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Regulation of Dendritic Cells and Macrophages by an Anti-Apoptotic Cell Natural Antibody that Suppresses TLR Responses and Inhibits Inflammatory Arthritis

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Although naturalAbs (NAbs) are present from birth, little is known about what drives their selection and whether they have housekeeping functions. The prototypic T15-NAb, first identified because of its protective role in infection, is representative of a special type of NAb response that specifically recognizes and forms complexes with apoptotic cells and which promotes cell-corpse engulfment by phagocytes. We now show that this T15-NAb IgM-mediated clearance process is dependent on the recruitment of C1q and mannose-binding lectin, which have known immune modulatory activities that also provide ‘‘eat me’’ signals for enhancing phagocytosis. Further investigation revealed that the addition of T15-NAb significantly suppressed in vitro LPS-induced TNF-α and IL-6 secretion by the macrophage-like cell line, RAW264.7, as well as TLR3-, TLR4-, TLR7-, and TLR9-induced maturation and secretion of a range of proinflammatory cytokines and chemokines by bone marrow-derived conventional dendritic cells. Significantly, high doses of this B-1 cell produced NAb also suppressed in vivo TLR-induced proinflammatory responses. Although infusions of apoptotic cells also suppressed such in vivo inflammatory responses and this effect was associated with the induction of high levels of IgM antiapoptotic cell Abs, apoptotic cell treatment was not effective at suppressing such TLR responses in B cell-deficient mice. Moreover, infusions of T15-NAb also efficiently inhibited both collagen-induced arthritis and anti-collagen II Ab-mediated arthritides. These studies identify and characterize a previously unknown regulatory circuit by which a NAb product of innate-like B cells aids homeostasis by control of fundamental inflammatory pathways. The Journal of Immunology, 2009, 183: 1346–1359.

To defend against infectious agents, yet also guard against autoimmune disease, complex activating and inhibitory pathways have evolved that interconnect the innate and adaptive immune systems and control their activation. The innate immune system senses for threats by recognizing microbe-associated molecular motifs using limited sets of cellular receptors, such as TLR, as well as soluble immune recognition opsonizing factors, such as complement, collagen-like lectins (i.e., collectins) and C-reactive protein. Some of these receptors also bind to stress-associate molecular motifs using limited sets of cellular receptors, such as TLR, as well as soluble immune recognition opsonizing factors, such as complement, collagen-like lectins (i.e., collectins) and C-reactive protein. Some of these receptors also bind to stress-associated proteins and other self-ligands (reviewed in Ref. 1). Professional phagocytic cells, macrophages (Mφ)5 and dendritic cells (DC) thereby respond to environmental stimuli, microbial Ags, and cytokines, which by facilitating or forbidding differentiation changes control the capacity of Mφ and DC for overall inflammatory responses as well as the immunogenicity of foreign and self-Ags.

Although the innate immune system is important or even essential for modulating lymphocyte responses, innate immune responses themselves are also reciprocally influenced by specialized tiers of the adaptive immune system, such as NK, NKT, and γδ T cells, which can recruit DC into proinflammatory responses (2). We have wondered how B lymphocytes might also affect innate responses, especially by B-1 cells, the primordial tier of the B lymphocyte compartment that is the major source of the ‘‘nonimmune’’ IgM NAbs constitutively produced throughout life and which are also involved in responses to nonprotein Ags (3). This distinct set of self-replenishing mature B lymphocyte have been described as innate-like as they express a restricted and recurrent Ab repertoire that arises by a programmed sequence during immune development (3, 4). Indeed, certain B-1 cell clones appear to have regulatory roles through effects on innate immune cells even at remote sites in the body (5), although how this might occur is not known.

The prototypic T15 B-1 cell clonotype, defined by H-L paired canonical Ab gene rearrangements without hypermutation, was first characterized 40 years ago (6) with later repeated independent isolations (e.g., S107 (7), HPCM2 (8), EO6 (9), and others). T15

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5 Abbreviations used in this paper: Mφ, macrophage; BM, bone marrow; CI1, collagen type II; DC, dendritic cell; LDL, low-density lipoprotein; MBL, mannose-binding lectin; MDA, malondialdehyde; MHC II, MHC class II; NAb, natural Ab; ODN, oligonucleotide; PC, phosphorylcholine; poly(I:C), polyinosinic-polycytidylic acid.
clonotypic B cells spontaneously arise and become highly represented within the first week of life, even in mice raised under germ-free conditions (10), which suggests that microbial ligands are not primary mediators of clonal selection. It has long been known that T15-NAbs bind to phosphorylcholine (PC) determinants and contribute to host defense to PC-containing pneumococci, and other microbes, and provide optimal protection from systemic infection (11). More recently, PC determinants were also identified on oxidatively modified low-density lipoprotein (LDL) generated during atherogenesis (9). Significantly, pneumococcal immunization, which induced active B cell responses that raised T15 Ab levels, greatly ameliorated the chronic inflammatory response in a murine model of hyperlipidemia and atherosclerosis (12). The mechanistic basis for these findings remains obscure, as the original hypothesis, that T15-NAbs might enhance clearance of the proinflammatory oxidatively modified LDL, has subsequently been ruled out (13). Although more recent studies suggest the possibility that immunization may induce regulatory B cells that serve as a source of inhibitory cytokines (14), we have suspected there are other Ab-mediated immune modulatory activities.

Other studies have previously shown that by immune recognition of the PC head group, T15-NAbs can discriminate dead/dying cells from healthy cells (15–17). This is because the PC head group, which is a ubiquitous component of cell membrane neutral phospholipids (e.g., phosphatidylcholine), is embedded within the lipid bilayer in healthy cells and therefore inaccessible to Abs unless exposed by membrane changes that occur during apoptosis (15–17). Importantly, we have shown that T15-NAbs is structurally and functionally representative of the PC-specific anti-apoptotic cell Abs that are induced in vivo by apoptotic cell infusions (18).

Herein, we show that the prototypic T15-NAbs can play a general role in modulating innate immune responses by inhibiting the activation of phagocytes and thereby suppressing in vitro and in vivo inflammatory responses. We further show that these regulatory properties derive from the capacity to complex with apoptotic cells and recruit soluble innate immune recognition molecules, which together enhance uptake and clearance of apoptotic cells and inhibit TLR-induced phagocyte activation and maturation.

Materials and Methods

Antibodies

T15-IgM (from the EO6 hybridoma) (9) and the IgM isotype control from the hybridoma, NC17-D8 (gift from L. Arnold, University of North Carolina, Chapel Hill, NC), both express J-chain transcripts. Hybridomas were grown under serum-free conditions in hollow fiber (10,000 MWCO) bioreactors in hybridoma serum-free media (Invitrogen) to a cell density of ~5 x 10^6/ml and then maintained for 30–45 days, by National Cell Culture Center. Supernatants were purified with a 300-kDa tangential flow filtration device, followed by a 10-kDa tangential flow filtration for further concentration, then dialyzed against PBS (pH 7.2), with documented low endotoxin (<0.5 EU/mg), then aliquots stored at 80°C. By native PAGE analysis and Western blot, the predominant IgM populations were pentamers with <10% hexamers, without monomeric IgM or low m.w. species.

Ab assays

Standard sandwich ELISA were performed with precoupled of goat anti-IgM-PC-albumin for control Ags, with detection with either biotinylated AB1-2 to detect T15-colonotypic Abs (19), or anti-IgM or anti-IgG, as described previously (20). Assays were adapted to buffer usage required to detect mannose-binding lectin (MBL) binding, as previously described (21), with limits of detection of ~5 ng/ml. In these studies, MBL binding by IgG could not be detected in sera either before or after thymocytes immunization. Array studies were performed as described previously (22).

Mice

Age and gender-matched adult C57BL/6, congenic B cell-deficient muMT, BALB/c, and DBA/1 mice were provided by The Jackson Laboratory or bred under specific pathogen-free conditions as supervised by University of California San Diego (UCSD) Animal Care Program. All animal protocols were approved by the UCSD Institutional Animal Care and Use Committee.

In vitro complement deposition

Apothecary thyocytes were incubated at 37°C with IgM at 20 μg/ml in TBS with 10 mM CaCl2 and/or TBS with 20% Ig-deficient plasma for complement or TBS alone. After 40 min, cells were washed and studied for apoptosis (7-aminoactinomycin D (7-AAD) and annexin V), and with allophycocyanin-labeled goat anti-IgM, and anti-C1q (goat; Cedarlane Laboratories), or human recombinant MBL (6 μg/ml) and biotinylated mouse anti-human MBL (clone 131-1), or biotinylated rat anti-murine MBL A (clone 2B4) anti-murine MBL C (clone1D14) (21), in the presence of Fc block (23).

In vivo apoptotic clearance assays

Using a standard in vivo approach (24), B cell-deficient mice received thyroglobulin treatment and, 3 days later, received i.v. PBS or 1 mg of IgM. After 16 h, 5 x 10^6 SNARF-1-labeled apoptotic or fresh thyocytes were instilled, then peritoneal cells recovered after 10 min. For immunofluorescence microscopic studies, cytospins were prepared, and MΦ were stained with FITC-anti-F4/80, with >800 MΦ counted per mouse and the proportion determined of recovered MΦ that had ingested (and not just surface bound) one or more labeled thyocytes. Although longer time periods were also examined, 10 min of in vivo exposure yielded the greatest differences between groups, as described previously (25). Although dexamethasone-treated thyocytes yielded similar results, most studies used etoposide for apoptosis induction due to >95% Annexin V+ (i.e., apoptotic) thyocyte yields by flow cytometry. In other studies, to quantitate MΦ uptake, flow cytometric analyses were performed with 7-AAD and annexin V staining of apoptotic thyocytes that were tracked via CD3 fluorescein label (C57BL/6) or peritoneal MΦ detected with FITC-conjugated F4/80 (Caltag Laboratories).

RAW264.7 cell cultures

Cells were grown to 80% confluence with ~10^6 cells per well in 48-well plates in RPMI 1640 and 10% FBS, glutamine, and 0.01 M HEPES, then serum-starved overnight. To some, replicates were preincubated with T15 IgM or isotype control for 1 h, followed by addition of LPS (Escherichia coli O55:B5; List Biological Laboratories) at 0.1 μg/ml or polyinosinic-cytidic acid (poly(I:C)) (Amersham Biosciences) at 3.3 μg/ml for overnight culture.

Bone marrow (BM)-derived DC

BM cells from C57BL/6 females/tibias were washed and cultured in RPMI 1640 containing 10% FBS 1% pen-strep-glutamine, GM-CSF (10 ng/ml), and IL-4 (400 pg/ml) and replenished on day 3 (26). On day 6, DC were selected in the presence of Fc block with magnetic anti-CD11c beads using LS magnetic columns (Miltenyi Biotec) to >94% CD11c purity. For phagocytosis assays, DC were cultured with GM-CSF but without IL-4 and harvested on day 5; CD11c+ cells were purified, then cultured at 0.5 x 10^6/100 μl in 96-well plates overnight in a 1:1 ratio with CFSE-labeled healthy or etoposide-induced apoptotic cells, as described previously (18). Some studies instead used STEMSPAN SF Expansion (StemCell Technologies) serum-free media. For heat inactivation, Ig-deficient sera were incubated for 30 min at 56°C.

For stimulation studies, DC were further cultured for 24–48 h without/ with agonists for TLR3, poly (I:C) at 3.3 μg/ml; TLR4, LPS at 0.1 μg/ml; TLR7, imiquimod (Invivogen) at 1 μg/ml; or TLR9, phosphorothioate CpG oligo 1018 at 0.5 μg/ml. Replicate cultures included serial concentrations of T15-IgM or IgG isotype control. Other cultures included blocking Ab to IL-10 or isotype control (R&D Systems) with Fc block, as per manufacturer’s directions. Cultures with T15-NAb blockade with Aβ1–2 anti-Id or isotype control also included Fc block. To assess DC maturation, cells were contained with PE-anti-mouse CD80 (clone 16-10A1) and for intracellular Alexa Fluor 647 anti-IL-12 p40 (clone C17-8), as per manufacturer’s protocol (eBioscience). qPCR was performed as described previously (20).

DC isolation

Splenic DC were isolated, as previously reported (26), then evaluated by flow cytometry for defined DC subsets and maturation/activation markers. Transcript analysis after 6 h of in vitro stimulation of CD11c-enriched BM-derived DC were performed by TaqMan (Applied Biosystems), using manufacturer’s directions.
In vivo challenge assays

On the basis of the pilot studies with outcomes assessed after weekly treat-
ments, we selected a 2-wk treatment period, which is also the turnover
period of most DC populations from stem cells (27). Hence, groups of adult
C57BL/6 received three i.p. infusions (days 0, 7, and 14) of 1.5 mg of
T15-IgM or isotype control. To assess the role of PC-binding specificity,
some groups received 1.5 mg of T15-IgM incubated with 2 mg of PC-BSA
for 30 min at room temperature before infusion. Other groups received i.v.
2.5 \times 10^7 freshly isolated (healthy), apoptotic, or necrotic (by repeated
freeze-thawing) thymocytes in PBS, with bleeds obtained on day 16. To
induce apoptosis, congeneric murine thymocytes either received 600 rad us-

ing a \( \frac{1}{3} \) Cs emission source or were treated with 10 \( \mu \)M eto-
poside, then incubated overnight in complete media at 37°C with 5% CO\(_2\),
then washed three times in media before use. Alternatively, mice received
T15-NAb with 2 mg of PC-BSA (Biosearch Technologies) or BSA as a
control. On day 17, at 18 h before sacrifice, mice received saline or chal-

lenge with 100 \( \mu \)g of poly(U/C), 30 \( \mu \)g of LPS, 100 \( \mu \)g of imiquimod, or
200 \( \mu \)g of PT Cpg ODN11018. As pilot studies did not demonstrate in vivo
activation after imiquimod treatment, we instead used 300 \( \mu \)g of
SM-360320 (28), because of 100-fold greater potency. Mice were bled at
sacrifice, and suspensions of splenocytes and other lymphoid organs were
evaluated by flow cytometry using standard Abs and methods (BD Phar-
ningen) (17, 20). Ab immunoassays and inhibitions were performed with
PC-BSA, ABA-BSA (Biosearch Technologies), or BSA (Sigma-Aldrich)
using IgG (sub)class and T15 clonotype-specific Abs, as described previ-
ously (20). Soluble factors in DC supernatants and sera were evaluated by
Luminex assay (Invitrogen) or ELISA (BioSource International).

Inflammatory arthritis models

For CIA studies, 8-wk-old DBA/1 male mice were immunized with avian
CII/CFCA (Chondrex) at the tail base on day 0 and i.p. boosted on day 21
with CII/IFA. Anti-CII Ab levels were assessed, per manufacturer’s instruc-
tions (Chondrex). For histologic analyses, paws and knees of mice sacri-
ficed on day 44 were decalcified, embedded, and sectioned. H&E-stained
slides were scored for inflammatory infiltrates and joint erosions, and sa-
franin O was stained for cartilage damage (29). Collagen Ab-induced ar-
thritis was induced in BALB/c mice with 2 mg of CII-specific monoclonal
IgG mixture injected i.v. on day 0, and 72 h later, each animal received 50
\( \mu \)g of PT CpG ODN1018. Anti-CII Ab levels were assayed, per manufac-
turer’s instructions (28), because of 100-fold greater potency. Mice were bled
after sacrifice, and suspensions of splenocytes and other lymphoid organs were
scored for inflammatory infiltrates and joint erosions, and sa-
franin O was stained for cartilage damage (29). Collagen Ab-induced ar-
thritis was induced in BALB/c mice with 2 mg of CII-specific monoclonal
IgG mixture injected i.v. on day 0, and 72 h later, each animal received 50
\( \mu \)g of LPS E. coli 011B4 i.p. (Arthogen-CIA kit; Chemicon International).
Different groups received T15-IgM or control IgM at 2 mg, or buffer, given
as a pretreatment and every 7 days thereafter. Clinical arthritis was scored
visually from 0 to 4 per paw, with a maximum score of 16 (29).

Statistical analysis

Values are reported as mean ± SEM unless otherwise stated. Significance
was assigned for \( p < 0.05 \) by two-tailed \( t \) test, with Welch correction, or
ANOVA, as appropriate (Instat; GraphPad).

Results

NAb enhances local deposition of Clq and MBL on apoptotic

To understand the immune-modulating properties of T15-NAb, we
first characterized its Ab-effector capabilities and then assessed
how these may affect the innate immune system. In earlier studies,
purified monoclonal T15 clonotypic Abs were shown to recognize
a subset of dying cells at both early ( Annexin V \(^{+}\)7-AAD\(^{+}\) ) and
late ( Annexin V \(^{+}\)7-AAD\(^{+}\) ) stages of apoptosis in a PC-inhibitable
fashion (17). As a physiologically relevant source of soluble op-
sonins, we used sera from B cell-deficient murine muMT mice that
are therefore deficient in Igs (30). Whereas incubation with Ig-
deficient sera results in low-level deposition of Clq on apoptotic
cells (31), we found that the addition of T15-NAb of the IgM
isotype increased the amount of Clq recruitment from Ig-deficient
sera onto apoptotic cells (18). Notably, although neither T15-NAb
nor Clq alone interacted with freshly isolated healthy thyocytes, T15-
NAb was responsible for \( >3\)-fold relative increases in Clq depo-
sition on cells at early stages of apoptosis, based on gathing on
7-AAD\(^{+}\) cells (Fig. 1A).

MBL is a multicellular collectin immune recognition protein that
initiates the lectin pathway of complement activation, which plays
a role in immune defenses but can also interact with certain self-gly-
coproteins (32). Although not well-known for contributions to Ab-
effector functions, because of the reported roles of MBL in apoptotic-
cell recognition (23) and modulation of inflammatory responses
(reviewed in Ref. 32), we also assessed the capacity of T15-NAb to
recruit MBL. Indeed, solid-phase immunoassays showed that both
T15-NAb as well as the IgM-isotype control had dose-dependent
binding to the labeled recombinant MBL used to detect binding (Fig.
1B). However, only T15-NAb recognized the PC-albumin coated
onto the wells and then also interacted with the labeled MBL reagent.
MBL binding to T15-NAb was inhibited by mannose or N-acetylglu-
cosamine, but not by N-acetylgalactosae, and was also calcium-depen-
dent (Fig. 1B), indicating that the carbohydrate recognition domain
of MBL is responsible for these IgM interactions presumably through
F\(_{\alpha} m\)-associated N-glycans (33). In contrast to a single report that bind-
ing of a recombinant IgM-Ab to an experimental Ag disallows cons-
istent region interactions with MBL (33), we found that T15-NAb, but
not the isotype control, was capable of concurrent binding interactions
with both PC and MBL (Fig. 1B). This indicated that binding inter-
actions with T15-NAb could potentially amplify recruitment of MBL
to immune complexes.

We therefore examined if T15-NAb could promote binding of
human recombinant MBL to apoptotic thyocytes. As previously
reported (23), incubation with MBL alone resulted in direct depo-
sition of only low levels of this opsonin, predominantly on thy-
ocytes at late stages of apoptosis and those undergoing second-
ary necrosis. By contrast, the addition of T15-NAb significantly enhanced
MBL deposition with the greatest increases on thy-
ocytes at early stages of apoptosis ( Fig. 1C and Supplemental Fig.
1 ). In further analyses, murine MBL-specific Abs (21) were used to
directly detect mouse MBL deposited on apoptotic cells from
Ig-deficient sera. In this study, these specific detection Abs showed
that T15-IgM similarly induced the recruitment of both MBL A
and C gene products from sera, either separately (data not shown)
or together, and the specificity was again confirmed as these in-
teractions were inhibited by mannose (Fig. 1D). Thus, a major
function of T15-NAb is the recruitment of both Clq and MBL to
primarily early, but also late, apoptotic cells.

NAb enhances in vivo MΦ clearance of apoptotic cells

To assess whether the T15-NAb can affect the phagocytic clear-
ance of apoptotic cells, we used a standard sterile peritonitis model
(24) with B cell/Ig-deficient muMT mice, which received pretreat-
ment infusions of either T15-IgM, control isotype IgM, or saline.
Mice were then injected i.p. with labeled apoptotic thyocytes, and
10 min later, peritoneal MΦ were recovered and examined for
phagocytosed thyocytes (24). We found that in saline- or IgM
isotype-treated mice, a mean of \( \sim23\% \) of recovered MΦ had en-
gulfed a labeled apoptotic cell or bleb, whereas after T15-IgM
treatment, the proportion of MΦ with ingested apoptotic thy-
ocytes/fragments increased to \( \sim36\% \). Hence, T15-IgM treatment
resulted in a 50–60% increase in the level of apoptotic phagocyt-
osis, compared with the isotype control ( \( p < 0.0004 \) ) or saline
treatments ( \( p < 0.0001 \); Fig. 1E). By contrast, we found that in
T15-IgM-treated mice, after injection of labeled healthy thy-
ocytes <3% of recovered peritoneal MΦ had engulfed a labeled
thyocyte (data not shown). Notably, increases in the efficiency
of apoptotic clearance in the same assay, akin to those mediated by
T15-NAb, have also been documented when wild-type mice were
compared with either C1q- or MBL-deficient mice (24, 34). In-
deed, we found that T15-NAb-coated apoptotic thyocytes
formed chains and clusters, which were engulfed by peritoneal MΦ

\(^{6}\) The online version of this article contains supplemental material.
Flow cytometric analysis of the recovered peritoneal cells demonstrated that T15-NAb enhanced the elimination of both early- and late-stage apoptotic cells (p < 0.004; supplemental Fig. 1). Thus, T15-NAb significantly enhances phagocytic clearance by peritoneal Mφ, with an influence akin to the individual contributions of MBL and C1q for phagocytic clearance.

NAb suppresses LPS-induced IL-6 secretion by RAW264.7 Mφ-like cell line

As interactions with apoptotic cells are reported to blunt inflammatory responses (35) and we found that T15-NAb enhances interactions of phagocytes with apoptotic cells, we also assessed whether this NAb can affect proinflammatory responses of the
RAW264.7 Mφ-like cell line. In a representative study, T15 IgM displayed a significant dose-dependent inhibition of induced TNF-α secretion (p < 0.0001), with 55% inhibition by 1 μg/ml, 74% at 4 μg/ml, and 83% inhibition at 20 μg/ml, whereas the IgM isotype control had no effect (Fig. 2A). T15-NAb similarly inhibited TLR-induced IL-6 secretion (Fig. 2B). However, TLR-mediated stimulation also caused a significant level of cell death (data not shown), so we could not control for a requirement for apoptotic cells for T15-mediated inhibition. Nonetheless, these findings document that the T15 NAb can induce a dose-dependent inhibition of TLR4-induced Mφ secretion of the key proinflammatory factors, TNF-α and IL-6.

NAb associates with MBL and C1q to enhance DC phagocytosis of apoptotic cells

At early stages of differentiation, immature DC share many cell surface receptors as well as the phagocytic capacities of Mφ (36). We therefore used a standard culture system to generate CD11c+ immature DC (26) and studied the phagocytic capacity of BM-derived conventional DC (18, 25, 37, 38). After incubation with labeled thymocytes, the purified CD11c+ immature DC were discriminated in flow cytometry studies based on size (i.e., forward light scatter) and/or staining for CD11c (Fig. 3), and the proportion of immature DC subpopulation that ingested CFSE-tagged apoptotic cells was quantified based on the associated shift in fluorescence (Fig. 3A), using methods previously confirmed with side-by-side microscopic quantitation (18). Importantly, under serum-free conditions (i.e., devoid of Ig and opsonins), we have recently shown that DC displayed the same low frequency of phagocytosis of apoptotic thymocytes as with labeled healthy thymocytes (Ref. 18 and data not shown), which is consistent with the notion that efficient phagocytosis of apoptotic cells, compared with viable cells, is dependent on the availability of specific serum factors.

As levels of the opsonins, C1q and MBL, are reported to directly correlate with the efficiency of apoptotic cell elimination (39), we also used this system to look for potential interactions of T15-NAb NAb in serum-free media with the addition of these opsonins. In this study, we found that addition of recombinant MBL provided a significant dose-dependent increase in DC phagocytosis, with dramatic increases seen only when T15-NAb was present (Fig. 3). Strikingly, in similar serum-free cultures with a fixed amount of T15-NAb, MBL conveyed much greater dose-dependent increases in the efficiency of phagocytosis than we have found was associated with supplementation with purified C1q (Fig. 3, C and D) (18). Furthermore, in cultures with T15-NAb and the highest level of MBL, the further addition of C1q resulted in only a minor additional increases in DC phagocytosis. In fact, the level of phagocytosis seen in cultures with MBL at 20 μg/ml were comparable to those instead supplemented with Ig-deficient sera (Fig. 3, C and D). Hence, this NAb-dependent influence on innate immune function was limited by the availability of MBL and C1q, which were redundant in their capacity to enhance the influence of T15-NAb on DC phagocytosis. Unexpectedly, in this assay, MBL appeared to be more potent than C1q, because MBL alone conveyed nearly the full level of phagocytosis associated with Ig-deficient sera. We also compared levels of phagocytosis with T15-IgM (at 20 μg/ml) without the addition of sera, or with supplementation with 10% Ig-deficient sera, or after heat inactivation, which was thereby shown to reduce by 83% the contribution of serum factors to T15-mediated enhancement of iDC phagocytosis of apoptotic cells (data not shown).

To confirm the requirement of the Ag-binding specificity of the NAb, we performed studies with saturating amounts of MBL and a limiting concentration of T15 IgM. In this study, we found that with T15-NAb and MBL, the frequency of DC that engulfed apoptotic cells was still nearly twice as high as with serum-free conditions alone (Fig. 3, C and D). Importantly, preincubation with PC-BSA significantly reduced (25%) the T15-IgM-mediated increase in the phagocytic engulfment of apoptotic cells (p < 0.004), whereas incubation with an irrelevant control BSA conjugate instead increased phagocytosis by ~25% (p = 0.0021) (Fig. 3A). Hence, the capacity of T15-NAb to enhance apoptotic cell engulfment was also shown to be dependent on its PC-binding specificity.

NAb in association with MBL and C1q inhibits TLR-induced DC maturation

To assess the effects of NAb-apoptotic cell complexes on DC maturation, we studied how different culture conditions can affect co-expression of the membrane-associated costimulatory molecule, CD80, and intracellular IL-12 p40 expression, which can be upregulated following TLR stimulation. Notably, control studies demonstrated that even without TLR agonists, these primary CD11c+ DC display a range in their phenotype (Fig. 4A), which in part reflects the persistent influence of GM-CSF and IL-4 (40). At first, we confirmed that either LPS or poly(I:C) induced the maturation of DC, based on evidence of decreased representation of less mature (i.e., CD80−/p40−) DC, compared with culture without these stimuli (p < 0.0001) (Fig. 4A).

We next evaluated whether the addition of a large number of apoptotic cells alone can affect DC maturation. Notably, we uniformly found that in DC cultures stimulated with LPS or poly(I:C), the addition of equal numbers of apoptotic thymocytes significantly inhibited the TLR-induced DC maturation. This result was
a consistent finding whether or not Ab and opsonin was added, and these differences were highly significant when we compared paired cultures without or with added apoptotic cells \((p / H11021 0.0001, \text{paired } t \text{ test, } n / H11005 3 \text{ replicate cultures})\) (data not shown). Hence, as previously reported, apoptotic cells themselves, when present in substantial numbers, inhibit TLR-induced conventional DC maturation \((41)\). Importantly, we found that after DC alone were in culture for 24 h, 10–15% of recovered cells were Annexin V \(\text{apoptotic cells and fragments (our unpublished data)}\) (Fig. 4A).

Predictably, when added to these DC cultures, T15-NAb coated these apoptotic DC (and their breakdown products) but not viable DC (supplemental Fig. 2).6 These T15-NAb-coated apoptotic DC were phagocytosed by viable immature DC (supplemental Fig. 2),6 whereas T15-NAb otherwise had no adverse effects on viability or proliferation. Thus, inherent to the biology of these primary cells, DC cultures contained apoptotic cells and debris that form into complexes with T15-NAb.

We therefore tested the hypothesis that T15-NAb can suppress TLR-induced DC maturation, in the presence of suitable opsonins, and only the limited number of the dead cells/fragments that are continuously generated in culture. We uniformly found that addition of T15-NAb increased the proportion of less mature DC, compared with cultures with isotype control or without IgM (Fig. 4). This was also found in T15-NAb-containing cultures without supplemented opsonic factors, may reflect the potential carryover of serum factors on the apoptotic thymocytes, or the production by immature DC of small amounts of C1q and MBL \((42, 43)\) and possibly other factors. Nonetheless, T15-NAb significantly suppressed DC maturation in cultures stimulated with either poly(I:C) or LPS \((p / H11021 0.05)\). There was also a trend toward less mature DC in cultures with T15-NAb but without TLR agonist (Fig. 4, B and C), presumably due to blunting of the residual influences of GM-CSF and IL-4.

There was a consistent hierarchy in the effects of C1q and MBL on T15-NAb suppression in these replicate LPS or poly(I:C)-stimulated serum-free cultures. The least inhibition of DC maturation was found in the absence of additional supplements, with greater inhibition with the addition of C1q, and even greater suppression with MBL. The greatest inhibition was seen when T15-NAb was added to TLR-stimulated cultures that had been supplemented with both C1q and MBL, because this resulted in significantly more immature DC than other T15-NAb-containing cultures, whether or not MBL or C1q were added \((p / H11021 0.015)\). Indeed, T15-NAb, in the presence of both C1q and MBL, effectively blocked DC maturation induced by LPS (Fig. 4B) or poly(I:C) (Fig. 4C) to the level found in cultures without TLR agonist and T15-NAb.

NAb inhibits in vitro inflammatory responses of DC

We next assessed whether T15-NAb, which binds apoptotic material in culture and enhances phagocytosis, can modulate other features of in vitro responses of DC in sera-containing media with a broad range of agonistic TLR ligands, including poly(I:C), LPS, imiquimod, and CpG DNA. Indeed, inhibition was again documented for surface maturation/activation markers, MHC class II \((\text{MHC II)}\), CD40, CD86, and CD80 (Fig. 5A and our unpublished data) and for secretion of proinflammatory cytokines (TNF-α,
By real-time PCR analysis, T15-NAb also inhibited LPS induction and CXC chemokine (IP-10) (Fig. 5).

T15-NAb inhibition of LPS-induced DC maturation is MBL and C1q dependent.

- Apoptotic cells induced anti-inflammatory NAb responses in vivo (Fig. 6).

**FIGURE 4.** T15-NAb inhibits in vitro TLR induced maturation of conventional DC. B.1 mononuclear cells were cultured with GM-CSF and IL-4 for 5 days, then purified with anti-CD11c beads before overnight culture in serum-free media. A. LPS induces maturation of DC, based on increased CD80 and IL-12p40 expression, which is inhibited by T15 IgM (20 μg/ml) in cultures supplemented MBL (20 μg/ml) and C1q (80 μg/ml). DC are shown to be heterogeneous in their level of maturation, even in the absence of LPS or poly(I:C). On the basis of a scattergram analysis, these overnight cultures contained 10–18% dead cells and fragments. B. T15-NAb, in the presence of MBL and C1q, inhibits LPS-induced DC maturation. Values for CD80+ IL-12p40+ DC, as gated in A, are shown from four or more replicate cultures (mean ± SEM). C. T15-NAb, in the presence of MBL and C1q, inhibits poly(I:C)-induced DC maturation. Data from four or more replicate cultures are shown. □ is without IgM, □ is with isotype control, and □ is with T15 IgM at 20 μg/ml. Significance as indicated: *, p < 0.05; **, p ≤ 0.01; ***, p ≤ 0.0001; and ****, p ≤ 0.0003. Representative of three independent experiments.

IL-6, and IL-12p70), CC chemokines (KC, MCP-1, and MIP-1α), and CXC chemokine (IP-10) (Fig. 5B and our unpublished data). By real-time PCR analysis, T15-NAb also inhibited LPS induction of TNF-α, IL-1β, IL-6, and IL-12 transcripts (Fig. 5C and our unpublished data). By contrast, at even high concentrations, the B-1 cell-derived IgM isotype control, which showed only minor binding to late-stage apoptotic cells, resulted in little or no inhibition. Further studies showed that T15-NAb-mediated inhibition of IL-6 production was >80% reduced by a T15-specific idiotypic Ab that blocks the T15 PC binding site (19). Hence, our findings support the hypothesis that the specific interactions of T15-NAb with dead and dying cells can inhibit DC maturation and suppress activation-associated expression of cytokine and chemokine factors.

To assess for potential pathways responsible for these T15-NAb-mediated inhibitory activities, we first examined expression of IL-10 and TGF-β1, which are both implicated in the inhibitory properties of regulatory DC responses. Neither, however, were induced, at either the transcript or protein level by T15 exposure, and in fact, T15-NAb inhibited the LPS-mediated induction of IL-10 (Fig. 5C and our unpublished data). The suppressive effects of T15-NAb were also unimpaired by IL-10-neutralizing Abs or in DC from IL-10-deficient mice (our unpublished data).

**T15-NAb inhibits in vivo inflammatory responses**

To determine whether T15-NAb can also inhibit in vivo inflammatory responses, we investigated the effects of infusions of purified T15-IgM on in vivo innate immune proinflammatory responses. Indeed, after 2 wk of T15-NAb exposure, which corresponds to the approximate turnover period for DC populations (27), the T15-NAb group had 17–21% less splenic CD11c+ DC (p < 0.02, n = 7–8/group) and significantly lower levels of surface-expressed MHC II (p < 0.02), which is consistent with evidence that T15-NAb can inhibit in vitro DC maturation. Importantly, responses to the TLR agonists poly(I:C) (TLR3), LPS (TLR4), and CpG nucleotides (TLR9) were also inhibited by T15-NAb pretreatment, with impaired induction of activation and maturation markers, CD86 and MHC II, on splenic MΦ and CD11c+ DC (Fig. 6, A and B). Furthermore, T15-NAb also significantly inhibited responses to the potent TLR7 agonist, SM-360320 (28) (Fig. 6A), as well as poly(I:C) induction of other costimulatory molecules such as CD40, CD80, and B7-DC (our unpublished data). T15-NAb treatment also blunted poly(I:C)-induced blood levels of proinflammatory cytokines (IL-6, IL-12, IL-17, and TNF-α) and chemokines (MIP1α, MCP-1, KC, and IP-10) (Fig. 6C). In addition, NAb treatment significantly reduced the production of IL-6 and IL-12 by peritoneal MΦ (our unpublished data). Confirming the role of the PC-binding specificity, preincubation with an excess of PC-conjugate before T15-NAb infusion antagonized >75% of the in vivo inhibitory effects. Hence, elevated levels of T15-NAb drastically reduced the in vivo responsiveness of the innate immune system to a range of proinflammatory stimuli.

**Apopotic cells induce anti-inflammatory NAb properties of T15**

We reasoned that apoptotic cells might be the main antigenic target for in vivo T15-related NAb responses. To examine the in vivo relationship between T15-NAb, apoptotic cells, and inflammation, we infused large numbers (25 × 10^7) of apoptotic thymocytes into naive mice that had low but detectable natural T15 levels (supplemental Fig. 3,A with data deposited in the GEO repository under accession number GSE14969; www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14969). Notably, this treatment also blunted MΦ and DC activation responses (Fig. 6, B and C). Moreover, cotreatments of apoptotic cells plus T15-NAb trended toward greater suppression of in vivo poly(I:C)-induced activation responses, compared with either apoptotic cells alone, or apoptotic cells plus isotype control (Fig. 6, B and C). Inhibition was also...
FIGURE 5. T15-NAb treatment blunts in vitro DC responses to TLR agonists. A, CD11c<sup>+</sup>-selected myeloid DC were cultured in replicate with agonists for TLR3 (poly(I:C)), TLR4 (LPS), TLR7 (imiquimod), or TLR9 (PT CpG ODN1018), without or with T15-IgM or isotype control at indicated concentrations. A, Histograms of MHC II and CD40 on DC after culture without or with stimulant (indicated above panel) are depicted. Mean fluorescence intensity is listed without and with IgM, at indicated concentrations. B, Supernatants from these overnight cultures of conventional BM-derived DC were assessed for levels of proinflammatory cytokines and chemokines, which were determined from standard curves (mean ± SEM). Results are shown without (none) or with stimulants (poly(I:C), pIC; imiquimod, imiq) without or with T15-IgM, isotype IgM control, at 10 μg/ml. The isotype control was associated with minor inhibition of MCP-1. C, Transcript levels were determined by real-time PCR for murine BM-derived CD11c<sup>+</sup> DC, under indicated cultured conditions over time (minutes). DC were preincubated with T15-NAb or isotype control before time “0” sampling, then LPS was added. Amplification for TGF-β is β1 isoform specific. Results are representative of three or more independent experiments.
observed for peripheral blood proinflammatory cytokines and chemokines (Fig. 6C). Hence, in vivo apoptotic-cell treatment unexpectedly produced a similar inhibitory effect as demonstrated for T15-NAb.

Although apoptotic cells were anti-inflammatory, primary necrotic thymocytes, which can exacerbate autoimmune disease (44), were not. In fact, infusion of necrotic cells alone significantly increased the expression of activation markers after poly(I:C).
challenge (Fig. 6, B and C). Strikingly, T15-NAb, albeit to a lesser extent, still blunted the proinflammatory effect of necrotic-cell treatment (Fig. 6, B and C), suggesting that the raised T15-NAb levels resulted in formation of inhibitory complexes with host apoptotic cells.

Extending recent observations (18), we also tested whether infusions of apoptotic cells in these studies induced relevant T15-like Ab responses similar to pneumococcal immunization (12). Indeed, 10 days after a single infusion of such cells without adjuvant, 8-fold increases in circulating IgM anti-PC levels were detected. Although responses varied in individual mice, three weekly apoptotic-cell infusions generally raised circulating levels of T15-clonospecific (19) and IgM anti-PC Abs >40-fold higher than in naive mice (p < 0.01) (supplemental Fig. 3, A and B)6 (18). In contrast, infusions of healthy or necrotic cells yielded only minor changes, which is consistent with findings that apoptotic cells induced ~20-fold increased numbers of PC-specific splenic IgM-secreting cells (18). In fact, apoptotic-cell infusions induced IgM Abs to PC-containing determinants to levels equivalent or higher than those that followed T15-NAb infusions (supplemental Fig. 3C), which required substantial doses due to the short half-life of IgM (45). Apoptotic cells also induced IgG anti-PC responses, but these levels were much lower (5.5 ± 2.5 μg/ml) and overwhelmingly of the IgG3 subclass, indicating a mainly T cell-independent response (supplemental Fig. 3D and our unpublished data).6 Hence, despite evidence that apoptotic cells may suppress some innate immune functions, i.e., treatment nonetheless induced robust B cell responses, even without the use of adjuvant.

To assess the relevance of these active immune responses to the above described T15-NAb studies, we have recently shown that incubation of sera from apoptotic cell-treated mice resulted in IgM binding to apoptotic cells and enhanced Ab-dependent C1q recruitment to apoptotic cells, both of which were inhibited by PC preincubation (18). We now used the solid-phase MBL recruitment anti-PC immunoassay and found that apoptotic cell treatments induced high levels of MBL-binding anti-PC Abs (384 ± 89 μg/ml, n = 4), whereas negligible levels were found before treatment (<2 μg/ml, p < 0.0001). Akin to the properties of the monoclonal T15-NAb, MBL recruitment by postimmune anti-PC responses was also >90% inhibited by preincubation with mannose or N-acetylglucosamine, or with EDTA in the media that demonstrated calcium dependence, whereas MBL binding was not inhibited by N-acetylgalactosamine (data not shown). Compared with naive sera, incubation in apoptotic-cell postimmune sera greatly increased levels of IgM binding to apoptotic cells, with similar increases in the recruitment of C1q and MBL. Notably, the greatest IgM-associated enhancement was seen on early apoptotic cells (i.e., 7-AAD) (18) (Fig. 6D). Preincubation of T15-NAb with PC-BSA also greatly reduced the deposition of IgM and also MBL (Fig. 6D) and C1q (18) on apoptotic cells. Furthermore, similar to purified T15-NAb (Fig. 3A), sera obtained after apoptotic-cell treatment, which had markedly increased levels of IgM Abs to apoptotic cells, also suppressed in vitro TLR-mediated activation of cultured DC (our unpublished data).

Suppression of inflammation by apoptotic cells requires B cells or T15-NAb

B cell-deficient muMT mice were next used to assess the requirement for IgM in the in vivo inhibitory properties of apoptotic cells. Strikingly, infusions of apoptotic cells alone had little or no effect on poly(I:C)-induced cellular activation or cytokine/chemokine responses in B cell-deficient mice (Fig. 7 and our unpublished data). In contrast, T15-NAb, but not isotype control, treatment of B cell-deficient mice induced the same blunting of TLR-induced cell activation and cytokine/chemokine production as demonstrated in C57BL/6 mice. Overall, these findings indicate that the suppressive effect of apoptotic cell infusions in vivo is dependent on the induction of Abs with certain specificities, which include anti-PC reactivity.

NAb protects from inflammatory arthritis

As inflammatory pathways involving Mφ, DC, and TLR have been implicated in the pathogenesis of autoimmune arthritis (46), we studied collagen-induced arthritis (CIA) in DBA/1 mice (47) to test the hypothesis that high levels of T15-NAb might suppress the development of inflammatory disease (Fig. 8 and supplemental Fig. 4). Significantly, pretreatment with the anti-PC NAb markedly reduced clinical disease activity, synovial leukocytic infiltrates, and bone and joint damage (Fig. 8, A–C). Notably, there were no differences in total IgG, or in IgG1 and IgG2a subclass anti-CII levels induced by collagen immunization in
the different treatment groups (our unpublished data), suggesting that T15-NAb was primarily inhibiting the end organ inflammatory response. In other studies, infusions of apoptotic cells into DBA/1 mice yielded increased IgM anti-PC levels and protection from clinical arthritis, while infusions of primary necrotic cells did not (Fig. 8A).

To further define the adaptive immune systems role in this process, we studied the effects of T15-NAb on passive transfer arthritis induced by anti-CII IgG, in which lymphocytes do not play a central role (48). In this study, we again found that T15-NAb treatment significantly diminished joint swelling (Fig. 8D). Taken together, these findings indicate that the regulatory properties of T15-NAb in these models of arthritis act through the blunting of proinflammatory effector mechanisms mediated by the recruitment of IgG-autoantibody immune complexes.

**Discussion**

In health, inflammatory responses are critical for combating infection and tissue injury, but of equal importance are control mechanisms that remove dying cells and prevent overexuberant responses detrimental to the host. In this study, we document several previously unrecognized and functionally important features of the prototypic anti-PC NAb, T15. First, by virtue of its capacities for PC-specific binding to apoptotic cells, in addition to C1q (18), T15-NAb also facilitates the deposition of MBL onto apoptotic cells, particularly early apoptotic cells that are generally less susceptible to direct opsonin recruitment. Second, T15-NAb, by recruiting the deposition of both C1q and MBL, enhances the phagocytosis of early and late apoptotic cells. Third, T15-NAb, by forming complexes with MBL and C1q on apoptotic cells, can effectively suppress TLR-induced maturation of conventional DCs. Fourth, T15-NAb inhibits Mφ and DC secretion of proinflammatory cytokines and chemokines in response to agonists for a broad range of TLR, and it is also capable of inhibition of in vivo phagocyte activation and suppression of potentially harmful inflammatory responses. Fifth, infusion of high doses of T15-NAb, or large numbers of apoptotic cells that induce IgM anti-PC Abs, can inhibit the development of autoimmune inflammatory arthritis. Taken together, these findings identify a hitherto unsuspected set of regulatory functions of this type of NAb.

Our findings therefore characterize the potential functional roles of T15-NAb, which is representative of a dominant Ab response induced by apoptotic cells. Multiplex autoantigen-microarray assays have shown that apoptotic-cell treatment of C57BL/6 mice induces a dominant IgM response directed toward PC-containing Ags, including PC-albumin, pneumococcal vaccine, and capsular polysaccharide alone, or the self-Ag, oxidized LDL, with little or no reactivity to a large panel of other autoantigens (18) (supplemental Fig. 3). In C57BL/6 mice, T15-related Abs represent up to half of all induced Abs that recognize apoptotic cells (18). In fact, apoptotic cells induced relatively low levels of anti-DNA IgM (18).
supplemental Fig. 3), which contrasted with an earlier report (49). Taken together, apoptotic cells do not appear to induce non-specific polyclonal IgM responses but instead provoke an Ab response with a strong bias toward immune recognition of PC-neo-determinants.

In contrast to the classical theory that a healthy immune system requires an absolute avoidance of self-reactive lymphocyte clones (i.e., ‘horror autotoxicus’) (50), the repertoire of innate-like B cells are known to incorporate some level of autoreactivity (3, 51), and self-ligands are believed essential for their positive clonal selection and survival (52). On the basis of evidence that T15 B cells recognize an immunodominant self-Ag in apoptotic cells/debris, it is plausible that the apoptotic-cell turnover occurring during ontogenesis provides the self-ligands for the early selection of these B-1 cell clones. Moreover, as antiapoptotic cell Abs are part of the physiologic repertoire, our findings may explain why mice without circulating IgM spontaneously develop IgG-autoantibodies and lupus-like disease (53, 54), as this may result in impaired clearance of apoptotic breakdown products, as well as the loss of an NAb regulatory influence that otherwise can suppress overexuberant responses of innate phagocytes.

Although the potential immunosuppressive roles of NAb have been generally overlooked, there is nonetheless overwhelming evidence that Clq and MBL have immune-modulating properties (32). On the basis of evidence that humans with a homozygotic deficiency in Clq have a high penetrance of lupus-like systemic autoimmunity (55), studies of murine models confirmed this also occurs on certain genetic backgrounds and is associated with an accumulation of apoptotic bodies in the kidney and glomerulonephritis in 25% of animals (56). These data are consistent with the hypothesis that Clq deficiency, resulting in inefficient clearance of apoptotic cells, leads to the release of intracellular components, exposure of the immune system to self-Ags, and subsequent autoimmunity. Like Clq deficiency, MBL-deficient mice also display defects in apoptotic clearance and are prone to inflammatory conditions (34), although it is controversial whether MBL deficiency also influences autoimmune predisposition or disease severity (57).

Although MBL is well-known for its contributions to the clearance of microbial pathogens and apoptotic cells, its potential role in IgM-Ab effector functions has been little explored. Although these opsonins can be directly deposited at low levels onto apoptotic cells, this is primarily on late-stage apoptotic and secondary necrotic cells (23). Our data show that MBL (and Clq) are recruited by T15-NAb to early-stage apoptotic cells, and this is associated with both enhanced phagocytosis and reduced inflammation. Significantly in vivo apoptotic-cell immunization was shown to induce IgM-anti-PC responses that also recruited MBL and Clq binding, confirming the physiologic relevance of their essential yet redundant roles in NAb-effector functions. The effector function for MBL recruitment has also been associated with B-1 cell-derived IgM Abs with a different self-specificity (58).

Apoptotic-cell phagocytosis by immature DC enables constant sampling and presentation of self-Ags in a manner believed essential for the maintenance of tolerance (59). Yet, if apoptotic cells are not quickly cleared, cellular progression to necrosis can lead to the release of proinflammatory substances and autoantigens that can lead to breaches in self-tolerance (60). Hence, the efficient elimination of the immense number of cell corpses generated each day is therefore indispensable for tissue homeostasis, resolution of inflammation, and prevention of autoimmune disease. We provide direct evidence that a monoclonal B-1 cell NAb can directly induce deposition of MBL and Clq, which can each directly enhance cell-corpse clearance, as well as inhibit immature DC activation and differentiation. The anti-inflammatory influences of T15-NAb are no doubt aided by the capacity to flag cells and fragments at earlier stages of apoptosis for clearance.

The association of MBL with an apoptotic cell alone has been reported to be inadequate for activation of the complement cascade (23), and host cells themselves express specific complement inhibitors that also provide protection from inappropriate complement activation (61). However, other recent reports (62, 63) have shown that polyclonal serum IgM can induce the deposition of C3 products, which enable recognition by Mac-1/C3R on Mφ also implicated in the clearance of apoptotic cells. We therefore repeated our in vitro challenge studies with TLR agonists in serum-free cultures of C3-deficient DC but found no reduced capacity of T15-NAb to enhance phagocytic clearance and inhibit inflammatory responses (18) (data not shown). Our pilot studies also indicate that the in vivo anti-inflammatory activities of T15-NAb are unimpaired in C3-deficient mice (our unpublished data). Taken together, we believe these findings rigorously rule out an absolute requirement for downstream propagation of complement pathway activation in the immune modulatory properties of T15-NAb.

Our studies highlight the otherwise overlooked capacity for Ag-specific IgM Abs to affect the fundamental process of apoptotic clearance and regulation of TLR responses. As discussed above, we postulate that T15-NAb, which recognizes apoptosis-specific determinants, by increasing the amount of Clq and MBL bound on apoptotic cells facilitates their phagocytosis and also enhances apoptotic cell-mediated inhibition of Mφ and DC activation. This process potentially involves receptors, such as CD36, CD93 αβγ, calreticulin, and the LDL receptor (LRP-1 and CD91) that have been implicated in MBL- and Clq-enhanced apoptotic clearance (39, 64, 65). However the topic remains controversial, especially in light of the recent report, which also used eotaxine-induced apoptotic thymocytes, to demonstrate that LRP-1 was not essential for the enhancement of phagocytosis by murine Mφ (38). In addition, T15-NAb also appears to enhance immunomodulatory cellular interactions of complexed apoptotic cells and fragments, for which there are also a number of candidate receptors and at times conflicting literature (reviewed in Ref. 66). Yet, even though T15-NAb enhanced the phagocytosis of cell corpses and also blunted TLR-induced DC maturation, the underlying pathways are not necessarily entirely identical, because these effects can be mediated by distinct residues in the intracellular domain of an apoptotic-cell receptor (67). Our preliminary studies indicate that the suppressive effects of T15-NAb on DC involve inhibition of inflammatory signaling pathways (our unpublished observations). However, although previous studies implicated IL-10 and TGF-β in the anti-inflammatory effects of apoptotic cells (reviewed in Ref. 66), we were unable to find evidence to support this for T15-NAb, but this may in part reflect that such responses may primarily occur at later time points. Albeit, it remains possible that IL-10 and TGF-β might contribute to the in vivo effects of T15-NAb.

We first appreciated the prohomeostatic properties of T15-NAb in the setting of the chronic inflammatory disease, atherosclerosis. Indeed, earlier in vitro studies initially suggested that T15-IgM might act by blocking apoptotic-cell binding to elicited Mφ (15). By contrast, we now document that these NAb-mediated properties are linked to the contributions of the opsonins, MBL and Clq, that were likely absent or in low amounts in the earlier study (15). Indeed, our current studies, which used more physiologically relevant assays, instead clearly showed that T15-NAb enhanced in vivo phagocytic clearance by Mφ and directly inhibited inflammatory responses. These mechanisms now appear to be a more likely explanation for previous evidence that immunization with a PC-vaccine-suppressed atherosclerosis (12).
The current studies of autoimmune arthritis models also showed that treatment with T15-NAb significantly reduced inflammatory joint injury despite grossly unimpaired collagen-specific lymphocyte responses. Our studies were limited to prevention of the initiation phase of disease. However, we believe these findings may still be clinically relevant, despite the fact that Clq and MBL deficiency do not specifically predispose to inflammatory arthritis, and such conditions are not known to result from defects in apoptotic clearance. Yet in vitro and in vivo studies in C57BL/6 did demonstrate a potent capacity to inhibit phagocyte production of TNF-α, IL-6, IL-17, IL-12, and key chemokines that recruit other cell types into inflammatory responses. Hence, we postulate that raising levels of such NAb may provide benefits in conditions as diverse as rheumatoid arthritis, and perhaps also atherosclerosis (12), that are associated with innate immune cell recruitment and uncontrolled activation at sites of pathologic inflammation. Overall, these findings suggest that administration of T15-NAb, or induction of T15-NAb by apoptotic cells or microbial products, might be of general use for the suppression of deleterious inflammatory conditions.

Earlier studies of T15 Abs suggested that the driving force for the recurrent expression of these germline-encoded NAb was linked to protection against microbial pathogens that bear PC molecular patterns, which is reminiscent of functions of receptors of innate immune cells. Our studies may now provide clues to unsuspected primary functional roles of the earliest B lymphocytes, the strong evolutionary pressure to maintain the anti-PC specificity, and why we are born with Abs in our bloodstreams. Indeed, the strong evolutionary pressure to maintain the anti-PC specificities elicited by elipidic macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. Proc. Natl. Acad. Sci. USA 96: 6353–6358.


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