stimulated monocytes are related to the extent of the arteriogenic response, implicating interferon-β signalling as an anti-arteriogenic process in both patients and experimental models.

Here, we conducted a study to identify additional targets that relate to collateral artery growth in patients with a chronic total coronary occlusion. We show that patients with a low capacity of the collateral circulation have increased monocytic mRNA expression of galectin-2, independent of different stimulations of these cells. Additionally, the mRNA expression of galectin-2 was significantly associated with the rs7291467 polymorphism in LGALS2 encoding galectin-2. Finally, we demonstrate that galectin-2 impairs arteriogenesis upon femoral artery occlusion in mice.

### Methods

#### Patient study and procedures

The present study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the institutional medical ethics committee. All patients gave informed consent. The patient study protocol and procedures have been described previously. Briefly, a total of 50 Caucasian patients that had a successful elective percutaneous coronary intervention (PCI) of a chronic total coronary occlusion were included. Exclusion criteria were previous myocardial infarction, cardiac surgery, depressed left ventricular function, diabetes mellitus, and inflammatory or neoplastic disease. During elective PCI, the pressure-derived collateral flow index (CFIp) was invasively measured using a 0.014″ pressure guide wire (BrightWire, Volcano, Rancho Cordova, CA, USA) to quantify the capacity of the collateral circulation. During 1 min balloon inflation, the pressure distal to the coronary occlusion (wedge pressure, Pw) as well as aortic pressure (Pao) was determined. The CFIp was calculated as (Pw−CVP)/(Pao−CVP), where the central venous pressure (CVP) was estimated to be 5 mmHg. It has been reported by Pohl et al. that CFIp values calculated by estimated CVP do not differ from those calculated by measured CVP in a cohort of 120 patients.

From each patient, heparin-coagulated blood was withdrawn just before PCI to obtain four monocytic cell types. Unstimulated monocytes were positively isolated from 5 mL whole blood using anti-CD14-coated immunomagnetic beads (Dynal, Invitrogen). Additionally, monocytes were isolated using a monocyte negative isolation kit (Dynal, Invitrogen) following Ficoll™ gradient centrifugation of 45 mL whole blood. Negatively isolated monocytes were either cultured for 3 h in the presence of 10 ng/mL lipopolysaccharide (LPS) (Sigma-Aldrich), or 10 ng/mL interleukin (IL)-4, or cultured for 20 h for differentiation towards macrophages. Interleukin-4 stimulation of monocytes was performed in only 31 patients. After isolation and culture, all cells were lysed and total RNA was micro-purified using the Absolutely RNA Microprep kit according to the manufacturer’s protocol (Stratagene, La Jolla, USA) and used for gene expression analysis.

### Whole genome transcriptome analysis

A total of 20 patients from both ends of the CFIp spectrum (10 patients with a high CFIp and 10 patients with a low CFIp) were carefully matched for sex, age, medication, and other factors that may influence arteriogenesis. From these patients, mRNA from unstimulated monocytes, LPS-stimulated monocytes, IL-4 stimulated, and macrophages was amplified and biotinylated using the Illumina TotalPrep RNA amplification Kit (Ambion). Samples were randomly hybridized to HumanRef-8 Expression bead chip arrays (Illumina) at ServiceXS (Leiden, The Netherlands), followed by scanning and feature extraction. Microarray data have been submitted to the Gene Expression Omnibus under accession number GSE13290.

### Validation of gene array results by real-time reverse transcriptase–polymerase chain reaction

From all 50 patients, RNA was reversed transcribed into cDNA using RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany) to assess the mRNA expression levels of LGALS2, C1QA, PHACS, RNF213, and LARP by real-time reverse transcriptase–polymerase chain reaction (RT–PCR) (see Supplementary material online, Table S1). mRNA expression levels were corrected for expression of GAPDH and displayed as relative expression values.

### Genotyping of the rs7291467 polymorphism

From each patient, DNA was amplified using nested primers, containing the rs7291467 polymorphism in intron 1 of LGALS2 encoding galectin-2; forward 5’-GGGAGTGTGCTTCTCTC-3’ and reverse 5’-CTCTGGAGATGAGGTTATC-3’ followed by amplification with forward 5’-CCTGCTGAGTCTGCTGCTG-3’ and reverse 5’-AGAAGACAGGTGTCCGCC-3’. The rs7291467 polymorphism was genotyped by HpyCH4IV digestion of PCR products (New England Biolabs, Inc.). One hundred and twelve and 39 bp fragments were generated for the T allele, while 21, 39, and 91 bp fragments were generated for the C allele.

### Animal experiments

The animal experiments were approved by the Institutional Animal Welfare Committee. A total of 32 male C57BL/6 mice (Charles River), 8–9 weeks of age, were allocated into two groups. Sixteen mice were treated twice daily with intraperitoneal injections of 10 μg human galectin-2 (R&D Systems) diluted in 100 μL phosphate buffered saline (PBS) and 16 mice served as control group, treated by 100 μL PBS. This dose of galectin-2 has been shown to be therapeutically effective in a murine model of colitis and a murine model of contact allergy. Two days after start of treatment, coagulation of the left femoral artery was performed, distal to the bifurcation of deep and superficial femoral artery, as described. Just before and immediately after the operation, all animals underwent laser-Doppler perfusion imaging (LDPI) (Moor Instruments Ltd.) of the right and left paw to document the reduction in blood flow in the left paw. At Day 2 after the operation, LDPI was repeated and six mice of each group were sacrificed. At Day 7, the remaining galectin-2 treated mice (n = 10) and control mice (n = 10) underwent LDPI and were sacrificed.

### Immunohistochemistry

At Day 7 after operation, left adductor muscles were collected, and tissue was formalin-fixed and paraffin-embedded. A total of three sections per animal were used for each staining (4 μm thick, with an interval of 20–24 μm). For the detection of smooth muscle cells, sections

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were stained using an antibody for smooth muscle actin (clone 1A4, Dako, 1:1000). The antibody for Mac-3 (clone M3/84, BD Pharmingen, 1:200) was used for the detection of macrophages. Stained sections were scanned with a Mirax slide scanner system using a 20× objective (3DHISTECH, Budapest, Hungary). Vessel diameters were measured for all arteries and mean arterial lumen (µm²) was calculated. Quantification of macrophages was performed by counting the number of positively stained cells around arterial vessels in a fixed frame of 326 µm by 272 µm. A minimum of five fields per section were analysed and expressed as number of cells per field.

Statistical analysis
Normalization and statistical analysis of the gene array data was performed with established methods as previously described, using the limma package and scripts in R/Bioconductor. Gene set enrichment analysis was used to identify genes that are most differentially expressed between the patients with a high and a low arteriogenic response. Genes were considered differentially expressed if the Bayesian statistics t-test (Cyber-T) P-value was <0.05, and the absolute fold change was >1.2. To test for differences between two groups, the Student’s t-test was used for data with a normal distribution and the nonparametric Mann–Whitney U-test was used for data with a non-normal distribution. The Fisher’s exact test was used for testing associations between categorical data. To test for differences between three groups, a one-way analysis of variance was used. The Chi-square test for trend was used to investigate a trend between the number of C alleles of the rs7291467 polymorphism and the capacity of the collateral circulation (high and low). All results were considered statistically significant if the two-sided P-value was <0.05. Statistical analysis was performed with Statistical Package for Social Sciences software (SPSS 15.0 for Windows, SPSS Inc).

Results
Patient demographic and clinical characteristics
In search of determinants of arteriogenesis, we investigated the gene expression profiles of circulating monocytes of 50 patients with a chronic total coronary occlusion. In all these patients, the CFIp was invasively measured, to quantify the capacity of the collateral circulation. The mean age in the study was 59 years and 75% of the patients was male. The mean CFIp was 0.37±0.11, and a wide range was observed in individuals in accordance with previous observations in larger cohorts. For patients with a chronic total coronary occlusion, no CFIp threshold has been defined to separate good from bad collateral responders. Therefore, we selected 20 patients from both ends of the CFIp spectrum (10 patients with a high and 10 patients with a low arteriogenic response; Figure 1A) for comparison of the transcriptome profiles of unstimulated monocytes, LPS-stimulated and IL-4-stimulated monocytes and macrophages. Based on our previous results, IL-4 was included as additional activating stimulus, as it does not induce an

Figure 1 Identification of genes that are differentially expressed in the four monocytic cell types between the patients with a high and a low arteriogenic response. (A) Distribution of CFIp among 50 patients with a chronic total coronary occlusion (n = 50). (B) The top 100 differentially expressed genes (50 upregulated and 50 downregulated) in the four monocytic cell types between 10 patients with a high and 10 patients with a low arteriogenic response are shown in a heatmap. Relatively highly expressed genes are depicted in red, whereas genes that are relatively low expressed are blue. (C) One-way clustering analysis showing genes that are differentially expressed in more than one monocytic cell type. A total of five genes (arrows) are differentially expressed in all four monocytic cell types between the patients with a high and patients with a low arteriogenic response.
interferon-β response, in contrast to LPS. The patient characteristics of these 10 and 10 selected patients were comparable (Table 1). Additionally, no differences were observed between the two groups regarding left ventricular function or the presence of wall motion abnormalities in the region of interest, as estimated by echocardiography (data not shown).

**Galectin-2 expression is increased in patients with a low arteriogenic response**

Within each monocytic cell type, comparative analysis of the transcriptome between the selected 10 patients with a low and 10 patients with a high arteriogenic response revealed 90 differentially expressed genes for unstimulated monocytes (50 genes upregulated and 40 genes downregulated), 120 for LPS-stimulated monocytes (65 genes upregulated and 55 genes downregulated), 167 for IL-4-stimulated monocytes (86 genes upregulated and 81 genes downregulated), and 60 for macrophages (32 genes upregulated and 40 genes downregulated), 120 for LPS-stimulated monocytes (50 upregulated and 50 downregulated) for each monocytic cell type. Bioinformatics analysis did not reveal specific molecular pathways or gene networks other than a confirmation of the differential anti-arteriogenic interferon-β signalling in this cohort, which was already reported elsewhere.

Strikingly, we observed five genes that were differentially expressed in all four monocytic cell types (Figure 1C and see Supplementary material online, Table S2). After we dichotomized the whole patient group (n = 50) according to the median CFIp (0.37), we measured the gene expression of these five genes in all 50 patients using real-time RT–PCR analysis. In the complete cohort, only the level of galectin-2 expression remained statistically different in the unstimulated monocytes (P = 0.03), the LPS-stimulated monocytes (P = 0.02) and macrophages (P = 0.02) between the patients with low and high arteriogenic response (Figure 2). A trend was found for the IL-4-stimulated monocytes (n = 31; P = 0.20) (Figure 2). When analysed continuously, trends in the same direction were found, although only significant for the LPS-stimulated monocytes (data not shown).

**Galectin-2 expression is associated with the rs7291467 polymorphism**

We hypothesized involvement of a genetic component, as increased galectin-2 expression was found in all four monocytic cell types in the patients with a low arteriogenic response, independent of different cell stimulations. Accordingly, we investigated the genotype of the rs7291467 polymorphism in intron 1 of

### Table 1 Patient characteristics (modified from Schirmer et al.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CFIp ≤ 0.37 (n = 10)</th>
<th>CFIp &gt; 0.37 (n = 10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collateral flow indexp</td>
<td>0.27 ± 0.06</td>
<td>0.47 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 ± 13</td>
<td>58 ± 6</td>
<td>0.93</td>
</tr>
<tr>
<td>Male gender</td>
<td>6 (60)</td>
<td>6 (60)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Body-mass indexa</td>
<td>24.7 [24.2–26.3]</td>
<td>27.0 [25.7–28.4]</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Coronary risk factors:

- Hypertension: 7 (70) vs. 6 (60), >0.99
- Family history of CAD: 7 (70) vs. 4 (40), 0.37
- Hypercholesterolaemia: 4 (40) vs. 6 (60), 0.66
- Current smoker: 1 (10) vs. 2 (20), >0.99
- Ex-smoker: 5 (50) vs. 4 (40), >0.99

Duration of anginal symptoms:

- <3 months: 3 (30) vs. 3 (30), >0.99
- ≥3 months, <1 year: 5 (50) vs. 5 (50), >0.99
- >1 year: 2 (20) vs. 2 (20), >0.99

Medication:

- Beta-blockers: 7 (70) vs. 8 (80), >0.99
- Statins: 9 (90) vs. 9 (90), >0.99
- Aspirin: 9 (90) vs. 10 (100), >0.99
- Clopidogrel: 9 (90) vs. 5 (50), 0.14
- Calcium antagonists: 3 (30) vs. 3 (30), >0.99
- Nitrates: 5 (50) vs. 6 (60), >0.99
- ACE-inhibitors/ARBs: 5 (50) vs. 4 (40), >0.99

Data are expressed as number (%), mean ± SD, or median [25th–75th percentile]. ACE denotes angiotensin converting enzyme; ARB, angiotensin II receptor blocker; NS, not significant.

*The body-mass index is the weight in kilograms divided by the square of the heights in metres.*
LGALS2 encoding galectin-2. This particular polymorphism has been shown to regulate the mRNA expression level of galectin-2 in *in vitro* studies.18 Interestingly, we found a strong association between the mRNA expression levels of galectin-2 and the rs7291467 polymorphism in all four monocytic cell types, with higher galectin-2 mRNA expression levels in patients with the CC genotype when compared with patients with the CT or TT genotype (*P* < 0.01) (Figure 3). Correspondingly, the number of rs7291467 C alleles was associated with a CFIp below the median value (P-value for trend = 0.04, Table 2).

**Galectin-2 is expressed on the surface of human monocytes**

Using flow cytometry, we observed galectin-2 expression on the cell surface of human monocytes (see Supplementary material online, *Figure S1*). Notably, galectin-2 could not be detected in supernatants of cultured monocytes (data not shown), suggesting that galectin-2 adheres to the monocytic cell surface, after secretion.

**Galectin-2 inhibits arteriogenesis in a murine hindlimb model**

Members of the galectin family have been implicated previously in (tumour) angiogenesis,19,20 but to our knowledge, galectin-2 has never been studied directly as modulator of either angiogenesis or arteriogenesis. Therefore, to determine whether galectin-2 impairs arteriogenesis in *vivo*, we investigated the effect of galectin-2 treatment on collateral artery growth in a murine hindlimb model (*Figure 4A*). Our data show that systemic treatment with galectin-2 markedly impairs the perfusion restoration in mice at 7 days.

*Figure 2* Galectin-2 mRNA expression is increased in the patients with a low capacity of the collateral circulation. After dichotomizing the whole patient cohort of 50 patients according to the median CFIp, real-time reverse transcriptase–polymerase chain reaction analyses of galectin-2 mRNA expression revealed increased mRNA levels of galectin-2 in the unstimulated monocytes (*n* = 25 vs. 25), lipopolysaccharide-stimulated monocytes (*n* = 25 vs. 25) and macrophages (*n* = 25 vs. 25) of the patients with a low capacity of the collateral circulation. A trend was found for the interleukin-4-stimulated monocytes (*n* = 18 vs. 13). Data are presented as mean ± SD.
Galectin-2 expression is dependent on the rs7291467 polymorphism

**Figure 3** The rs7291467 polymorphism is associated with galectin-2 mRNA expression. In all four monocytic cell types, the galectin-2 mRNA level was associated with the rs7291467 polymorphism ($n = 50$ for the unstimulated monocytes, $n = 50$ for the LPS-stimulated monocytes, $n = 31$ for the IL-4-stimulated monocytes, and $n = 50$ for the macrophages). For all four monocytic cell types, increased mRNA expression levels of galectin-2 are found in patients with the CC genotype when compared with patients with the CT and TT genotypes. SNP denotes single nucleotide polymorphism.

![Galectin-2 expression graphs](image)

**Table 2** Genotype distribution of the rs7291467 polymorphism

<table>
<thead>
<tr>
<th>rs7291467 SNP</th>
<th>Low CFI$_p$ ($n = 25$)</th>
<th>High CFI$_p$ ($n = 25$)</th>
<th>Total ($n = 50$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>9 (36)</td>
<td>3 (12)</td>
<td>12 (24)</td>
</tr>
<tr>
<td>CT</td>
<td>11 (44)</td>
<td>13 (52)</td>
<td>24 (48)</td>
</tr>
<tr>
<td>TT</td>
<td>5 (20)</td>
<td>9 (36)</td>
<td>14 (28)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%). SNP denotes single nucleotide polymorphism.

Following left femoral artery coagulation (54.5% in the galectin-2-treated mice vs. 74.6% in the control mice, $P < 0.01$) (Figure 4B). In addition, mean arterial lumen tended to be lower in the galectin-2-treated mice ($P = 0.07$) (Figure 4C). Furthermore, we found a decreased number of macrophages in close proximity to the arterial vessels in the galectin-2 group ($P = 0.049$) (Figure 4D). Notably, no signs of necrosis of the paws were detected in the galectin-2- and placebo-treated mice. No mice died during this animal study, and the galectin-2-treated mice exhibited the same weight gain as the control mice, demonstrating an absence of any major short-term side effects of galectin-2 treatment.
Galectin-2 does not change the percentage of several mononuclear cell subsets in the murine hindlimb model

Previous studies have indicated a potential impact of galectin-2 on T-cell survival.\textsuperscript{11,12,21} Since T-cells also play an important role in arteriogenesis,\textsuperscript{22,23} we examined the effect of galectin-2 on T-cells in our model of arteriogenesis. Galectin-2 did not affect the percentages of T-cells (including CD4\textsuperscript{+} T-cells and naive and active CD8\textsuperscript{+} T-cells) in the regional and the peripheral inguinal lymph nodes at Days 2 and 7 after left femoral artery coagulation. In addition, the percentage of circulating blood T-cells (see Supplementary material online, Table S3), monocytes and natural killer cells was also not affected by galectin-2 treatment.

**Figure 4** Galectin-2 impairs arteriogenesis in the murine hindlimb model. The effect of galectin-2 treatment on arteriogenesis in a murine hindlimb model was investigated using laser-Doppler perfusion imaging. (A) Representative laser-Doppler perfusion images of the left and the right paw of the control mice and galectin-2-treated mice at different time points are shown [before left femoral artery coagulation (pre-OK), after coagulation (post-OK) and at day 2 and 7 after coagulation]. (B) At each time point, the perfusion restoration of the galectin-2-treated mice was compared with the control mice (n = 16 vs. 16 mice pre-OK, post-OK and at day 2; n = 10 vs. 10 mice at day 7). Each bar represents the mean ± SD value. (C and D) Paraffin sections from left adductor muscle were stained for SMA and Mac-3. Counterstaining was performed with haematoxylin, visualizing nuclei in blue. Arrow heads indicate positive macrophages. The mean arterial lumen (μm\textsuperscript{2}) and the number of MAC-3 positive cells per field (placebo, n = 8; galectin-2, n = 9) was quantified as described in the Methods section.
cells (data not shown) did not differ significantly between the galectin-2-treated mice and the control mice. All together, these data indicate an absence of systemic derangement of the immune system by galectin-2 treatment in the hindlimb model.

Discussion

The clinical benefit of a well-developed collateral circulation in patients with CAD has been well recognized. Monocytes play an important role in the modulation of collateral artery growth. In search of determinants of collateral artery growth, we therefore investigated gene expression profiles of monocytes from CAD patients with a chronic total coronary occlusion, and to relate the monocyte characteristics to the capacity of their collateral circulation. In this cohort of CAD patients, collateral artery growth has been maximally triggered and has reached its plateau phase over time, in contrast to our initial studies on patients with a partial coronary occlusion.9 In the present study, we found increased mRNA expression of galectin-2 in monocytes of patients with a low capacity of the collateral circulation, pointing at an inhibitory role of galectin-2 in arteriogenesis. Additionally, galectin-2 mRNA expression was associated with the rs7291467 polymorphism in all four monocytic cell types. Finally, we demonstrate that galectin-2 inhibits the arteriogenic response upon femoral artery occlusion in mice. In accordance with our initial study on patients with a partial coronary occlusion,9 we also found increased interferon signalling in patients from the current cohort with a lower than median arteriogenic response, data which have been reported previously by Schirmer et al.10 We did not find a correlation between interferon response and galectin-2 levels (data not shown), indicating that these are two apparently independent regulators of the multifactorial arteriogenic response.

Arteriogenesis requires the recruitment of monocytes. Upon transmigration into the arteriolar wall, the monocytes differentiate into macrophages. Macrophages create space for the arteriolar vessel to expand, promote an inflammatory environment, and stimulate the proliferation of vascular cells.24 It is unknown whether the accumulation of one or several types of macrophages is necessary to perform these different functions, or whether macrophages are functionally flexible and are able to alter their phenotype during the process. To find arteriogenesis-related differences in gene expression we therefore not only investigated the transcriptome of circulating monocytes but we also ex vivo activated the monocytes by LPS or by IL-4, as the latter stimulus does not induce an interferon-β response. Additionally, monocytes were cultured for differentiation into macrophages. Our results show distinct gene expression profiles in all four monocytic cell types, separating the patients with a low arteriogenic response from the patients with a high arteriogenic response. Interestingly, we observed increased mRNA expression of galectin-2 in all four monocytic cell types from the patients with a low arteriogenic response, raising the possibility of an intrinsic mechanism determining the monocytic galectin-2 mRNA level, independent of environmental cues.

In 2004, Ozaki et al.18 reported the rs7291467 polymorphism in intron 1 of LGALS2 encoding galectin-2 to affect the transcriptional level of galectin-2 in their in vitro studies using HeLa and HepG2 cells. Therefore, we investigated the genotype of the rs7291467 polymorphism in the present study. For the first time, we show that the mRNA expression level of galectin-2 is associated with the rs7291467 polymorphism in patients. Patients with the rs7291467 CC genotype displayed higher mRNA expression levels of galectin-2 in all four monocytic cell types when compared with the patients with the CT or TT genotype. Accordingly, patients with the rs7291467 CC genotype and higher mRNA expression of galectin-2 also tended to have a lower capacity of the collateral circulation. It is well known that patients with a low arteriogenic response are at increased risk of adverse cardiac events.2 The association between the rs7291467 polymorphism and susceptibility to acute myocardial infarction has been investigated extensively in clinical studies; however, it was concluded that a direct association with the rs7291467 polymorphism is unlikely.25 Association of this polymorphism with collateral remodelling has not been studied directly to date.

To evaluate a direct effect of galectin-2 on arteriogenesis, we used a well-established murine model of arteriogenesis. We show that systemic treatment with galectin-2 significantly inhibits perfusion restoration in mice, after 7 days following left femoral artery coagulation. In line with this, mean arterial lumen tended to be lower in the galectin-2-treated mice. Interestingly, we also found decreased numbers of macrophages in close proximity to the arterial vessels, suggesting that galectin-2 may impair arteriogenesis through modulation of monocyte/macrophage responses. Experimental studies have demonstrated that galectin-2 affects T-cell homeostasis.11,12,21 However, in the hindlimb model, galectin-2 did not reduce the percentages of several T-cell subsets, indication an absence of systemic derangement of the immune system by galectin-2 treatment. Given our observation that galectin-2 remains associated with the monocyte surface, these results prime future studies to investigate the mechanism through which galectin-2 exerts its anti-arteriogenic effect, and in this regard, whether galectin-2 modulates monocyte/macrophage responses. A potential limitation to the study is that we did not measure left ventricular filling pressure which is related to collateral flow.26,27 Probably, in the present study, this influence was low, since the majority of patients had sufficient collaterals (chronic total coronary occlusion without signs of myocardial ischemia at rest) and a preserved left ventricular function.

This study establishes galectin-2 as a novel inhibitor of arteriogenesis, and shows for the first time an important association between galectin-2 expression and the rs7291467 polymorphism in patients with CAD. In a murine hindlimb model, treatment with galectin-2 markedly impaired arteriogenesis. Taken together, these combined clinical and experimental findings indicate galectin-2 as a novel target for the modulation of arteriogenesis in patients with CAD.

Supplementary material

Supplementary material is available at European Heart Journal online.
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