Molecular mechanisms of complement activation by damaged cells
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Citation for published version (APA):

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Levels of natural IgM antibodies against phosphorylcholine in healthy individuals and in patients undergoing isolated limb perfusion.

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Journal of Immunological methods
In press
Abstract

Natural IgM antibodies against phosphorylcholine (anti-Pc IgM) resemble C-reactive protein (CRP) regarding specificity and have gained increasing attention because of their supposed role in clearance of damaged cells and in cardiovascular disease. In order to quantify these antibodies in human plasma, we have developed an ELISA system, in which p-aminophenylphosphorylcholine (PCH) coupled to human serum albumin (HSA) was coated on microtiter plates. Human plasma or serum samples were incubated in the plates, after which bound anti-Pc IgM was detected with mouse anti-human IgM-HRP. Pre-incubation of plasma samples with competitors such as phosphorylcholine, phosphorylethanolamine or glycine-HSA, confirmed that the ELISA was specific for anti-PC IgM. Levels of anti-Pc IgM in a cohort of healthy donors differed by more than 100-fold, whereas the fluctuation of anti-Pc IgM levels in individuals over time was small (coefficient of variation between 6 to 25%). Furthermore, there was no correlation between CRP and anti-Pc IgM in this cohort. Levels of anti-Pc IgM in the normal donors correlated significantly with IgM binding to apoptotic cells. To test the hypothesis that anti-Pc IgM can bind to neo-antigens expressed on necrotic or apoptotic cells, anti-Pc IgM was also quantified in patients with tumors undergoing isolated limb perfusion with tumor necrosis factor-α. Following this procedure a significant decrease of circulating anti-Pc IgM relative to total IgM was found in all 5 patients tested.

In conclusion, we have developed a specific and reproducible ELISA for anti-Pc IgM. Fluctuation of levels of these natural antibodies over time in healthy individuals was limited, although the variation among individuals was large. Significant decreases of levels of anti-Pc IgM were found to occur during tissue damage.

Introduction

Antibodies against phosphorylcholine (anti-Pc Abs) have been studied for a few decades and are thought to constitute a first line of defense against infections by virulent *Streptococcus pneumonia* and possibly other bacteria [3, 16]. These antibodies (abs) belong to the class of natural antibodies [11], and in mice frequently carry the so-called T15 idiotype, which initially was identified in an IgA paraprotein secreted by the plasmacytoma line T (EPC) 15 [24]. More recently, high titers of anti-Pc Abs have been described in hypercholesterolemic apolipoprotein E-deficient mice that develop severe atherosclerotic disease [19]. Most of these anti-Pc abs are of the IgM class, and are produced by CD5+ B1-
cells, the predominant B lymphocytes in the newborn. Further characterization of these abs revealed that they are structurally and genetically related, if not identical, to the classic T15 idiotype-carrying abs [15]. Both T15 anti-Pc abs as well as the abs occurring in atherosclerotic mice recognize the Pc headgroup moiety of phosphatidylcholine in oxidized low density lipoprotein particles (OxLDL) [23,12,19,7]. Both types of abs also bind to oxidized Pc-containing phospholipids on apoptotic cells but only poorly, if at all, to native LDL or non-oxidized Pc-containing phospholipids [13,4,22,23,7,19].

Anti-Pc abs presumably play a role in the pathogenesis of atherosclerosis although their precise role is not known [21]. Among their supposed functions is blockade of uptake of LDL by macrophages [10]. Accordingly, anti-Pc abs may slow down progression of atherosclerosis by inhibiting the transformation of macrophages into foam cells. Recently, Binder et al. [1] have shown that circulating levels of natural anti-Pc IgM in LDL receptor-deficient mice increase upon vaccination with *Streptococcus pneumoniae*. Importantly, this immunization attenuated progression of atherosclerosis, suggesting a protective effect of anti-Pc IgM in this murine model.

Based on their supposed role in murine models of atherosclerosis, studies on the relationship between circulating levels of anti-Pc IgM and the risk of cardiovascular disease are warranted. To this end specific and reproducible assays for these abs are needed. In the present study, we developed a specific and reproducible ELISA for the quantification of anti-Pc IgM in humans. Using this assay, the distribution of anti-Pc IgM in a cohort of healthy donors was estimated. In addition, the correlation between plasma levels of anti-Pc IgM and those of IgM binding to apoptotic cells was assessed. Finally, levels of this IgM were studied in 5 patients with malignant tumors undergoing isolated limb perfusion with tumor necrosis factor-α (TNF-α), to assess whether levels of anti-Pc IgM decrease in situations of enhanced tissue destruction.

**Materials and Methods**

**Patients and healthy individuals**

For the present study, the following sets of plasma samples were tested: 1) samples from healthy lab donors obtained at 1-week interval during a period of 6 weeks; 2) samples from healthy blood donors; 3) serial samples from 5 patients with a limb tumor (sarcoma or melanoma) who were treated with isolated limb perfusion with TNF α (Boehringer Ingelheim; 4 mg/leg or 3 mg/arm). These patients participated in studies on the effect of isolated limb...
perfusion on local and systemic activation of coagulation and inflammatory systems [29]. TNF-α was administered during a 3.5 h-limb perfusion. Blood samples were drawn before and at several times points after the perfusion up to 48 h. Baseline samples and samples obtained at 24 or 48 h after the perfusion, were used for the present study.

Collection of blood samples

The blood samples were collected in siliconized vacutainer tubes containing sodium citrate, EDTA or sodium heparin (Greiner, Kremsmunster Bad Haller, Austria). Plasma was obtained after centrifugation at 1,300 g for 10 min at 4°C. To obtain recalcified plasma, sodium citrate or EDTA plasma were recalcified by incubation with 12 mM CaCl₂ for 15 min at 37°C. The formed fibrin clot was removed by centrifugation as described above. Fresh normal human serum was obtained by collection of blood in glass tubes. Blood was allowed to clot for 1 h at room temperature (RT), and centrifuged as described above. Serum and plasma samples were stored in aliquots at -70°C.

Reagents, proteins and antibodies

P-aminophenylphosphorylcholine (Pc) was obtained from Sigma Chemical Co. (St Louis, MO, USA). O-phosphorylethanolamine (PE) and O-phospho-L-serine (PS) were obtained from Sigma-Aldrich (St Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as well as gel containing immobilized p-aminophenylphosphorylcholine was obtained from Pierce Biotechnology Perbio (Rockford, IL, USA). The conjugation buffer, (2-[N-morpholino] ethanesulfonic acid) Hydrate (MES), was obtained from Sigma Aldrich (St. Louis, MO, USA).

Human serum albumin (HSA) was obtained from the Business Unit Immune Reagents of our institute (Sanquin). Recombinant human C-reactive protein (rhCRP) was from BiosPacific (Emeryville, CA 94608, USA). Streptavidin-coupled to peroxidase (strept-PO) was purchased from Amersham-Pharmacia Biotech (Buckinghamshire, UK). L-C-biotin-N-hydroxysuccimide ester was from Pierce. Streptavidin coupled to polymerized horseradish peroxidase was obtained from the Business Unit Immune Reagents (Sanquin).

The mouse monoclonal antibody (mAb) against human CRP (5G4) was produced in our laboratory [30] and biotinylated (mAb 5G4bt) according to established procedures. MAb M15 against human IgM, coupled to horseradish peroxidase or to biotin, was obtained from the Business Unit Immune Reagents (Sanquin).
ELISA for anti-Pc IgM antibodies

p-Aminophenylphosphorylcholine was coupled to HSA according to the procedure provided by Pierce (Pierce Biotechnology, www.piercenet.com). Briefly, 4 mg of HSA was coupled to 4 mg of PCh in 0.1 M MES buffer, pH 4.5, containing 2 mg of EDC in a final volume of 1.6 ml. The mixture was incubated for 2 h at RT. The conjugated HSA was then dialyzed against phosphate buffered saline, pH 7.4 (PBS), at 4°C. As a control, glycine instead of PCh was coupled to HSA (glycine-HSA).

Polystyrene microtiter plates (Dynatech, Plochingen, Germany) were incubated o/n at 4°C with Pc-HSA (2.5 μg/ml) in 0.1 M carbonate/bicarbonate buffer, pH 9.6. Glycine-HSA was also included as a control. Final volume of this as well as of all other steps was 100 μl per well, unless stated otherwise. After washing the plates twice with PBS, residual binding sites were blocked (1 h at RT) with 200 μl per well of 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 CaCl2, 0.1 %, w/v, Tween 20, pH 20, pH 7.4), and incubated for 1h at RT. After this and the subsequent incubation steps the plates were washed with PBS containing 0.1 %, w/v, Tween-20. IgM bound to Pc-HSA was quantified with peroxidase-labelled anti-human IgM mAb diluted in assay buffer. Finally, peroxidase activity was visualized by incubation with 3,3', 5,5'-tetra-methyl-benzidine, 100 μg/ml in 0.11 M sodium acetate, pH 5.5, containing 0.003 %, v/v, H2O2. The reaction was stopped after 10 minutes by addition of 2 M H2SO4, and the absorbance at 450 nm was measured in a Titertek plate reader.

Dilutions of a pool of normal plasma, obtained from 40 healthy volunteers, were used to generate a standard curve in each microtiter plate. This standard was arbitrarily proposed to contain 100 U per ml of anti-Pc IgM antibodies. Results with plasma samples were related to this standard and expressed as U/ml of anti-Pc IgM.

The specificity of the binding of anti-Pc IgM to Pc-HSA was determined by competition immunoassay. The standard curve was pre-incubated with increasing amounts of the competitors (Pc, PE, and PS) or glycine-HSA. After 1-h incubation, the standard with or without competitors was added to the Pc-HSA coated plates and tested as described above. As another control, four normal sera were diluted 1 to 2 in VB, and absorbed batch wise onto PCh-Sepharose gel (1 volume of 1:2 diluted serum, with 1 volume of Sepharose suspension (capacity 5-11 mg of human CRP per ml gel, 5 ml gel and 5 ml buffer) o/n at 4°C. Glycine-Sepharose was used as a negative control. The supernatants were then tested in the ELISA.

Binding of CRP to Pc-HSA coated plates
Plates with Pc-HSA were prepared as described above, and incubated with recalcified NHP or rhCRP diluted in assay buffer (see previous paragraph) for 60 min at RT. After a washing procedure, bound CRP was detected by incubation for 60 min with biotinylated mAb 5G4 against human CRP (mAb 5G4bt), diluted in assay buffer followed by a subsequent incubation with polymerized peroxidase dissolved in veronal buffer containing 2 mM CaCl₂ and 2 %, v/v, milk (Campina, The Netherlands). Finally, peroxidase activity was visualized with tetra-methyl-benzidine as described above. As a control, sample incubations were prepared in the presence of 10 mM EDTA.

Quantification of total IgM

The concentration of total IgM was determined by nephelometry (Behring Nephelometer Analyzer, Marburg, Germany), according to standard procedures.

ELISA for human CRP

CRP concentration was determined by ELISA as described [30]. Briefly, polyclonal rabbit anti-human CRP (KH61) was used as capture abs and mAb 5G4bt was used as the detecting antibody. Results were obtained by reference to a standard from Behringwerke AG (Marburg, Germany).

Binding of IgM to apoptotic cells

Levels of IgM binding to apoptotic cells was assessed with FACS using Jurkat cells as previously described [32]. Briefly, Jurkat cells were made apoptotic by incubation with etoposide (Sigma). Cells were washed with serum-free medium and incubated with 10%, v/v, recalcified human plasma for 30 min. IgM binding to the cells was assessed by incubation with biotin-labeled anti-human IgM monoclonal antibody. Results were analyzed by flow cytometry, and expressed as mean fluorescence intensity (MFI). Plasma samples were also tested with vital Jurkat cells, as a control. Under the conditions used, binding of IgM to these vital cells was negligible.

Statistical analysis

Data were analyzed with the GraphPad software Prism, Inc (San Diego, CA, USA). The distribution of anti-Pc IgM in healthy donors was analyzed using a normality test. Groups of data were compared with repeated measures ANOVA. The variation of levels of anti-Pc IgM and CRP was evaluated by comparing intra-individual variation over time with
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the paired Student's t test (two-tailed). Correlations between parameters were assessed by estimating Spearman's rank correlation coefficient. A two-sided p-value < 0.05 was considered to indicate statistical significance.

Results

Quantification of anti-Pc IgM with ELISA

To quantify natural IgM antibodies against phosphorylcholine in humans, an ELISA was developed using PCh coupled to HSA as capture antigen on the plates. The coating antigen was validated by assessing binding of CRP, which has a similar specificity as anti-Pc IgM [25, 26, 28, 5]. Upon incubation of the plates with rhCRP or NHP, calcium-dependent binding of CRP was observed to Pc-HSA plates but not to glycine-HSA plates (data not shown). Thus, Pc-HSA was probably a suitable antigen for anti-Pc IgM. This was further assessed by incubation of Pc-HSA plates with serial dilutions of NHP followed by detection of bound IgM with biotinylated anti-IgM. A dose-dependent binding of IgM was observed (Fig. 1A). In general, IgM is notorious for antigen non-specific sticking to solid phases. Hence, we included a number of controls to rule out the possibility that the observed binding of IgM to Pc-HSA was specific. Binding of IgM to HSA- or non-coated plates upon incubation with dilutions of NHP was negligible. However, significant binding of IgM to glycine-HSA was observed, though this binding was less than that to Pc-HSA coated plates (Fig.1A). We did competition experiments to further substantiate the specificity of the ELISA for anti-Pc IgM. Binding of IgM to Pc-HSA coated plates was almost completely inhibited in the presence of increasing concentrations of Pc during the sample incubation, whereas PE, PS or glycine-HSA had no effect (Fig.1B). Conversely, binding of IgM to glycine-HSA was inhibited by glycine-HSA but not by Pc, PE or PS (data not shown). In addition, four sera absorbed onto PCh-Sepharose yielded negative results in the ELISA with Pc-HSA coated plates, whereas the same sera absorbed onto glycine-Sepharose exhibited unaffected IgM binding to Pc-HSA coated plates (Fig.1C).
Figure 1: Specificity of the ELISA for anti Pc IgM antibodies. (A) Microtiter plates were coated with Pc-HSA (filled square) glycine-HSA (diamond) HSA (triangle) or coating buffer (open square). Dilutions of pooled normal human plasma were then added to the plates. Bound IgM was detected using mouse anti-human IgM conjugated to HRP. Peroxidase activity was visualised with TMB. (B) Pooled normal plasma was pre-incubated for 1h with Pc (filled square), PE (triangle), PS (filled circle) or glycine-HSA (diamond), and tested in the ELISA. (C) Four sera were adsorbed onto Pc-Sepharose and tested in the ELISA. Bars represent the amount of specific IgM against Pc relative to the total IgM (U/g) in the sera absorbed with Pc-Sepharose (open bars), glycine-Sepharose (black chequered bar), or without absorption (black bar). Results in the figure are the means of triplicate determinations. The experiment was repeated twice with similar results.

Thus, these experiments together demonstrated the specificity of the ELISA with Pc-HSA coated plates for anti-Pc IgM. We next tested the effect of the method of blood collection, and of freezing and thawing of samples, on levels of anti-Pc IgM as measured with the ELISA. In Table 1 it can be seen that anti-Pc IgM levels were similar in plasma samples collected in EDTA, sodium citrate or in sodium heparin, as well as in serum samples. Moreover, Table 1 also shows that five additional cycles of freezing at -70°C and thawing did not affect levels of anti-IgM. We also tested with plasmas from 10 different donors whether binding of IgM in the Pc-HSA ELISA was calcium-dependent. The results showed no difference in levels when EDTA was present during the sample incubation step (data not shown).
Variation of anti-Pc IgM levels in healthy donors

To quantify results obtained with plasma samples, a pool of plasma samples from healthy donors was arbitrarily assigned 100 U of anti-Pc IgM per ml. Results with samples to be tested were then compared with this plasma pool and expressed as U/ml. In 40 healthy donors, the median concentration of anti-Pc IgM was 108 U/ml, the 25th percentile being 55 U/ml, and the 75th percentile 162 U/ml. The lowest concentration of anti-Pc IgM in these healthy donors was 3 U/ml and the highest was 469 U/ml. Thus, levels of anti-Pc IgM in the healthy donors differed considerably, i.e. more than 100-fold. The distribution of anti-Pc IgM in the donors according to age is shown in Fig 2A, and that according to sex is shown in Fig 2B. Younger or older persons had similar levels of anti-Pc IgM. Moreover, men had similar levels as women. To estimate variation of anti-Pc IgM levels over time, plasma samples from 20 healthy donors were collected weekly during a period of 6 weeks, and tested for anti-Pc IgM with the ELISA. Small fluctuations in anti-Pc IgM levels were observed (coefficient of intra-individual variation over time: 6-25 %; Fig. 3A). For comparison, CRP plasma levels
were also measured in the same samples and appeared to differ significantly (p< 0.0001) more during the observation period of 6 weeks than anti-Pc IgM levels (coefficient of intra-individual variation over time for CRP: up to 114%; Fig. 3B). These differences in variation of plasma levels of anti-Pc IgM CRP were not explained by different reproducibility of the assays, since the coefficient of inter-assay variation was less than 10 % for either assay.

Figure 3: Fluctuation of individual levels of anti-Pc IgM over time. Anti-Pc IgM (A and B) and CRP concentrations (C and D) were measured in plasma obtained weekly from healthy controls.

Because of the functional similarities (specificity for PC; complement activation) between anti-Pc IgM and CRP, we studied the correlation between levels of either parameter in the healthy individuals. However, the levels of anti-Pc IgM and CRP, did not correlate (Spearman’s r: 0.115, p = 0.2429). Furthermore, we also assessed the relationship between anti-Pc IgM and total IgM concentration. These parameters showed only a borderline correlation (Spearman’s r 0.44; P = 0.051).
Anti-Pc IgM in healthy donors correlate with IgM binding to apoptotic cells

IgM with specificity for Pc has been shown to bind to apoptotic cells [4,5,13,23]. In order to examine the relation between levels of natural IgM abs as measured with Pc-HSA coated plates, and IgM binding to apoptotic cells, 30 sera were tested both in the anti-Pc IgM ELISA as well as for binding of IgM to apoptotic Jurkat cells. As seen in Fig. 4 anti-Pc IgM correlated positively with the amount of IgM that bound to the apoptotic cells (p <0.0001).

Circulating levels of anti-Pc IgM antibodies decrease during tissue damage

We postulated that during tissue damage, neo-antigens are exposed on the membranes of jeopardized cells, which may be recognized by the anti-Pc IgM antibodies. Consequently, a decrease in plasma levels of anti-Pc IgM is expected in situations of sufficient tissue damage.
To test this hypothesis, the kinetics of anti-Pc IgM in plasma samples from five patients undergoing isolated limb perfusion with TNFα because of sarcoma or melanoma was determined. In all patients regression of the tumor was observed, and was accompanied by high levels of several pro-inflammatory cytokines such as IL-6 (data not shown). The anti-Pc IgM concentration was related to total IgM levels in order to adjust for hemodilution. A moderate decrease of anti-Pc IgM levels was observed in all patients at 24 h after perfusion (p>0.05). At 48 h after perfusion, this decrease was up to 60% and had become significant (p<0.05; Fig. 5).

Table 1: Repeated freezing/thawing has no effect on levels of anti-Pc IgM in plasma or serum samples. EDTA, citrated or heparin plasma or serum samples were frozen at -70°C and thawed one (t1) to six (t6) times, and then tested in ELISA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>t1</th>
<th>t6</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>141.91±28.63</td>
<td>145.08±28.13</td>
</tr>
<tr>
<td>Citrate</td>
<td>128.39±21.74</td>
<td>117.15±19.57</td>
</tr>
<tr>
<td>Heparin</td>
<td>139.58±22.17</td>
<td>143.16±24.35</td>
</tr>
<tr>
<td>Serum</td>
<td>133.96±23.85</td>
<td>151.13±29.43</td>
</tr>
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Values represent mean ± SEM of the anti-Pc IgM concentration in U/ml of 6 healthy donors.

Discussion

Some decades ago, anti-Pc IgM was considered to constitute a first-line defense against microorganisms. More recently, interest in these antibodies was renewed because of their potential involvement in processes such as atherosclerosis [1,17-19,31] or removal of apoptotic cells and cellular debris [13]. As a consequence levels of anti-Pc IgM may constitute a risk marker for human diseases such as cardiovascular disease [21]. Here we describe a specific and reproducible ELISA for the quantification of anti-Pc IgM. Levels of these antibodies differed by more than 100-fold among healthy donors, whereas the intra-individual variation over time was limited. Furthermore, anti-Pc IgM levels correlated well with IgM binding to apoptotic Jurkat T cells and decreased in patients with sarcoma or melanoma upon isolated limb perfusion with TNF-α.

Quantification of IgM with ELISA in general is associated with an increased risk of non-specific binding of IgM to the plates. However, the ELISA for the quantification of natural anti-Pc IgM in humans described here was considered specific since the signal was
abolished when plasma was pre-incubated with Pc, but not PE or PS. In addition, pre-absorption of sera with Pc-Sepharose reduced IgM binding in the ELISA. Furthermore, pre-incubation of plasma with modified albumin (glycine-HSA) did not affect the response of plasma samples in the anti-Pc IgM ELISA, ruling out the possibility that IgM detected in this assay had interacted with the chemical linker used to prepare Pc-albumin. Thus, the IgM detected in the ELISA was IgM specifically binding to Pc. A limitation of the use of Pc-albumin as a coating antigen in the ELISA is that Pc conjugated to HSA may not resemble Pc presented by injured cells. However, CRP, which has a well known specificity for Pc [25,28] and is able to bind to apoptotic and injured cells [5,9], easily bound to the Pc-HSA. Thus, Pc conjugated to HSA resembled Pc exposed on apoptotic or injured cells. This was confirmed by the observation that levels of anti-Pc IgM in healthy donors correlated with IgM binding to apoptotic cells.

Studies on the relationship between biochemical markers and cardiovascular events are more credible when markers to be measured in blood samples are stable during repeated freezing and thawing. As with anticardiolipin IgM [2], we found no significant difference in mean concentration of anti-Pc IgM after repeated freeze-thaw cycles.

Notably, the levels of anti-Pc IgM differed by more than 100-fold among healthy donors, though there was no relationships between these levels and age or sex. In animal models for atherosclerosis anti-Pc IgM increase over time [1]. Our data do not support the notion that anti-Pc IgM in humans is increased in atherosclerosis since in that case some relationship between plasma levels and age would be expected. Conversely, anti-Pc IgG1 was significantly higher in children than in adults [6]. In addition, anti-Pc IgM levels did not correlate with total IgM concentration, as has been found earlier for anti-Pc IgG [6]. We speculate that the inter-individual differences in anti-Pc IgM levels among healthy donors reflect genetic variations as well as exposure to different microbes during life.

Anti-Pc IgM and CRP share similarities in biological function in that they can bind to Pc exposed on injured cells and activate complement [9,20]. Nevertheless, plasma levels of these moieties did not correlate with each other. Hence, one can speculate that IgM and CRP constitute two independent mechanisms taking care of injured cells. The relative contribution of anti-Pc IgM and CRP to complement activation could differ between individuals depending of their relative concentration. Indeed, we (Krijnen P. et al., unpublished observations) have recently shown that the relative amounts of CRP and IgM deposited on cardiomyocytes in infarcted myocardium may differ from patient to patient.
Complement activation by apoptotic cells in plasma is mainly mediated by IgM binding to neo-epitopes exposed in the membranes of these cells [13,32]. Though it has been claimed that most if not all of these IgM antibodies recognize Pc exposed in the lysophosphatidylcholine in the cell membrane [13], we have observed that this only holds for some individuals, whereas others also have IgM recognizing additional, unknown epitopes (Ciurana C. et al., in press). In this study we report a good correlation between the levels of anti-Pc IgM as measured by ELISA and the amount of IgM binding to apoptotic Jurkat cells. This finding is consistent with the notion that part of the IgM binding to apoptotic cells recognizes Pc. The specificity of the rest of the IgM recognizing apoptotic cells is currently under investigation.

To further verify that anti-Pc IgM can bind to apoptotic or damaged cells we measured plasma levels of these antibodies in patients with sarcoma or melanoma and treated by isolated limb perfusion with TNF-α. Most of these patients respond well to this therapy within a few days [14,27], and exhibit massive death of both malignant cells and endothelial cells of the tumor vasculature. Ischemia and subsequent reperfusion of the perfused limb may also add to cell death. A decrease of anti-Pc IgM relative to total IgM levels was observed in all of the patients tested, in agreement with the notion that anti-Pc IgM can bind to oxidized or hydrolyzed phospholipids of damaged cells. We speculate that this mechanism contributes to the clearance of damaged cells and cellular debris.

In conclusion we describe a specific and reproducible ELISA for anti-Pc IgM. Levels of this IgM differ by up to 100-fold among healthy persons. Future studies on anti-Pc IgM should delineate its role in human diseases such as cardiovascular disease.

Abbreviations

abs: antibodies; anti-Pc IgM: anti-phosphorylcholine IgM antibodies; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HSA: human serum albumin; mAb: monoclonal antibody; NHP: normal human plasma; o/n: overnight; oxLDL: oxidized low density lipoprotein; PBS: phosphate buffered saline, pH 7.4; Pc: phosphorylcholine; PCh: p-aminophenylphosphorylcholine; PE: O-phosphoryl-ethanolamine; PS: O-phospho-L-serine; rhCRP: recombinant human C-reactive protein; RT: room temperature; strept-PO: streptavidin-coupled to peroxidase; TNF-α: tumor necrosis factor-α; VB: veronal-buffered saline containing 2 mM CaCl₂, 10 mM MgCl₂, pH 7.4.

Reference List

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33. Complement activation by apoptotic cells occurs predominantly via IgM and is limited to late apoptotic (secondary necrotic) cells. Autoimmunity 37 (2): 95.