Molecular mechanisms of complement activation by damaged cells
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Relation of IgM antibodies against apoptotic cells and phosphorylcholine to the inflammatory response and infarct size in patients with acute myocardial infarction.

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# Both authors contributed equally to the study.
Abstract

Background: Natural IgM antibodies, particularly anti-phosphorylcholine IgM (anti-PCh IgM), are suspected to be involved in cardiovascular disease as they possibly modulate atherosclerosis or acute myocardial infarction (AMI).

Aim: To assess the relationship between plasma levels of anti-PCh IgM and IgM that binds to damaged cells, with infarct size or post-infarct inflammatory responses in patients with AMI.

Methods: Plasma samples from 50 patients with AMI and 46 healthy controls were analysed. IgM binding to damaged cells was measured by incubating plasma samples with apoptotic Jurkat cells with subsequent detection of bound IgM by FACS. Anti-PCh IgM was measured with a specific ELISA. The post-infarct inflammatory response was quantified by measuring C-reactive protein (CRP), secretory phospholipase A2 (sPLA2), IL6, IL8 and activated complement.

Results: On admission, patients with AMI had similar levels of IgM binding to apoptotic cells but lower levels of anti-PCh IgM, also when corrected for hemodilution, than healthy controls. These levels were constant during 48 hours. To analyse the relation of IgM species to apoptotic cells and inflammatory or clinical parameters, patients with levels above the median were compared to those with levels below the median. Patients with higher levels of IgM binding to apoptotic cells had similar inflammatory responses and infarct size as those with lower levels. Patients with higher levels of anti-PCh IgM, however, had larger infarcts as assessed with ECG, and a more pronounced response of the acute phase protein sPLA2.

Conclusion: Plasma levels of natural IgM, in particular anti-PCh IgM, may modify inflammatory responses and infarct size in patients with acute myocardial infarction.

Introduction

Patients with acute myocardial infarction (AMI) frequently develop fever and have increased plasma levels of cytokines and acute phase proteins such as C-reactive protein (CRP) and secretory phospholipase A2 (sPLA2) in the first days following the onset of infarction. Thus, myocardial infarction induces an inflammatory response in the ischemic tissue which may lead to systemic symptoms. Indeed expression of cytokines and infiltration of polymorphonuclear granulocytes (PMN) occurs in the jeopardized myocardium during infarction [1-4]. Furthermore, acute phase proteins such as CRP and sPLA2 are deposited in
the infarcted myocardium and the complement system is activated locally [5-7]. Cardiomyocytes in the ischemic area may show evidence for apoptosis [8]. To what extent this post-infarct inflammatory response in the heart contributes to the infarct size in humans is unknown. Animal studies suggest that this contribution may be substantial [9].

Though local ischemia obviously is the primary stimulus for the inflammatory changes ensuing in the infarcted myocardium, the molecular mechanisms explaining the link between ischemia and inflammation are far from clear. Possibly, changes in membrane phospholipids such as oxidation, hydrolysis and membrane flip-flop due to the formation of oxygen radicals, the decrease of intracellular ATP and the increase of intracellular calcium, stimulate the binding of extracellular proteins such as sPLA2 and CRP [10;11], which in turns activate complement thereby triggering inflammation. Some evidence suggests that also natural IgM antibodies may be among proteins that bind to altered phospholipids in the membranes of ischemic cells, and via subsequent activation of complement contribute to the post-infarction inflammatory response. For example, studies in knock-out mice have convincingly shown that natural IgM is involved in ischemia-reperfusion injury by binding to ischemic endothelial cells and by triggering reperfusion-induced complement activation [12]. The specificity of this IgM is still unknown. In immunohistochemical studies we recently observed that IgM becomes deposited in the ischemic myocardium during infarction in humans who died from AMI. This IgM is co-localized with CRP and activated complement (P. Krijnen et al., manuscript submitted). Thus, in humans IgM, in addition to CRP [5;6] and possibly other molecules, may contribute to local complement activation in the ischemic myocardium. The nature of this IgM is also unknown.

A number of studies have shown that IgM present in normal serum can bind to apoptotic cells [13;14], including natural IgM against phosphorylcholine (anti-PCh IgM) [15]. In the present study we tested the hypothesis that IgM that binds to damaged cells and or anti-PCh IgM may enhance the post-infarct inflammatory response in patients with AMI. To test this hypothesis, we developed an assay to measure plasma IgM that binds to apoptotic cells, and we used this assay, as well as an ELISA for anti-PCh IgM, to measure plasma levels of this IgM in patients with AMI. These levels were compared to those in healthy controls and were also related to parameters for cardiac damage and of inflammatory mediators such as IL6, IL8 and activated complement.
Patients, material and methods

Blood samples

Blood samples from 50 patients with AMI were collected in 10 mM EDTA, final concentration, at various time points after admission to the hospital. Samples were centrifuged at 1300 g after which the supernatant was stored in aliquots at -70°C until tested. All patients fulfilled the criteria for AMI, which included typical chest pain, typical electrocardiographic abnormalities in combination with elevated cardiac markers such as creatine-kinase (CK) [16]. Infarct size was calculated from the cumulative release of lactate dehydrogenase (LDH) or CK [17;18], and also from electrocardiographical infarct scores (Selvester scores) in patients with a first AMI before therapy [19;20]. Patients had participated in earlier studies on the role of complement in AMI. All patients had given informed consent. The study was approved by the Medical Ethical committee of the VU Medical Centre.

Plasma samples from a group of 46 healthy donors were collected and processed in the same way. In addition, serial samples were obtained from 5 healthy donors weekly for a period of 6 weeks. During this period none of the volunteers suffered from an intercurrent illness.

Proteins and antibodies

Anti-human IgM monoclonal antibody (mAb) MH-15 (IgG1 subclass) was obtained from Sanquin, Business Unit Reagents (Amsterdam, The Netherlands). The mAb was biotinylated with LC-biotin-n-hydroxysuccinimide ester (Pierce, Rockford, IL) according to the manufacturer’s instructions. AnnexinV-FITC and propidium iodide (PI) were obtained from Bender Med System (Vienna, Austria) and streptavidin-allophycocyanin (Strep-APC) conjugate was obtained from BD Biosciences Pharmingen (San Diego, CA). Human serum albumin (HSA), purchased at Sanquin Business Reagent and p-aminophenylphosphorylcholine (Sigma Chemicals Co., St Louis, MO) were coupled following a protocol described earlier [21].

Assay for IgM binding to late apoptotic cells (apo-IgM)

Jurkat cells were cultured in IMDM supplemented with 5%, v/v, heat-inactivated foetal calf serum (Bodinco, Alkmaar, The Netherlands), 20 µg/ml human apo-transferrin (Sigma), 100 U/ml penicillin and 100 µg/ml streptomycin (Life Technologies, Grand Island, NY) at 37°C in a humidified atmosphere (5%, v/v, CO2 / 95% air). Apoptosis was induced in Jurkat cells by incubation in 96 wells round-bottom plates at 2.5 x 10^5 cells per well in serum-
Association of IgM antibodies and inflammation in patients with AMI

free IMDM containing 100 μM etoposide (Sigma) for 48 hours at 37°C. After apoptosis induction, cells were washed with serum-free culture medium to remove etoposide and incubated with various amounts of plasma in veronal buffered saline, pH 7.4 (VB), containing 10 mM CaCl\(_2\) and 2 mM MgCl\(_2\) (VB++), final volume 100 μl. Plasma samples from patients were tested in this system at a concentration of 5%, v/v, unless otherwise indicated. After an incubation for 30 minutes at 37°C, cells were washed thrice with Heps buffer containing calcium (Heps 10 mM, NaCl 150 mM, KCl 5 mM, CaCl\(_2\) 1.8 mM and MgCl\(_2\) 1 mM, pH 7.4) and incubated with biotin-labelled monoclonal anti-IgM (5 μg per ml in 50 μl of heps buffer, final volume) for 30 minutes at 4°C in darkness. After a second washing procedure, cells were incubated with streptavidin-APC (1 to 750 diluted) or annexin-V-FITC (1 to 100 dilution) in 100 μl final volume for 30 minutes at 4°C in darkness. After washing with Heps buffer, cells were resuspended in Heps buffer containing calcium as well as PI (at a final concentration of 500 ng per ml). Results were analysed by flow cytometry, and expressed as mean fluorescence intensity (MFI). Data were stored in Cellquest acquisition program and analysed with WinMDI 2.8 program.

**ELISA for anti-phosphorylcholine IgM**

IgM antibodies directed against phosphorylcholine (PCh) were measured by specific ELISA as described [21]. Briefly, p-aminophenylphosphorylcholine coupled to human serum albumin (PCh-HSA; 2.5 μg/ml in 0.1 M carbonate/bicarbonate buffer, pH 9.6) was coated onto polystyrene microtiter plates (Dynatech, Plochingen, Germany) overnight at 4°C. After washing the plates twice with PBS, residual binding sites were blocked (1 hour at room temperature) with 200 μl per well of phosphate buffered saline, pH 7.4 (PBS) containing 2 %, w/v, HSA. Human plasma or sera were appropriately diluted in assay buffer (veronal buffer containing 0.1 %, w/v, HSA, 2 mM CaCl\(_2\), 0.1 %, w/v, Tween 20, pH 7.4), and incubated for 1 hour at room temperature. Thereafter, the plates were washed with PBS containing 0.1 %, w/v, Tween-20. IgM bound to PCh-HSA was quantified with peroxidase-labelled anti-human IgM mAb diluted in assay buffer. Finally, peroxidase activity was visualized with tetramethyl-benzidine. Dilutions of a pool of normal plasma, obtained from 40 healthy volunteers, were used as standard curve in the assay. This standard was arbitrarily said to contain 100 U per mL of anti-PCh IgM antibodies. Results of plasma samples were related to this standard and expressed as U of anti-PCh IgM per mL.
Biochemical and inflammatory parameters

IgM concentration was assessed with a nephelometric assay. Secretory phospholipase A$_2$ (sPLA2), CRP, interleukins-6 and -8 (IL6 and IL8, respectively), and activated complement fragments C4b/c and C3b/c were determined with specific ELISA as described earlier [22-25]. Note that the ELISAs do not discriminate between C4b, C4bi or C4e, and C3b, C3bi and C3c, and that the activation products detected by these assays are hence referred to as C4b/c and C3b/c, respectively. Results were expressed as µg/L (sPLA2)[22], mg/L (CRP) [23], ng/L (IL6 and IL8) [24;25] and nmol/L (C3b/c and C4b/c) [26].

LDH and CK concentrations were determined in the routine clinical chemistry laboratorium of the VU Medical Centre.

Analysis of data

Data were analysed with Graph Pad Instat® (version 3.0). Distribution of data was analysed with the method of Kolmogorov and Smirnov. When normally distributed, data were analysed with Student’s t test or one-way analysis of variance (ANOVA) multiple comparison test with Bonferroni. Correlation between parameters was assessed by calculating the Pearson’s correlation coefficient. In case of non-normal distribution, Mann-Whitney’s and Kruskal-
Wallis with Dunn's multiple comparison tests were used to assess differences between groups. A P-value <0.05 was considered to represent a significant difference or correlation.

Results

Patients included

The patients with AMI, 8 females and 42 males, included in the analysis had a median age of 60 years, range 34 to 76 years. Seventy % of the patients were treated with thrombolytic agents, 20% with acute percutaneous transluminal coronary angioplasty. Twenty-four patients had a first infarction; the others had suffered from a myocardial infarction before. None of the patients had an underlying illness, none died during the observation period.

IgM binding to apoptotic cells

IgM that binds to damaged cells was measured by incubating apoptotic Jurkat cells with plasma samples, and detection of bound IgM with FACS [14]. A triple staining procedure allowed the determination of specific IgM that bound to the late apoptotic population, which was identified by its characteristic side and forward scatter dot plot (figure 1A), and by its capacity to bind annexinV and propidium iodide (figure 1B). IgM bound to the cells was then assessed with the third marker, streptavidin-APC that bound to biotinylated anti-IgM (figure 1C). Thus, the mean fluorescence intensity (MFI) of the streptavidin-APC of the gated population was determined as a measure for IgM binding to apoptotic cell (anti-apo IgM). Cells not incubated with plasma were used as control for aspecific staining (figure 1D). IgM binding to apoptotic cells appeared to vary among donors. For example, figure 1D shows the results, expressed as MFI, obtained when plasma samples from 2 different healthy donors...
were tested. To get insight into the variation in time of levels of anti-apo IgM, plasma samples were obtained weekly from 5 healthy lab donors during a period of 6 weeks, and measured for anti-apo IgM. Intra-individual levels of anti-apo IgM hardly varied during this period with a variation coefficient (CV) of 11 % ± 1.0, (figure 2). Previously we have observed that the variation of levels of anti-PCh IgM in time is also limited in healthy individuals [21]. In order to reduce assay variation as much as possible, all plasma samples were tested within the same experiment.

Figure 3: Anti-apo IgM in patients with myocardial infarction and controls. Apoptotic Jurkat cells were used to measure levels of anti-apo IgM. Plasma samples from patients on admission (t=0; n=50) or 48 hours later (t=48; n=45), and from healthy controls (n=46) were tested for anti-apo IgM as described in material and methods. Levels are expressed as MFI, data were analysed with Kruskal-Wallis and Dunn's multiple comparison test. *P<0.05 and ***P<0.001.

Anti-apo IgM and anti-PCh IgM in the AMI patients

Levels of anti-apo IgM were determined as described above in blood samples from patients with AMI or from healthy controls (figure 3). Levels were somewhat lower in the AMI patients as compared to those in the healthy controls (230 MFI ±34, mean ±sem, n=50, versus 264 MFI ±24, n=46; p<0.05). Moreover, levels of anti-apo IgM 48 hours after the onset of complaints had further decreased compared to those on admission (169 MFI ±15 versus 230 MFI ±34, n=45; p<0.001). These differences in levels between patients and controls, however, were lost when levels were corrected for hemodilution by calculating the ratio of anti-apo IgM to total IgM (patients t=0: 93 ±8 and 114 ±10 at t=48, versus healthy controls 86 ±5, figure 4A).

In addition to anti-apo IgM we also determined the concentration of anti-PCh IgM by ELISA as described in materials and methods. Similarly as for anti-apo IgM, values were corrected for hemodilution by estimating the ratio of anti-PCh IgM to total IgM. Although there was no significant difference between the ratios of the patients on admission versus those at 48 hours (84 ±7, mean ±sem, versus 87 ±10, respectively), the ratio at either time point was significantly lower than that in the control group of healthy donors (141 ±17, respectively p<0.01 and p<0.001, figure 4B).
Relation of anti-apo IgM or anti-PCh IgM with infarct size

Infarct size in the patients was estimated from the course of LDH and CK-MB. This course was quantified by calculating a cumulative concentration over the first 48 hours after the onset of complaints [17]. To establish the relation of levels of anti-apo IgM or anti-PCh IgM to infarct size, we compared the cumulative release of LDH or CK-MB in patients with anti-apo IgM levels lower or higher than the median value at the onset of the infarction (Figure 5 A, B and Table 1).

No significant difference in cumulative release of LDH or CK was observed between the groups. The cumulative release of these cardiac enzymes during the first 48 hours in patients having high anti-PCh IgM on admission was higher than that in the patients with low levels of anti-PCh IgM (figure 5 D, E and Table 1), although the difference between the groups did not reach a statistical significance. In patients without a previous infarction infarct, size can also be measured from the electrocardiographic criteria, as indicated by the Selvester score. Twenty-four patients could be evaluated in this way. There was no difference in Selvester score of patients on admission with high or low levels of anti-apo IgM on admission (figure 5C). However, the score was significantly higher in patients with high levels of circulating anti-PCh IgM on admission, as compared to those with low levels (figure 5F).
<table>
<thead>
<tr>
<th>Marker</th>
<th>IgM level</th>
<th>Low anti-apo IgM</th>
<th>High anti-apo IgM</th>
<th>Low anti-PCh IgM</th>
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<td>Anti-PCh IgM</td>
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<td>LDH (U/L)</td>
<td>552.9 ±85.9 (n=22)</td>
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<td>CK (U/L)</td>
<td>595.4 ±114.4 (n=22)</td>
<td>566.3 ±82.7 (n=21)</td>
<td>521.4 ±77.5 (n=21)</td>
<td>638.2 ±116.4 (n=22)</td>
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<td>ECG (selvester score)</td>
<td>28.6 ±3.2 (n=13)</td>
<td>27.6 ±2.9 (n=11)</td>
<td>23.2 ±3.1 (n=11)</td>
<td>32.3 ±2.5 (n=13)</td>
<td>*P=0.03</td>
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Table 1: Relation of anti-apo IgM or anti-PCh IgM on admission to cardiac markers. Anti-apo IgM levels were determined with apoptotic Jurkat cells and anti-PCh IgM on admission was determined by ELISA. Patients with low (<median) or high (>median) levels of anti-apo IgM or anti-PCh IgM were compared regarding cumulative concentration over 48 hours of creatine kinase (CK), lactate dehydrogenase (LDH) and their electrocardiographic (ECG) score. Data are given as mean value ± sem, and the difference between groups was analyzed with two-tailed Student’s t test when they appeared to be normally distributed.

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<td></td>
<td>Anti-apo IgM</td>
<td>Anti-PCh IgM</td>
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<td>sPLA2 (*g/L)</td>
<td>943.8 ±316.0 (n=22)</td>
<td>943.3 ±180.1 (n=23)</td>
<td>860.7 ±308.1 (n=23)</td>
<td>1031.0 ±175.1 (n=22)</td>
<td>*P=0.03</td>
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<td>CRP (mg/L)</td>
<td>87.6 ±15.5 (n=22)</td>
<td>148.5 ±35.5 (n=22)</td>
<td>100.7 ±21.7 (n=23)</td>
<td>145.9 ±33.5 (n=21)</td>
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<td>C4b/c (nmol/L)</td>
<td>101.2 ±20.6 (n=22)</td>
<td>136.5 ±33.48 (n=23)</td>
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<td>125.3 ±35.4 (n=22)</td>
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<td>C3b/c (nmol/L)</td>
<td>156.7 ±44.5 (n=22)</td>
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<td>IL6 (ng/L)</td>
<td>11.2 ±4.5 (n=22)</td>
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<td>IL8 (ng/L)</td>
<td>12.1 ±2.1 (n=19)</td>
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<td>8.1 ±1.7 (n=22)</td>
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Table 2: Relation of anti-PCh IgM on admission to inflammatory markers. Anti-apo IgM levels were determined with apoptotic Jurkat cells and anti-PCh IgM on admission was determined by ELISA. Patients with low (<median) and high levels of anti-apo IgM or anti-PCh IgM (>median) were then compared regarding cumulative concentration over 48 hours of sPLA2, CRP, complement activated products (C4b/c and C3b/c) and cytokines IL6 and IL8. Data are given as mean value ± sem, and were analysed with two-tailed Student’s t test as they appeared to be normally distributed. *P<0.05 was considered significant.
Figure 5: Relation between apo-IgM, anti-PCh IgM and infarction size.
(A, B, C): Levels of anti-apo IgM were measured with apoptotic Jurkat cells and expressed as MFI. Patients were said to have low or high levels when they had levels below or above, respectively, the median of the group. Electrocardiographic (ECG) results evaluated by cumulative release values of cardiac enzyme over 48 hours LDH (A) and CK (B) expressed as units per litre (U/L) and the Selvester score (C), and A similar analysis was made for anti-PCh IgM (D, E, and F). Data were analysed with Student’s t test. ns=non significant, *P<0.05.

Relation of anti-apo IgM or anti-PCh IgM with post-infarct inflammatory response

A number of parameters including the acute phase proteins CRP and sPLA2, the cytokines IL6 and IL8, and activated complement fragments C4b/c and C3b/c, were determined to assess the inflammatory response in the patients. The cumulative concentration of the acute phase protein, sPLA2, over 48 hours after admission was comparable between patient groups having low or high levels of anti-apo IgM. However, the patients with increased circulating anti-PCh IgM on admission had a significant increase in their sPLA2 concentration when compared to patients with lower anti-PCh IgM (1031 µg/L ±175 versus 860 µg/L ±308, P=0.03, respectively; Table 2). Pro-inflammatory cytokines IL6 and IL8 concentrations 48 hours after admission were comparable in patients having anti-apo IgM values or anti-PCh IgM below or above the median values (Table 2). The cumulative concentration of CRP over the 48 hours following admission was increased in the patients having both anti-apo IgM and anti-PCh IgM above the median values but did not reach a statistical significance (88 mg/L ± 16 versus 149 mg/L ± 36, p=0.12, in patients with low or
high anti-apo IgM, respectively, and 101 mg/L ± 22 versus 146 mg/L ± 34, p=0.26, in patients with low and high anti-PCh IgM, respectively, mean ± sem).

Similarly, the activated complement fragments, C4b/c and C3b/c 48 hours after admission were higher in the group of patients with the higher anti-apo IgM or anti-PCh IgM, but none of these differences reached statistical significance (Table 2).

Discussion

Recently, the role of IgM antibodies in cardiovascular disease has received considerable attention. It has been shown to play a role in the course of atherosclerosis [27;28], although their function has not been completely elucidated. Furthermore, our own studies show that IgM antibodies localize in infarcted human myocardium together with activated complement and CRP (Krijnen et al., submitted). Co-localization with CRP, which can bind to PCh, suggests this IgM in infarcted myocardium in part is directed against PCh. Interestingly, IgM against PCh can bind to apoptotic cells [15]. In the present study we measured IgM binding to apoptotic cells, as well as anti-PCh IgM in patients with AMI and found that levels of the latter antibodies on admission were related to the post-infarct response as well as to infarct size.

Though anti-PCh IgM has been found to bind to apoptotic cells [15;29], competition experiments with fluid-phase PCh indicate that not all IgM binding to these cells is directed against PCh (C.Ciurana.et al., manuscript submitted). Hence, to cover other specificities of IgM binding to apoptotic cells, we developed an assay in which apoptotic Jurkat cells are used as solid phase antigen and IgM bound to the cells is detected with FACS. The variability of levels of the IgM binding in this assay, in time was limited in healthy individuals (see figure 2) indicating a limited variation of levels in individuals in time. Notably, in an earlier study we have also observed a similar limited variation of levels of anti-PCh IgM in time [21].

Levels of IgM binding to apoptotic cells at first glance were lower in the patients than in the healthy controls. However, as patients with AMI receive (intravenous) fluid infusions, we decided to correct levels for hemodilution by calculating the ratio to IgM. Indeed ratios of anti-apo IgM and total IgM appeared to be similar in patients versus healthy controls. Hence, these data did not support the possibility that binding of this IgM to the ischemic myocardium had resulted in lower levels in the circulation. In contrast, levels of anti-PCh IgM, even when corrected for total IgM, were significantly lower in the patients on admission as compared to levels in healthy controls. Presumably this different behaviour of anti-PCh IgM versus anti-
apo IgM reflects that absolute levels of the former are lower than those of the latter, making anti-PCh IgM a more sensitive parameter in case of consumption. Preliminary experiments indeed indicate that anti-PCh IgM constitutes less than 1% of total IgM (N. Diaz Padilla, unpublished observation). Furthermore, we did not observe differences in anti-apo IgM or anti-PCh IgM, corrected for total IgM, on admission versus after 48 hours. In another study we observed in patients deceased after AMI that IgM binding to ischemic cardiomyocytes, appears in the infarcted myocardium approximately 24 hours after the occlusion of a coronary vessel as based on pathological criteria (P. Krijnen et al., submitted). Therefore, we expected a further decrease of anti-PCh IgM in the patients with AMI during the first 48 hours after admission, but this was not observed. Further studies should reveal whether increased production of anti-PCh IgM has masked a decrease of levels due to localization in the infarcts.

Several studies have shown significant changes in plasma IgM and IgG levels upon tissue injury for examples in case of burns [30]. In contrast we found no significant change of anti-apo IgM or anti-PCh IgM levels in patients with AMI during the first 48 hours. Though these data do not exclude that also AMI may induce an immunoglobulin response, such a response at least does not seem to occur within the observation period. Furthermore, similarity in levels on admission and after 48 hours, indicate that the analysis of levels on admission was not blurred by ongoing responses. Therefore, we decided to analyse whether patients with high levels on admission had a more intense inflammatory response than patients with lower levels of anti-apo or anti-PCh IgM.

Though patients with high levels of anti-apo IgM or anti-PCh IgM on admission had similar levels of IL6 and IL8 as patients with low levels (Table 2), their concentrations of CRP, sPLA2 and of complement activation products tended to increase. When the analysis was limited to the anti-PCh IgM specificity alone, sPLA2 concentrations were significantly higher in the group above median value. These data suggest that IgM with specificity for PCh is associated with the inflammatory response in patients with MI.

Infarct size is determined by a number of parameters including localization of the occlusion, collateral circulation, treatment, and others [31;32]. Therefore, we did not expect to find an association between levels of anti-apo IgM or anti-PCh IgM and parameters of cardiac damage in this limited number of patients. Yet, patients with anti-PCh IgM on admission tended to have higher cumulative release of CK and LDH. Assessment of infarction size using electrographical score is only possible in case of a first infarction. Among patients with a first infarction, those who had higher anti-PCh IgM had a significantly higher Selvester score on admission than those with lower anti-PCh IgM. IgM is a known activator of complement and
has been shown to bind apoptotic surfaces, in particular to phosphorylcholine [15] and activate complement [14]. Taken together, these observations would suggest that IgM antibodies particularly anti-PCh IgM may participate in the activation or amplification of the inflammation after AMI via the activation of complement.

Anti-PCh IgM can bind to apoptotic cells [15;27]. Competition experiments with soluble phosphorylcholine, however, indicate that not all IgM directed against apoptotic cells is against PCh (C.Ciurana, manuscript submitted). Our results do not point to a role of anti-apoptotic cell IgM with other specificities in myocardial infarction. Yet it should be noted that a relationship between plasma levels of this IgM and inflammatory responses and infarct size only become clear when plasma levels are limiting. Thus, our data do not definitely rule out a role for IgM binding to apoptotic cells in local inflammatory reactions ensuing in the infarcted myocardium.

In conclusion, levels of anti-PCh IgM in patients with AMI are associated with a somewhat more intense inflammatory response and a larger infarct size. These data fit with the hypothesis that this IgM species by enhancing inflammatory damage to the heart during infarction, may contribute to infarction size.

**Abbreviations**


**References**

Association of IgM antibodies and inflammation in patients with AMI


