The Autoreactivity of Anti-Phosphorylcholine Antibodies for Atherosclerosis-Associated Neo-Antigens and Apoptotic Cells

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The Autoreactivity of Anti-Phosphorylcholine Antibodies for Atherosclerosis-Associated Neo-Antigens and Apoptotic Cells\textsuperscript{1,2}

Peter X. Shaw,\textsuperscript{3*} Carl S. Goodyear,\textsuperscript{7} Mi-Kyung Chang,\textsuperscript{*} Joseph L. Witztum,\textsuperscript{4*} and Gregg J. Silverman\textsuperscript{3,4**}

Abs specific for phosphorylcholine (PC) are known to contribute to the immune defense against a variety of microbial infections. To assess for other types of binding interactions, we performed surveys of anti-PC Abs of diverse biologic origins and structural diversity and demonstrated a common autoreactivity for oxidatively modified low density lipoprotein and other oxidation-specific structures containing PC-Ags. We also found that cells undergoing apoptosis sequentially express a range of oxidation-specific neo-self PC determinants. Whereas natural Abs to PC recognized cells at early stages of apoptosis, by contrast, an IgG anti-PC Ab, representative of a T cell-dependent response, recognized PC determinants primarily associated with late stages of apoptosis. Cumulatively, these results demonstrate a fundamental paradigm in which Abs from both the innate and the T cell-dependent tiers of the B cell compartment recognize a minimal molecular motif arrayed both on microbes and as neo-self Ags linked to atherosclerosis and autoimmune disease. The Journal of Immunology, 2003, 170: 6151–6157.

The functional capacities of the immune system were viewed earlier as a delicate balance between the activities contributing to the defense from infectious agents and other threats, and the avoidance of autoreactivity that can damage the host. However, it is now generally accepted that certain aspects of autoimmunity may occur in, and perhaps even contribute to, health (1). This is especially true for the B cell compartment for which absolute immunologic tolerance apparently does not occur. In fact, some level of autoreactivity may be required for the selection of newly generated B cell clones into the peripheral pool of mature lymphocytes (2).

In recent reports we have shown that B lymphocytes secrete autoantibodies capable of recognizing a distinct class of neo-self Ags, which are newly generated on oxidatively modified phospholipids (OxLDL)\textsuperscript{3} and on cells that have undergone apoptosis (3, 4). These autoantibodies specifically bind to oxidized phosphorylcholine (PC)-containing phospholipids, such as 1-palmitoyl-2-(5-oxo-

ovalleryl)-sn-glycero-3-phosphocholine (POVPC), and also POVPC-protein adducts, but not to native low density lipoprotein (LDL) and nonoxidized phosphatidylcholine such as in 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (PAPC) (5). Such autoantibodies also recognize these immune determinants in atherosclerotic lesions, a condition involving chronic inflammation and lipid abnormalities that is the most common source of morbidity and mortality in the western world. In animal models of atherosclerosis, Ab titers to these OxLDL-specific epitopes often correlate with the extent and severity of disease. In addition, these POVPC-binding Abs can block the recognition and uptake of OxLDL by macrophages, a rate-limiting step in the development of foam cells that are major contributors to atherosclerotic lesions, indicating that oxidized phospholipids can serve as ligands on OxLDL to mediate such uptake (5).

Characterization of the OxLDL-specific B cell hybridomas from the spleens of atherosclerosis-prone apolipoprotein E-deficient (ApoE\textsuperscript{−/−}) mice led to an unexpected discovery; a dominant set of these anti-OxLDL Abs, as exemplified by EO6, was found to be encoded by nonmutated variable region genes completely identical to those of T15 Abs (6) that were long known for their phosphorylcholine (PC) specificity. It has been known for years that T15 Abs play a central role in immune defense from infection by microbial pathogens like pneumococci and salmonella for which PC is an immunodominant antigenic determinant. The T15 clonal set are natural Abs, (i.e., those which arise without specific immunization), which solely derive from B-I cells that represent an innate tier within the adaptive immune system (7). We found that T15 Abs, like EO6, recognize the PC headgroup in OxLDL and in oxidized PC-containing phospholipids such as POVPC and POVPC-protein adducts, but not in native LDL and nonoxidized PC-containing phospholipids (6, 8). Cumulatively, these findings suggest that for T15 B cell responses there is a functional molecular mimicry of PC-containing epitopes occurring in common microbial pathogens on the one hand, and in oxidatively altered determinants on OxLDL and apoptotic cells on the other (4, 6, 9).

In the current studies we have explored whether the cross-reactivity between PC-containing microbial determinants and certain oxidatively modified neo-self Ags is a property specific to the T15 B cell clone or whether it is common to many anti-PC Abs. Our panel included group I anti-PC Abs that are “natural Abs” and...
group II anti-PC Abs that are hypermutated Abs from T cell-dependent responses to PC-protein conjugates (10). These analyses indicate that autoreactivity for atherosclerosis and apoptosis-associated neo-determinants is a property of many anti-PC Abs of diverse origins, demonstrating a fundamental paradigm of dual functionality that is integral to the immune recognition of a simple minimal molecular hapten.

Materials and Methods

Ags and ligands

POVPC was synthesized as previously described (5, 8), and POVPC-BSA adducts were generated in the presence of cyanoborohydride as described (5). PC chloride (PC-C) was from Sigma-Aldrich (St. Louis, MO). Alkaline phosphatase-labeled goat anti-mouse-IgM, anti-IgG, and anti-IgA were from Sigma-Aldrich, and LumiPhos 530 was from Lumigen (Southfield, MI). P-diazonium phenyl-PC conjugated to keyhole limpet hemocyanin (PC-KLH) was from Biosearch Technologies (Novato, CA). PC-BSA was the kind gift of Dr. N. Kliman (The Scripps Research Institute, La Jolla, CA). Purified pneumococcal cell wall polysaccharide (C-PS) was obtained from the Statens Serum Institut (Copenhagen, Denmark). LDL was prepared in the presence of EDTA and protease inhibitors, and copper-oxidized LDL (Cu-OxLDL) and malondialdehyde (MDA)-modified LDL (MDA-LDL) were prepared as previously described (3, 11).

Monoclonal Abs

Monoclonal autoantibodies against OxLDL were generated from ApoE-deficient mice on high-fat diets with very high titers of autoantibodies to Cu-OxLDL (3). In brief, B lymphocytes from the spleens of two ApoE/−/− mice, which had not been immunized exogenously, were fused to generate hybridomas, which were screened for binding to model oxidation-associated epitopes of OxLDL, including Cu-OxLDL and MDA-LDL. Thirteen hybridoma cell lines were isolated and designated as E/O autoantibodies to indicate their ApoE/−/− source. All were IgM isotype and characterized as described (3, 5). Ab EO6 bound to OxLDL was specific for PC and displayed the T15 idiotype (6). In contrast, EO14 specifically bound to MDA-LDL (3). Abs from our lab used in this report were purified from ascites by fast protein liquid chromatography, as described (3). The T(EPC)15, murine monoclonal IgA anti- phosphophorylcholine Ab was the kind gift of Dr. H. Kohler (University of Kentucky, Lexington, KY). Group I anti-PC mAbs, H(OPC)8, M(OPC)167, M511, M(OPC)603, and the group II anti-PC mAbs, PGCl-14 and M3C56, were gifts of Dr. J. J. Kenny (National Institute of Aging, Baltimore, MD) and Dr. M. Rittenberg (University of Oregon, Portland, OR) (see Table I). Isotype controls were purchased from Sigma-Aldrich.

Chemiluminescent immunoassay

For direct binding chemiluminescence studies, microtiter wells were coated with Abs dissolved in PBS with antioxidants as described (3, 5, 12). In parallel, replicate wells were also coated with affinity-purified goat anti-mouse Ig at 5 μg/ml (Jackson ImmunoResearch Laboratories, West Grove, PA) to document loading of primary Abs. A total of 50 μl of primary Abs (in BSA-PBS) were added to wells and incubated for 1 h at room temperature. The amount of bound primary Ab was detected with alkaline phosphatase-labeled secondary Ab, followed by 25 μl of 50% LumiPhos 530 solution, and measured as relative light units (RLU) over 100 ms using a Dynex Luminometer (Dynex Technologies, Chantilly, VA) (12). For the competitive inhibition assay, 25 μl of primary Ab (10 μg/ml in BSA-PBS) was mixed with 25 μl of the indicated concentration of competitors and then incubated on the Ag-coated plate for 1 h at room temperature, with detection as described (6).

Immunohistochemistry

Rabbit aortas were perfusion fixed, and segments containing large atherosclerotic lesions were paraffin embedded and then sectioned. The immunostaining for oxidation-specific epitopes and macropathies was performed as described (13). Briefly, the tissue sections were deparaffinized and incubated in 3% hydrogen peroxide to block endogenous peroxidase activity. After blocking nonspecific binding with nonimmune antisera, the sections were incubated with 20 μg/ml murine primary monoclonal anti-PC Abs and appropriate isotype controls. The Ig bound to rabbit lesion was detected using biotinylated secondary Abs followed by an avidin-biotin-peroxidase complex and the diaminobenzidine substrate (Vector Laboratories, Burlingame, CA). As an additional control, adjacent sections were also stained without the primary Ab. The sections were then counterstained with hematoxylin.

Assays of Ab binding to apoptotic cells

Thymocytes from 4- to 6-wk-old C57BL/6 mice were isolated, dissociated, and cultured in RPMI 1640 containing 5% FCS, with apoptosis induced by 1 μM dexamethasone for 16 h. In replicate cultures we added the pan-caspase inhibitor Z-Val-Ala-Asp(Ome)-fluoromethylketone (Z-VAD) at 25 μM or control compound (Enzyme Systems Products, Livermore CA). Aliquots of 10^6 cells were incubated with mAb at 4°C for 20 min, or in certain studies mAbs were first preincubated with an inhibitor overnight at 4°C. Cells were then washed and incubated with 1 μg/ml isotype-specific fluorescein-conjugated Ab (BD PharMingen, San Diego CA) for 20 min. Thereafter, cells were washed and incubated with propidium iodide (PI), or 7-aminoactinomycin D (7-AAD) and annexin V-PE (BD PharMingen). Data were acquired with a FACSCaliber (BD Biosciences, Mountain View, CA).

Results

All group I anti-PC Abs bind OxLDL epitopes

To extend our earlier findings regarding T15 and EO6 Abs, we evaluated four additional group I mAbs to PC, H8, M167, M511, and M603 (Table I). Confirming classical reports (14), all group I anti-PC mAbs bound strongly to the pneumococcal C-PS, for which PC is the immunodominant epitope, and PC-KLH (Fig. 1). These Abs were also strongly reactive with a conjugate of the hapten, POVPC, a PC-containing oxidation-specific compound identified as an immunodominant epitope in atherosclerotic vascular lesions (5, 6). In addition, except for M511 these Abs all bound strongly to Cu-OxLDL but display little or no activity for native LDL. Also reiterating our earlier findings (6), the binding of group I Abs to OxLDL was strongly inhibited by PC-C1 salt or PC-KLH or OxLDL (Fig. 2), except for M603 for which OxLDL was a somewhat weaker inhibitor. In contrast, neither native LDL,

<table>
<thead>
<tr>
<th>Group I</th>
<th>V_H</th>
<th>V_L</th>
<th>C-PS</th>
<th>POVPC</th>
<th>OxLDL</th>
<th>Staining of Atheroma</th>
<th>Binding to Apoptotic Thymocytes</th>
<th>Refs.</th>
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<tr>
<td>T15</td>
<td>IgA</td>
<td>S107.1</td>
<td>V_22</td>
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<tr>
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<td>IgM</td>
<td>S107.1</td>
<td>V_22</td>
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<tr>
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<td>IgA</td>
<td>S107.1</td>
<td>V_22</td>
<td>++++</td>
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<tr>
<td>M511</td>
<td>IgA</td>
<td>S107.1</td>
<td>V_22</td>
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<tr>
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<td>J558</td>
<td>V_24</td>
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<td>IgG2b</td>
<td>PJ14</td>
<td>V_L</td>
<td>–</td>
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</table>

* H8 has been reported to express more than a single L chain.
* For studies of H8 and M167, the only available samples were shown to be degraded by PAGE analysis, which may have impaired the detection of their binding activities in the flow cytometric studies of apoptotic thymocytes.
nor malondialdehyde modified LDL (MDA-LDL), another oxidation-specific epitope of OxLDL structurally unrelated to PC, inhibited the interactions of the group I Abs with OxLDL. These findings suggest that the binding of the group I Abs is mediated by the recognition of the same, or closely related, oxidation-specific PC-containing epitope(s) on OxLDL, with limited differences in their relative reactivities.

Group II anti-PC Abs also bind oxidation-associated epitopes

The two group II anti-PC Abs, PCG1-14 (γ1/κ) and M3C65 (γ2b/λ1), bound strongly to PC-KLH, the immunogen used in their generation, with somewhat less activity for POVPC-BSA, an oxidation-specific PC-containing compound representative of POVPC-like compounds found in atherosclerotic lesions (5, 15) (Fig. 3). These group II Abs also had significant but weaker reactivity with Cu-OxLDL, but little or no direct binding reactivity with native-LDL or the control protein BSA. As reported earlier (10), these group II Abs were nonreactive with pneumococcal C-PS. The binding of PCG1-14 or M3C65 to PC-KLH was completely inhibited by PC-KLH, but with little or no inhibition by PC-Cl, native LDL, MDA-LDL, Cu-OxLDL (Fig. 4), or pneumococcal C-PS (data not shown). Furthermore, direct binding of the group II Abs to Cu-OxLDL was strongly inhibited by PC-KLH, whereas Cu-OxLDL was a good inhibitor for PCG1-14, but not M3C65; PC-Cl, native LDL, or MDA-LDL did not compete at all (Fig. 4). These studies indicate that group II Abs recognize Cu-OxLDL via PC-containing neo-determinants that share features with PC-KLH.

Anti-PC Abs recognize determinants in atherosclerotic lesions

The IgM group I Ab, EO6, and IgA group I Abs, T15, H8, and M167, specifically immunostained macrophage-rich areas of atherosclerotic lesions (Fig. 5, A and B), with relative reactivities akin to their ELISA activities for PC and OxLDL epitopes. Although M603 had the similar binding properties in solid phase immunoassays, it displayed specific but somewhat weaker recognition of these lesions. The IgA group I Ab, M511, which did not bind to Cu-OxLDL, also failed to immunostain these vascular lesions (Fig. 5A). Of the IgG group II anti-PC Abs, PCG1-14 also strongly stained these macrophage-rich lesions, whereas M3C65 displayed only weak immunostaining (Fig. 5B). These results suggest that reactivity of anti-PC Abs with atherosclerotic vascular lesions generally mirrors their activities for PC-related epitopes on Cu-OxLDL (Table I).

Binding of anti-PC mAbs to thymocytes undergoing apoptotic cell death

Extending earlier findings (4, 6), the group I Abs, T15 Ab and EO6, displayed a similar pattern of binding to apoptotic cells (Fig. 6A) but not to cells cultured without dexamethasone (data not shown). Another group I Ab, M603, also specifically recognized apoptotic thymocytes with a pattern of reactivity nearly identical to that of...
T15 (Fig. 6A), whereas the H8 and M167 Abs displayed a lower level of reactivity. Only M511, which also displayed weaker PC binding activities in other assays, was devoid of detectable reactivity with apoptotic thymocytes (Table I). These group I Abs reacted primarily with thymocytes that were PI-dim or negative (and 7-AAD dim or negative), indicating dying cells that maintained cell membrane integrity (Fig. 6A). They also preferentially recognized cells with little or no reactivity with annexin V that is specific for PS, which has “flipped” to the outer membrane during apoptotic death (16). Also reiterating our earlier results with T15 and E06 (6), the binding of group I Abs with apoptotic thymocytes was blocked by either PC-Cl salt, PC-KLH, pneumococcal C-PS, or by OxLDL, but not by native LDL (data not shown).

The group II IgG anti-PC Ab, PCG1-14, was also strongly reactive with dexamethasone-induced thymocytes (Fig. 6B) because of the recognition of an annexin V<sup>high</sup> and 7-AAD<sup>high</sup> subpopulation (Fig. 6C), which represents a set of dying thymocytes distinct from those recognized by the group I anti-PC mAbs. Moreover, conaining with annexin V did not interfere with Ab binding (data not shown), suggesting recognition of an epitope coexpressed with membrane-accessible PS on these dying thymocytes. This Ab reactivity for apoptotic thymocytes was completely inhibited by PC-KLH, the immunogen used in its generation, whereas there was somewhat lesser efficiency with OxLDL, presumably because of differences in epitopic density. There was no significant inhibition, however, by PC-Cl salt or by native LDL (Fig. 6D). These studies suggest that unlike the group I Abs that recognized cells at a relatively early stage of programmed death, the PCG1-14 Ab primarily recognized cells at later stages. Moreover, the inhibition patterns suggest that these Abs recognize PC-related determinants on apoptotic cells with the same Ag-binding sites used for PC epitopes in solid phase assays, and their recognition of neo-self epitopes in vascular lesions and apoptotic cells is limited by the same structural biases.

To assess how alteration of apoptotic pathways may influence expression of neo-determinants recognized by anti-PC Abs, we also cultured the dexamethasone-induced thymocytes with Z-VAD, a pan-caspase inhibitor, and found a slowing in the progression to cytolysis, resulting in greatly increased representation of PI-dim/negative (Fig. 6, A and B) and annexin V-negative thymocytes (data not shown). Significantly, the group I Abs, T15 and M603, had enhanced binding reactivity for the apoptotic thymocytes cultured with Z-VAD (Fig. 6A) (e.g., 4% of control-treated thymocytes were T15 reactive, which increased to 8% after Z-VAD treatment), whereas binding of the group II Ab, PCG1-14, was significantly reduced (Fig. 6B) (e.g., 25% of control treated induced thymocytes were PCG1-14 reactive, which decreased to 3.4% after Z-VAD treatment). These opposite effects of the caspase inhibitor on the binding of group I and group II anti-PC Abs to apoptotic thymocytes was highly reproducible in more than five independent studies. Together, these studies suggest that there are inherent differences in the specific PC-associated neo-determinant(s) recognized by the group I Abs and the group II Ab, PCG1–14, and/or that the determinants recognized by these two types of Abs are differentially generated, or revealed, in the cell membrane at distinct stages of programmed cell death.

Discussion

Our studies have elucidated a primordial Ag-Ab system of potent dual functional capabilities. Extending our earlier findings, we found that each of six group I Abs, which all utilize VHS107.1 gene rearrangements, displayed strong and specific binding interactions with POVCPC, an immunodominant oxidation-specific PC-containing epitope in atherosclerotic lesions and also with oxidatively modified LDL (i.e., Cu-OxLDL), and binding was specifically inhibited by simple PC-Cl salt. Significantly, the relative levels of anti-PC reactivities were also roughly proportional to that for recognition of determinants in atherosclerotic vascular lesions.

The two group II anti-PC Abs, derived from secondary immune responses to a T cell-dependent PC-protein conjugate, use structurally diverse Ag-binding sites (17) to bind with high affinity to PC-KLH, a derivatized PC compound; but unlike group I Abs they do not recognize PC alone (10). Therefore, we were surprised to discover that group II anti-PC Abs also recognized determinants on OxLDL and in atherosclerotic lesions, with reactivities that were proportional to that for POVPc-BSA and Cu-OxLDL. Whereas we found that group I and group II Abs recognize neo-determinants on apoptotic thymocytes, there were distinct differences in their preferences for the binding of PC-determinants exposed during induced cell death, suggesting that a range of different membrane-associated PC neo-determinants may be revealed during the course of apoptosis.

The autoreactivities conveyed by certain Ab transgenes have been reported to drive B cells into one mature lymphocyte subset or another, and T15 Ab transgenes have been shown to drive mature B cells to accumulate in the B-1 compartment (18). Moreover,
even in immunologically intact naive mice raised under germ-free conditions, T15 B cells become clonally expanded during the perinatal period, to later dominate Ab responses to PC, the immunodominant moiety in nonencapsulated *Streptococcus pneumoniae* and several other microbes. Murine B cells expressing transgenes for group I anti-PC Abs have also been reported to display altered activation, trafficking, and peripheral accumulation (19), which may suggest that these Ig transgene-expressing B cells were being selected by an autoantigen. However, until our recent demonstration of PC neo-determinants on apoptotic cells and on atherosclerosis-associated oxidation-specific Ags (20), the true nature of such postulated selecting self-Ags was unsuspected (21).

For the M167 group I Ab, expression of such Ig transgenes leads to a characteristic surface phenotype, CD21\(^{\text{high}}\)CD23\(^{\text{low}}\)CD1\(^{\text{mid}}\)/CD1\(^{\text{high}}\), on B cells that accumulate in the splenic marginal zone compartment (22). Significantly, Kearney and colleagues (22) have demonstrated that M167-expressing marginal zone B cells act in concert with B-1 cells to produce the most effective anti-PC responses to pneumococcal infection. Our studies indicate that these natural Abs also recognize PC-containing autoantigens, and we speculate that the special autoreactive qualities of these specific clones or the context of their autoantigen exposure contributes to their preferential and recurrent selection into different innate-like B cell compartments.

The group II Abs, PCG1-14 and M3C65, generated by immunization with a form of PC linked to a carrier protein, are encoded by hypermutated genes bearing the hallmarks of conventional B cells (i.e., follicular B cells) recruited into T cell-dependent germinal center reactions, which are the sources of memory B-cells. We found that these Abs also exhibit inherent autoreactivity with

**FIGURE 4.** Group II anti-PC Ab binding to Cu-OxLDL inhibited by PC-KLH. Studies were performed as described for Fig. 2.

**FIGURE 5.** Anti-PC mAbs immunostain atherosclerotic lesions. Rabbit aortas containing atherosclerotic lesions were immunostained with anti-PC followed by biotinylated secondary Abs, so epitopes recognized are indicated in brown; the nuclei are counterstained with hematoxylin. A, Immunostaining with IgA group I Abs or isotype control. B, Immunostaining with IgM group I Abs or isotype control, or with IgG group II Abs or isotype control.
PC-containing neo-self determinants, but this apparently does not prevent clonal entry into the peripheral B cell pools, even for follicular B cells. However, it is also possible that cell debris bearing these late-stage apoptosis-associated determinants may not be accessible to the immune systems of healthy individuals.

Our observations bear several important parallels with those of Radic and coworkers (23, 24), who demonstrated that 3H9, a classic lupus IgG autoantibody to native DNA, also recognizes PS, a distinct phospholipid determinant expressed on lymphocytes at advanced stages of apoptotic death. Moreover, as 3H9 has replacement mutations that enhance PS binding affinity, these structural and functional properties are akin to the hypermutated PCG1-14 group II Ab that recognized determinant(s) on thymocytes that also co-stained with markers for the loss of membrane integrity. By contrast, group I Abs recognized a different type of apoptosis-associated PC-containing neo-determinant(s), which are instead predominantly expressed during early stages of cell death when membrane integrity is retained and PS determinants are not accessible to annexin V. The group I Abs also displayed enhanced reactivity with caspase-blocked apoptotic thymocytes, and this recognition may not be linked to shifts between the membrane leaflets, as these cells were predominantly annexin V negative. By contrast, akin to the reactivity of 3H9, the inhibition of the caspase pathway also diminished the reactivity of the group II Ab. Hence, we found that these two anti-PC groups primarily recognize thymocytes at different phases of apoptosis, via PC-containing neo-self antigen(s) with different molecular features and cellular distributions. Whether this differential self-recognition by the

**FIGURE 6.** Binding of anti-PC Abs to thymocytes undergoing dexamethasone induced apoptosis. A, Group I anti-PC Abs T15 and M603 are shown to selectively bind a subpopulation that is PI-dim/negative, and reactivity is enhanced for thymocytes also cultured with Z-VAD, a pan-caspase inhibitor. Herein, PI identifies dexamethasone-induced thymocytes with breached cellular membranes, which are associated with advanced stages of apoptosis. At bottom, equivalent studies were performed on induced thymocytes incubated overnight with Z-VAD, a pan-caspase inhibitor, which resulted in an increased representation of PI-dim/negative cells. Reactivity with these group I anti-PC Abs is detected with an anti-IgA reagent. B, Group II anti-PC Ab, PCG1–14 selectively binds a subpopulation that is PI positive. Binding reactivity is decreased for thymocytes also cultured with Z-VAD, a pan-caspase inhibitor. Herein, reactivity with this group II anti-PC Ab is detected with an anti-IgG reagent. C, Group II Ab, PCG1-14, is strongly reactive with apoptotic thymocytes and displays little reactivity with thymocytes cultured without dexamethasone. Reactivity is greatest with the R3 subset (7-AAD-positive and annexin V-positive) cells, which are at an advanced stage of apoptotic death. At the top, different stages of apoptotic death are displayed for thymocytes cultured overnight without (left) and with (right) dexamethasone. At the bottom, binding of the PG1-14 Ab is detected with an anti-IgG reagent, with comparisons for noninduced (shaded) and induced thymocytes (bold line) for annexin V-negative 7-AAD-negative cells (R1), Annexin V-positive 7-AAD-negative cells (R2), and annexin V-positive 7-AAD-positive cells (R3). D, The binding reactivity of the group II anti-PC Ab PCG1-14 is strongly inhibited by preincubation with PC-KLH or Cu-OxLDL, but not by PC-CI salt or native LDL. Herein, compared with the IgG isotype control, the group II anti-PC Ab, is shown to be reactive with dexamethasone-induced thymocytes at later stages of apoptosis. At the bottom, binding of the isotype control (shaded area) is compared with PCG1-14, in the absence (bold line) or presence (dashed line) of different inhibitors, as indicated.
distinct types of anti-PC Abs applies to all primary cells undergoing apoptosis remains to be determined.

Recent studies have demonstrated an enrichment of oxidized PC-containing phospholipids in apoptotic cells (25). Therefore, we speculate that, in part by slowing the progression to cytolysis, the influence of a caspase inhibitor also indirectly alters the degree to which membrane components are oxidatively modified during programmed cell death or are affected by apoptosis-associated phospholipase activity (26). Hence, the most likely explanation of why distinct major cellular apoptotic subpopulations are recognized by the group I or group II anti-PC Abs is that these are each specific for different variations in the density or structural features of PC-containing determinants that arise during sequential stages of apoptosis. Furthermore, our other studies indicate that OxLDL and atherosclerotic vascular lesions also express a similar range of PC determinants.

In the innate immune system, C-reactive protein (CRP), a member of the highly conserved pentraxin family, also recognizes PC-containing determinants as a pathogen-associated molecular pattern that marks diverse microbial pathogens for immune recognition (27). However, CRP may play a dual role as it can bind apoptotic cells, potentially aiding clearance and preventing immune sensitization (28). CRP is also deposited in atherosclerotic lesions, and an elevated circulating level represents a potent non-classical risk factor for atherosclerosis (29). Importantly, CRP recognizes PC moieties in both OxLDL and apoptotic cells (30).

In conclusion, our investigations indicate that Abs to PC haptens recognized related Ags in divergent biologic contexts, whether associated with pathogens, the membranes of cells undergoing apoptosis, or expressed in the lipid-rich deposits of disease-associated atherosclerotic vascular lesions. Given its elegant structural simplicity and common display on pathogenic microbes, it is provocative that PC determinants are recognized by elements of both the innate and adaptive immune systems. Specifically, CRP and anti-PC natural Abs that represent an innate tier of the adaptive immune system may have parallel and perhaps complementary roles in the recognition of phosphocholine determinants.

C-reactive protein and anti-PC Abs to phosphocholine contribute to the pathogenesis of conventional autoimmune diseases. Recent studies have demonstrated an enrichment of oxidized PC-containing phospholipids in apoptotic cells (25). Therefore, we speculate that, in part by slowing the progression to cytolysis, the influence of a caspase inhibitor also indirectly alters the degree to which membrane components are oxidatively modified during programmed cell death or are affected by apoptosis-associated phospholipase activity (26). Hence, the most likely explanation of why distinct major cellular apoptotic subpopulations are recognized by the group I or group II anti-PC Abs is that these are each specific for different variations in the density or structural features of PC-containing determinants that arise during sequential stages of apoptosis. Furthermore, our other studies indicate that OxLDL and atherosclerotic vascular lesions also express a similar range of PC determinants.

In the innate immune system, C-reactive protein (CRP), a member of the highly conserved pentraxin family, also recognizes PC-containing determinants as a pathogen-associated molecular pattern that marks diverse microbial pathogens for immune recognition (27). However, CRP may play a dual role as it can bind apoptotic cells, potentially aiding clearance and preventing immune sensitization (28). CRP is also deposited in atherosclerotic lesions, and an elevated circulating level represents a potent non-classical risk factor for atherosclerosis (29). Importantly, CRP recognizes PC moieties in both OxLDL and apoptotic cells (30).

In conclusion, our investigations indicate that Abs to PC haptens recognized related Ags in divergent biologic contexts, whether associated with pathogens, the membranes of cells undergoing apoptosis, or expressed in the lipid-rich deposits of disease-associated atherosclerotic vascular lesions. Given its elegant structural simplicity and common display on pathogenic microbes, it is provocative that PC determinants are recognized by elements of both the innate and adaptive immune systems. Specifically, CRP and anti-PC natural Abs that represent an innate tier of the adaptive immune system may have parallel and perhaps complementary roles in the clearance of apoptotic cells and oxidatively modified atherosclerosis-specific moieties. Furthermore, defects in such housekeeping functions or in B cell clonal regulation may lead to IgG autobody responses and the development of atherosclerosis, or contribute to the pathogenesis of conventional autoimmune diseases.

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