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Complement protein C1q bound to apoptotic cells suppresses human macrophage and dendritic cell-mediated Th17 and Th1 T cell subset proliferation

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ABSTRACT

A complete genetic deficiency of the complement protein C1q results in SLE with nearly 100% penetrance in humans, but the molecular mechanisms responsible for this association have not yet been fully determined. C1q opsonizes ACs for enhanced ingestion by phagocytes, such as Mϕ and iDCs, avoiding the extracellular release of inflammatory DAMPs upon loss of the membrane integrity of the dying cell. We previously showed that human monocyte-derived Mϕ and DCs ingesting autologous, C1q-bound LALs (C1q-polarized Mϕ and C1q-polarized DCs), enhance the production of anti-inflammatory cytokines, and reduce proinflammatory cytokines relative to Mϕ or DC ingesting LAL alone. Here, we show that C1q-polarized Mϕ have elevated PD-L1 and PD-L2 and suppressed surface CD40, and C1q-polarized DCs have higher surface PD-L2 and less CD86 relative to Mϕ or DC ingesting LAL alone, respectively. In an MLR, C1q-polarized Mϕ reduced allogeneic and autologous Th17 and Th1 subset proliferation and demonstrated a trend toward increased Treg proliferation relative to Mϕ ingesting LAL alone. Moreover, relative to DC ingesting AC in the absence of C1q, C1q-polarized DCs decreased autologous Th17 and Th1 proliferation. These data demonstrate that a functional consequence of C1q-polarized Mϕ and DC is the regulation of Teff activation, thereby "sculpting" the adaptive immune system to avoid autoimmunity, while clearing dying cells. It is noteworthy that these studies identify novel target pathways for therapeutic intervention in SLE and other autoimmune diseases. *J. Leukoc. Biol.* 97: 147–160; 2015.

Abbreviations: AC = apoptotic cell, APC = antigen-presenting cell, CD40/86L = cluster of differentiation 40/86 ligand, CFSE = carboxyfluorescein succinimidyl ester; DAMP = damage-associated molecular pattern, DC = dendritic cell, Foxp3 = forkhead box p3, FSC = forward-scatter, IC = immune complex , iDC = immature dendritic cell, LAIR-1 = leukocyte-associated Ig-like receptor 1, LAL = late apoptotic lymphocyte, Mϕ = macrophage(s), MFI = mean fluorescence intensity, PD-L1/2 = programmed death ligand 1/2, PI = propidium iodide, rh = recombinant human, SCARF1 = scavenger receptor class F, SLE = systemic lupus erythematosus, SSC = side-scatter, Teff = T-effector cell (Th1, Th17, or $CD8^+IFN\text{-}\gamma^+$), Treg = regulatory T cell ($CD4^+Foxp3^+$ or $CD8^+Foxp3^+$), UCI = University of California, Irvine

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

Introduction

C1q is the recognition component of the classic complement pathway with a prominent role in initiating complement-dependent killing of invading microbes and clearance of ICs. It is well established that a genetic deficiency of C1q in humans leads to the development of SLE, with nearly 100% penetrance [1]. In mice, complete genetic C1q deficiency (or reduced Mϕ C1q production) results in autoantibody production and murine lupus nephritis on certain strain backgrounds [2–4], consistent with the function of this protein as a regulator of inflammation and autoimmunity. Moreover, in murine Mϕ, C1q increases Mer tyrosine kinase, a critical receptor tyrosine kinase for the uptake of ACs [5, 6]. Given that C1q binds to ACs via its globular head domains [7], it is hypothesized that C1q functions in this context by opsonizing ACs for more rapid removal by phagocytes before they undergo secondary necrosis (reviewed in refs. [8, 9]), which avoids the release of inflammatory DAMPs and dangerous alarmins [10].

However, additional molecular mechanisms responsible for the C1q-mediated protection against autoimmunity in humans are beginning to be elucidated. In humans, C1q bound to ICs markedly shifted IC binding to monocytes and away from plasmacytoid DCs, thereby reducing the expression of the majority of IFN-response genes induced by ICs [11]. Our laboratory and others have demonstrated that C1q modulates phagocyte production of cytokines, enhancing anti-inflammatory and reducing proinflammatory cytokines [12–14]. In addition, Lu and colleagues [15] demonstrated that primary human monocytes, differentiated to DCs in the presence of immobilized C1q, showed reduced induction of allogeneic Th1 and Th17 cells. Our previous studies have shown that human monocyte-derived iDCs, ingesting C1q-bound autologous ACs

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(C1q-polarized DCs), increase secretion of anti-inflammatory cytokines, such as IL-10, and reduce proinflammatory cytokines, such as TNF-α [13]. However, many of these initial studies had evaluated the effect of C1q on the ingestion of ACs generated from transformed cell lines [13] or assessed C1q-cytokine responses and signaling in primary human monocytes or Mϕ by use of plate-bound presentation of C1q [5, 15, 16]. Recently, we developed a model in which primary human Mϕ ingest more physiologically relevant, autologous LALs to which C1q is bound. In this system, we have found that Mϕ ingesting C1q-bound LAL promote the successive gene expression and production of type 1 IFN followed by the anti-inflammatory cytokines IL-27 and IL-10 while decreasing inflammasome activity and secretion of mature IL-1β [17]. These data suggest that C1q is crucial, not only for the effective clearance of dying cells but also for suppressing the inflammatory environment in a human autologous system.

Regulation of the adaptive immune response is critical for the avoidance of autoimmunity. For instance, T cells can contribute to SLE pathogenesis, causing B cells to produce pathogenic autoantibodies in the inductive phase, as well as producing proinflammatory cytokines during the effector phase [18]. Polarized Mϕ, programmed by pathogen associated molecular patterns, DAMPs and the molecular environment secrete different cytokines and chemokines and express different surface markers, which together activate or suppress different populations of T cells, thus impacting the immune response. For example, the costimulatory CD40L is known to enhance Th1 responses [19], and CD86 can trigger Th1 [20] and Th17 [21] cell skewing. Th17 cells produce IL-17, which causes follicular Th cells to promote autoantibody-producing B cells [22] and contribute to pathology [23] in human and mouse models of SLE. In contrast, IL-27 is a direct and indirect negative regulator of the Th17 cell lineage [24–27]. Increases in IL-27 have been shown to be a result of, at least in part, Mϕ increase in type I IFNs acting back on the Mϕ in an autocrine fashion [28, 29]. Thus, the sequential increase in type 1 IFN, IL-27, and IL-10 gene expression and protein production by Mϕ ingesting C1q-bound LAL [17] is consistent with the hypothesis that C1q could attenuate T cell-mediated autoimmunity by increasing levels of these cytokines. Additionally, IL-27, acting on DCs, has been shown to up-regulate CD39, an ectoenzyme that decreases the extracellular concentration of ATP and thus attenuates ATP-dependent activation of the NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing 3) inflammasome and ultimately suppresses DC-mediated Th17 proliferation [24]. PD-L1, whose expression is induced by IL-27 [30] on human monocyte-derived DCs, and PD-L2, elevated on alternatively activated mouse Mϕ [31], are known to suppress antigen-dependent Teff activation via interaction with the T cell-inhibitory receptor PD-1 [32, 33].

Tregs play an essential role in maintaining immune homeostasis and preventing autoimmunity [34]. Defects in Treg development, maintenance, or function have been associated with SLE [35]. Surfactant protein A (SP-A), a lung tissue-specific defense collagen with similar structure and function to C1q, dramatically increases the proliferation of the Treg lineage in a MLR [36]. More recently, a novel type of Treg, $CD8^+Foxp3^+$ ($CD8^+$ Tregs), has been identified that completely prevented mortality because of graft-versus-host disease after allogeneic stem cell transplantation in mice in the absence of $CD4^+$ Tregs [37]. Thus, these $CD8^+Foxp3^+$ cells may reduce inflammatory T cell responses and promote tolerance.

In this study, we discovered that human Mϕ and DCs, ingesting autologous C1q-bound LAL (C1q-polarized Mϕ and DC), suppressed the induction of allogeneic and autologous Th17 and Th1 cell proliferation. In addition to the previously reported enhanced production of IL-27 and IL-10, C1q-polarized human Mϕ exhibit decreased levels of CD40 and increased levels of PD-L1 and PD-L2 on the cell surface. Furthermore, primary human C1q-polarized DCs up-regulated PD-L2, down-regulated CD86, and enhanced IL-27 expression relative to DC ingesting LAL alone. Taken together, these data identify a novel pathway by which C1q interaction with APCs modulates the adaptive immune response, can prevent the initiation and propagation phases of autoimmunity, suppress human autoimmune inflammation and potentially promote tolerance.

MATERIALS AND METHODS

Media and reagents

RPMI 1640 medium, penicillin/streptomycin, trypsin-EDTA, and L-glutamine were purchased from Invitrogen (Carlsbad, CA, USA), whereas X-VIVO 15 medium was purchased from Lonza (Basel, Switzerland), and defined FBS was from HyClone (Logan, UT, USA). rhM-CSF, rhIL-2, rhIL-7, rhIL-4, and rhGM-CSF were from PeproTech (Rocky Hill, NJ, USA). HSA, used for monocyte elutriation, was obtained from Talecris Biotherapeutics (Research Triangle Park, NC, USA). Ultrapure LPS was from List Biological Laboratories (Campbell, CA, USA). R848 (TLR7/8 agonist) was purchased from Enzo Life Sciences (Farmingdale, NY, USA). CFSE was from Molecular Probes/Life Technologies (Carlsbad, CA, USA). C1q was isolated from plasma-derived normal human serum by ion-exchange chromatography, followed by size-exclusion chromatography, according to Tenner et al. [38] and modified as described [39]. All C1q preparations were homogeneous (determined by SDS-PAGE and Coomassie Blue staining) and had <0.03 EU/ml endotoxin by *Limulus* amebocyte lysate clot assay (Lonza).

Human peripheral blood leukocyte isolation and culture

All blood samples were collected into CPDA1 (citrate phosphate dextrose adenine 1 solution) at the UCI Institute for Clinical and Translational Science (Irvine, CA, USA), in accordance with guidelines and approval of the UCI Institutional Review Board. Human peripheral blood lymphocytes and monocytes were isolated from PBMCs by countercurrent elutriation, as described previously [17], with 80% $CD3^+$ cells in the lymphocyte fraction and 90–98% $CD11b^+$ in the monocyte fraction. Human monocyte-derived Mϕ were generated from monocytes by culture at 2×10^6 cells/ml for 6–8 days in RPMI 1640 medium, 10% FBS, 2 mM L-glutamine, and 1% penicillin/streptomycin (complete media), containing 25 ng/ml rhM-CSF, with addition of fresh media + rhM-CSF at day 3. Human monocyte-derived DCs were generated from monocytes by culturing at 2×10^6 cells/ml for 6–8 days in complete media containing 25 ng/ml rhGM-CSF + rhIL-4, with addition of fresh media + rhGM-CSF + rhIL-4 at day 3. For MLR and surface expression experiments, the adherent Mϕ or DCs were harvested by washing twice with 1× HBSS and incubating with nonenzymatic Cellstripper (CellGro; Mediatech, Manassas, VA, USA) for 20–30 min. Autologous lymphocytes were maintained for 6–8 days in complete media containing 50 U/ml rhIL-2 and then γ-irradiated (10 Gy) and maintained overnight in media without FBS to generate LALs (40–60% Annexin V^+/PI^+), respectively [17]. For every experiment, apoptosis (apoptosis detection

kit from BioVision, Milpitas, CA, USA), C1q binding to LAL, and Mϕ phenotype were assessed by flow cytometry, as described previously [17].

Phagocytosis of LAL

Mϕ or DC ingestion of LAL in 96-well round-bottom plates (Nalge Nunc, Rochester, NY, USA) was evaluated as described previously [17]. In brief, LALs [in some cases, prelabeled with PKH26 (Sigma-Aldrich, St. Louis, MO, USA)], precoated or not with C1q, were incubated with 100 μl containing 3×10^4 Mϕ or DCs at a 5:1 ratio for 1 h in phagocytosis buffer (RPMI 1640 medium, 25 mM HEPES, and 5 mM $MgCl_2$). The Mϕ or DCs were then washed twice in 1× HBSS to clear away the uningested LAL and C1q-LAL. For phagocytosis quantification, cells were washed, harvested with trypsin/EDTA (Life Technologies), stained with anti-CD11b-FITC antibodies (Invitrogen) for flow cytometry, and scored for $PKH26^+$ $CD11b^+$ double-positive cells. The percentage of Mϕ and DCs phagocytosing at least 1 LAL within 1 h ranged from 48% to 64% and 27% to 44%, respectively, by $CD11b^+PKH26^+$ double-staining.

Detection of surface-marker expression

To evaluate CD40, PD-L1, PD-L2, CD86, and CD39 expression, Mϕ or DCs were cultured for 24 h after the ingestion of ACs (as described above) in 1 ml X-VIVO 15 in the presence or absence of 10 ng/ml LPS, where indicated. Mϕ or DCs were harvested with 1 ml Cellstripper (Mediatech), washed in HBSS containing 3% (w/v) BSA and 0.05% (w/v) NaN_3, and then incubated for 30 min with α-CD40-PE (Invitrogen); α-PD-L1-PE or α-PD-L2-PE (eBioscience, San Diego, CA, USA); and α-CD11b-FITC or CD86-FITC, α-CD39-APC, and α-CD11b-PE (BioLegend, San Diego, CA, USA), according the manufacturer's instructions. Cells were washed and analyzed by use of a FACSCalibur (BD Biosciences, San Jose, CA, USA). All $CD11b^+$ cells were included in the analysis.

MLR

T cells used for MLR experiments were purified from PBMCs to ≥95% $CD3^+$ using EasySep negative-selection T cell isolation kit (Stemcell Technologies, Vancouver, BC, Canada). Allogeneic T cells were used on the same day as isolated, whereas autologous T cells were maintained for 7 days (during the differentiation of monocytes into Mϕ or DCs) in complete media containing 50 U/ml rhIL-7. In some experiments, CD45RO-PE and α-CD45RA-PerCP-Cy5.5 (eBioscience or Tonbo, Biosciences, San Diego, CA, USA) were used to characterize the resulting populations of T cells. Allogeneic or autologous primary human T cells (50 ml; 1×10^5 cells), prelabeled with 5 μM CFSE, were added to 200 μl Mϕ or DC (a 3:1 ratio), 24 h after the phagocytes had ingested LAL or C1q-bound LAL (and treated with or without 10 ng/ml LPS or 5 μM R848, as indicated). After 5 and 6 days, the nonadherent cells were removed (routinely, 12 replicate wells were pooled/condition) and restimulated with 50 ng/ml PMA (Sigma-Aldrich) and 50 μg/ml ionomycin (EMD Millipore, Billerica, MA, USA) in the presence of 3 μg/ml brefeldin-A (eBioscience) for 6 h. After washing, cells were first stained for surface markers CD8-Pacific Blue (BioLegend) and α-CD4-PE and subsequently, for intracellular markers with α-Foxp3-APC, α-IL-17-PE-Cy7, and IFN-γ-APC-Cy7 (all from eBioscience). Intracellular antigens were detected by blocking with 2% normal mouse serum and staining with the Foxp3 staining buffer set from eBioscience (following the manufacturer's instructions). In initial experiments, T cells were incubated with IL-23, IL-12p70, or IL-2 and stimulated anti-CD3 and anti-CD28 for 3 days to generate positive controls for the detection of Th17 ($CD4^+IL\text{-}17^+$), Th1 ($CD4^+IFN\text{-}\gamma^+$), and Treg ($CD4^+Foxp3^+$) phenotypes, respectively.

For MLR flow cytometry experiments, we used the following gating strategy. With the use of an equivalent number of cells/sample, a sample of T cells that had been prestained with PI and subsequently washed was then fixed, permeabilized, and processed identically to all other samples. A gate was drawn around the PI-negative cells (live cells), and the live gate was expressed as forward scatter/side scatter and applied to the other samples. In subsequent experiments, this forward scatter/side scatter "live" gate was applied to each sample. The live cells were then gated on α-CD4-PE or α-CD8-Pacific Blue, and MFI of Foxp3, IL-17, or IFN-γ, and CFSE was assessed by flow cytometry on a BD LSRII (BD Biosciences). Flow cytometric data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA) or FlowLogic software (eBioscience). The influence of C1q on T cell lineage was assessed by calculating the ratio (fold change) of percent proliferating-specific T cell subsets in the MLR containing Mϕ or DC that had ingested C1q-LAL versus Mϕ or DCs that ingested LAL in the absence of C1q. The two-tailed Student's *t*-test in Prism version 6 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. For each analysis, error bars represent SD, and asterisks indicate the *P* value, referring to the difference between the mean fold change value of each subset and a theoretical mean of 1.0 by "one sample *t*-test" (two-tailed).

RESULTS

C1q-polarized Mϕ express elevated levels of PD-L1 and PD-L2 and reduced levels of CD40 relative to Mϕ ingesting LAL

To begin to explore the functional consequences of C1q interaction with Mϕ, the induction of PD-L1 and PD-L2 molecules in Mϕ, 24 h after ingesting ACs, was evaluated. To provide a mild inflammatory stimulus to the Mϕ that could be present in the area of dying cells, a low level of the TLR4 agonist LPS (10 ng/ml) was added immediately after the removal of uningested ACs. LALs were used, since due to the late stage of apoptosis, these cells are beginning to lose their membrane integrity and thus, are releasing proinflammatory molecules (such as high-mobility group box 1, a TLR ligand [40]). Whereas all Mϕ that had ingested LAL up-regulated PD-L1 expression significantly, Mϕ that had ingested LAL to which C1q was bound, i.e., C1q-polarized Mϕ, expressed significantly more PD-L1 relative to Mϕ that had ingested LAL without C1q, in the presence and absence of LPS (**Fig. 1A** and **B**). C1q-polarized Mϕ also significantly increased levels of PD-L2 relative to Mϕ ingesting LAL in the presence of LPS (Fig. 1C and D). The magnitude of the increase of PD-L2 expression on C1q-polarized Mϕ in the presence of added LPS was less than that of PD-L1 but was still statistically significant. C1q also promoted a slight trend toward increased expression of the surface regulatory molecule CD39 (data not shown).

To determine whether C1q-polarized Mϕ influence the expression of costimulatory molecules, which could have an impact on antigen-dependent T cell activation, we assessed the levels of surface CD40 in these Mϕ. In the absence of LPS, CD40 expression on Mϕ increased 3-fold upon ingestion of LAL, whereas addition of C1q-bound LAL dampened this response (Fig. 1E and F). A similar effect was noted with the addition of LPS to cultures in which C1q-bound LAL limited the up-regulation of CD40 on Mϕ after ingestion (Fig. 1E and F); in contrast, C1q did not influence Mϕ expression of the costimulatory CD86L relative to LAL in the presence or absence of LPS (data not shown).

C1q-polarized DCs express elevated levels of PD-L2 and reduced levels of CD86 relative to Mϕ ingesting LAL

To define the functional phenotype of C1q-polarized DCs, we evaluated surface-marker expression of DCs ingesting C1q-bound LAL. In the presence of LPS, C1q triggered significantly more

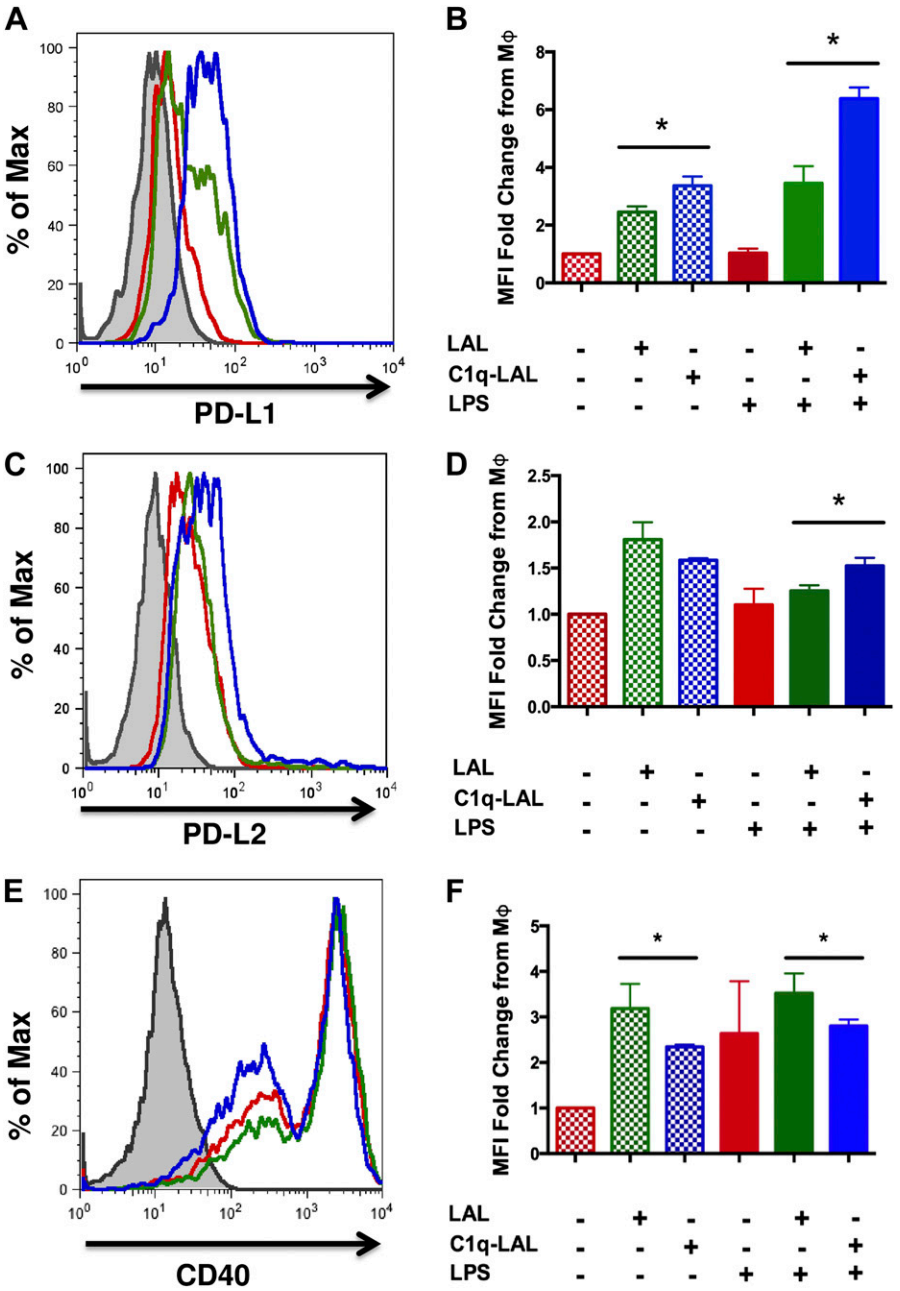

Figure 1. Mϕ s ingesting C1q-bound ACs are polarized toward an anti-inflammatory phenotype. C1q-coated autologous apoptotic lymphocytes (C1q-LAL; blue) or LAL only (green) were added to M-CSF monocyte-derived Mϕ (A–F) in a 5:1 ratio for 1 h, after which, the uningested ACs were washed away. Mϕ were then stimulated or not with 10 ng/ml LPS in X-VIVO 15 for 24 h. The adherent cells were harvested; stained with CD11b-FITC and anti-PD-L1-PE (A and B), anti-PD-L2-PE (C and D), or anti-CD40-PE (E and F); and read by flow cytometry. Controls were Mϕ without LAL (red). Gray shading (A, C, and E) indicates PE-isotype control. Representative FACS plots, gated on CD11b-positive cells, are shown for samples with LPS added (A, C, and E). Average fold change MFI relative to Mϕ only (B, D, and F) for 3–4 independent experiments. $*P < 0.05$ by Student's *t*-test. Error bars are SD.

PD-L2 expression relative to DCs ingesting LAL alone (**Fig. 2A** and **B**). Interestingly, however, C1q-polarized DCs did not exhibit levels of PD-L1 that differed from DCs ingesting LAL without C1q (data not shown). C1q-polarized DCs exhibited slightly elevated (but statistically significant) surface-expression levels of the anti-inflammatory ectoenzyme CD39 relative to DCs ingesting LAL alone (data not shown). In addition, whereas DCs ingesting LAL without C1q had a pronounced induction of CD86, C1q-polarized DCs attenuated this LAL-triggered enhancement of CD86 expression in the presence of LPS (Fig. 2C and D). However, C1q did not affect the levels of CD40 on DCs relative to LAL without C1q (data not shown).

C1q-polarized Mϕ suppress the proliferation of human allogeneic inflammatory T cells and trend toward enhanced proliferation of human allogeneic Tregs

Given that PD-L1 and PD-L2 binding to PD-1 on the surface of the T cell results in inhibition of T cell activation and that CD40 acts as a costimulatory ligand known to activate T cells during autoimmunity (reviewed in ref. [41]), we assessed the ability of primary human Mϕ that ingested LAL with C1q bound to their surface to direct the differential stimulation of primary human T cell subsets in a MLR. After the ingestion of LAL, Mϕ were treated with a low level of LPS (10 ng/ml), as described above for 24 h, followed by the addition of purified

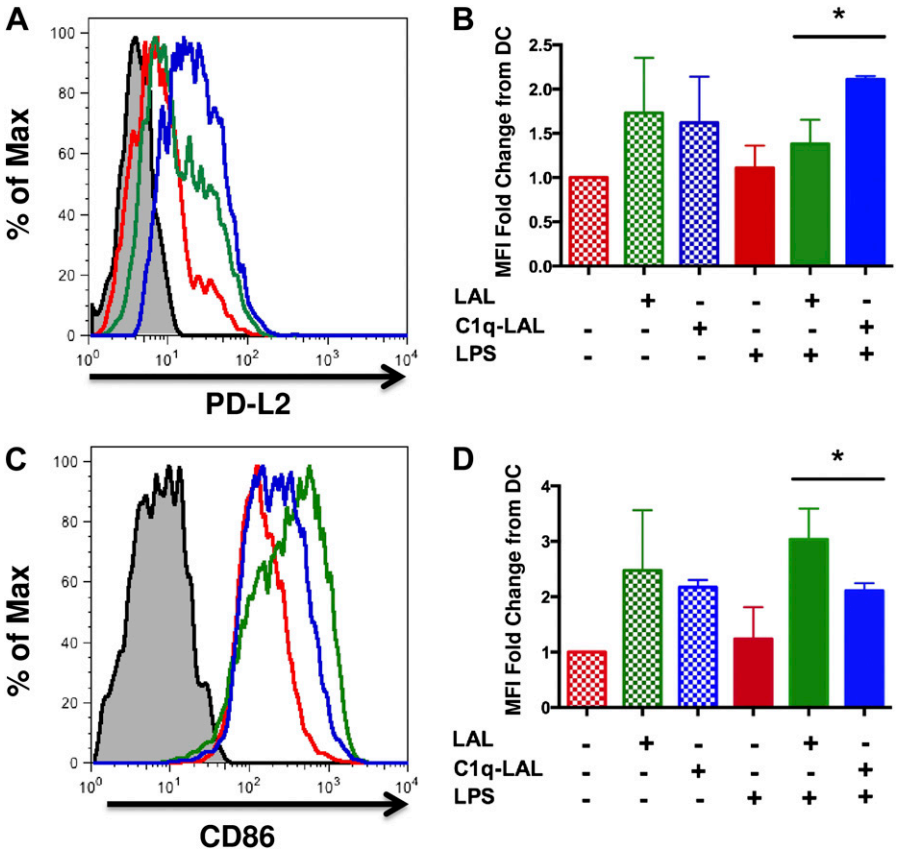

Figure 2. iDCs ingesting C1q-bound ACs are polarized toward an anti-inflammatory phenotype. C1q-coated autologous apoptotic lymphocytes (C1q-LAL; blue) or LAL only (green) were added to GM-CSF + IL-4 monocyte-derived DCs (A–D) in a 5:1 ratio for 1 h, after which, the uningested ACs were washed away. DCs were then stimulated or not with 10 ng/ml LPS in X-VIVO 15 for 24 h. The adherent cells were harvested; stained with CD11b-FITC and anti-PD-L2-PE (A and B) or CD11b-PE and anti-CD86-FITC (C and D); and read by flow cytometry. Controls were DCs without LAL (red). Gray shading (A and C) indicates PE-isotype control. Representative FACS plots, gated on CD11b-positive cells, are shown for samples with LPS added (A and C). Average fold change MFI relative to DCs only (B and D) for 4–5 independent experiments. $*P < 0.05$ by two-tailed Student's *t*-test. Error bars are SD.

allogeneic T cells to the Mϕ for 6 days. In this entirely human primary cell system, with its inherent diversity of individual donor responses, C1q-polarized Mϕ consistently suppressed the proliferation of the inflammatory T cell subsets. Specifically, the increase in proliferation of $CD4^+IL\text{-}17^+$ (Th17) cells, triggered by Mϕ that had ingested LAL, was suppressed by LAL with bound C1q by an average of 29% ($n = 5$, $P < 0.02$) relative to Mϕ that had ingested LAL in the absence of C1q (**Fig. 3A–C, J, and K**). It is noteworthy that this suppression resulted in a level of proliferation that was often back to the baseline level of proliferation seen with T cells cocultured with Mϕ that had not ingested ACs.

Likewise, C1q-polarized Mϕ decreased the allogeneic proliferation of IFN-γ-producing inflammatory subsets. CFSE dilution of $CD4^+IFN\text{-}\gamma^+$ (Th1) cells was decreased by an average of 32% ($P < 0.03$; Fig. 3D–F, J, and K). Proliferation of $CD8^+IFN\text{-}\gamma^+$ cells was also decreased by C1q-polarized Mϕ by an average of 22%, although this trend was not statistically significant ($P < 0.1$; Fig. 3G–K).

C1q-polarized Mϕ also exhibited a trend toward enhanced proliferation of $CD4^+Foxp3^+$ (Treg) cells in 3 of 5 sets of donors (1.43- to 2.68-fold increase; $P = 0.36$; $n = 5$) and $CD8^+Foxp3^+$ cells in 4 of 5 sets of donors (1.17- to 3.61-fold increase; $P = 0.29$; $n = 5$) relative to LAL after 6 days of coculture with allogeneic T cells (Fig. 3J and K). These data suggest that the microenvironment generated by the C1q-polarized Mϕ may favor proliferation of these regulatory cells but that other factors also influence these subpopulations.

In the absence of an extrinsic TLR ligand, the effect of C1q on Mϕ-mediated allogeneic T cell subset proliferation was not statistically significant for any subset (data not shown), consistent with previous reports demonstrating that low levels of LPS provide a level of proinflammatory stimulation that is then reduced by C1q, such as that seen with its effect on the modulation of cytokines and surface-marker expression [13, 17, 42, 43].

C1q-polarized Mϕ suppress proliferation of autologous inflammatory T cells

To investigate whether C1q might reduce inflammatory T cell subset activation and enhance Treg lineages in situations of autoimmune inflammation, as may be involved in SLE or similar diseases, we modified our experimental design to use autologous rather than allogeneic T cells in our T cell activation assay. To maintain T cells from the same donor during Mϕ differentiation, purified $CD3^+$ cells were maintained in IL-7 for 7 days, which results in nearly 100% $CD45RA^+$ cells (data not shown), consistent with previous reports [44, 45] that comprise naïve and/or effector populations [46]. Under these conditions, C1q-polarized Mϕ suppressed inflammatory Th17 autologous T cell proliferation in all donors, with the average decrease of nearly 50% ($P < 0.0001$; $n = 13$; **Fig. 4 A–C, M,** and **N**). Likewise, C1q-polarized Mϕ initiated less Th1 proliferation relative to Mϕ ingesting LAL in all donors (average of 39% decrease; $P < 0.0001$; Fig. 4 G–I, M, and N). However, C1q did not have a consistent effect on $CD8^+IFN\text{-}\gamma^+$ cell proliferation (Fig. 4M and N).

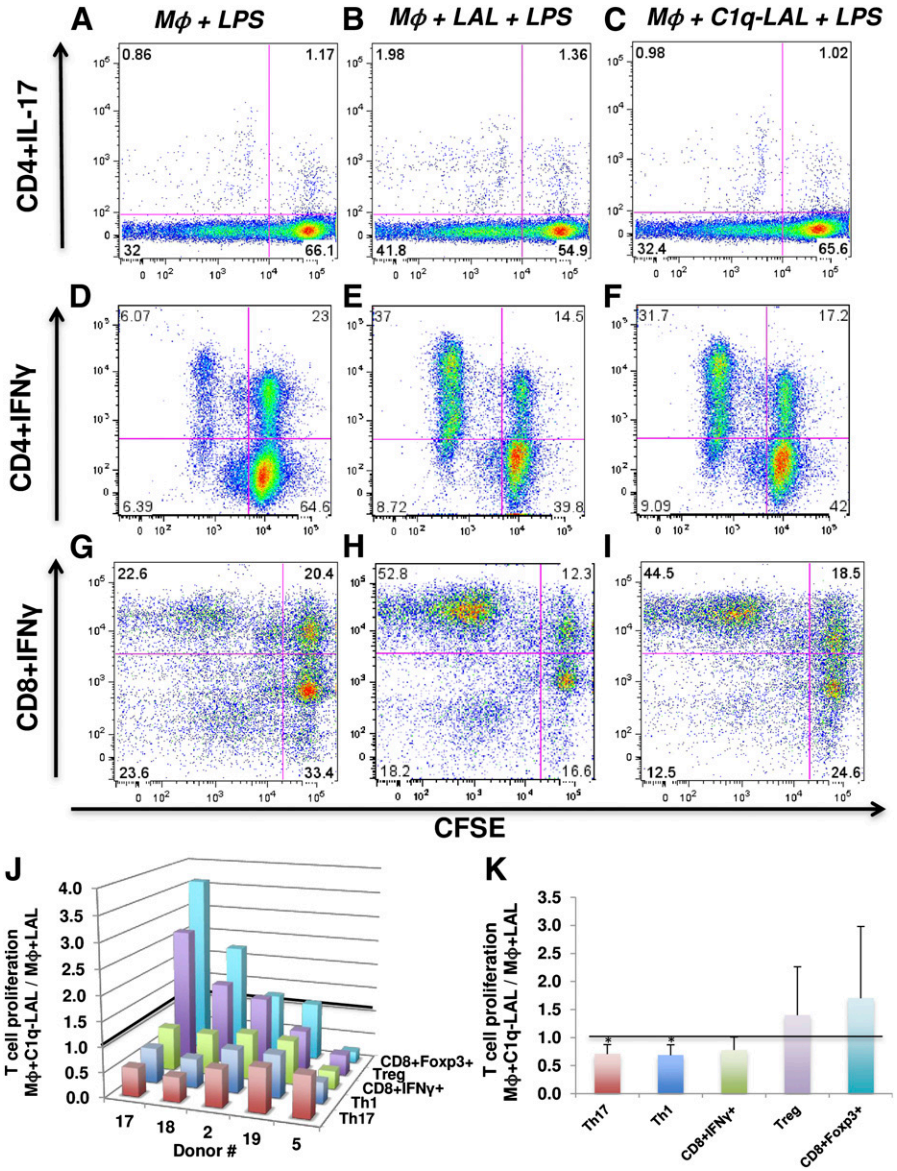

Figure 3. C1q suppresses Mϕ-mediated allogeneic T cell proliferation of inflammatory T cell subsets. CFSE-stained allogeneic primary human T cells (95% $CD3^+$) were added (3:1), 24 h after LPS treatment of Mϕ that had ingested LAL (B, E, and H) or C1q-bound LAL (C, F, and I) or without ACs (A, D, and G), as described in Fig. 1. After 6 days, the nonadherent cells were removed; restimulated with 50 ng/ml PMA and 50 μg/ml ionomycin in the presence of 3 μg/ml brefeldin-A for 6 h; then stained with α-CD4-PE, α-CD8-Pacific Blue, IL-17-PE-Cy7, and IFN-γ-APC-Cy7; and assessed by flow cytometry on a BD LSRII. With the use of FlowLogic, events were gated on α-CD4-PE (A–F) or α-CD8-Pacific Blue (G–I), and MFI of IL-17-PE-Cy7 (A–C), IFN-γ-APC-Cy7 (G–I), and CFSE dilution (percent proliferating and nonproliferating cells) is depicted. Data are representative of 5 independent experiments. Percent of total events is indicated in each quadrant. (J) The ratio of Mϕ + C1q-LAL/Mϕ + LAL for the percent proliferating Th17, Th1, $CD8^+IFN\text{-}\gamma^+$, Treg, and $CD8^+Foxp3^+$ cells for 5 donors is shown for Mϕ-stimulated LPS. Darkened line at 1.0 equals no difference for Mϕ + C1q-LAL/Mϕ + LAL for the percent proliferating subsets. (K) Average fold change Mϕ + C1q-LAL/Mϕ + LAL for the percent proliferating subsets of 5 donors (1.0 would indicate no difference between Mϕ + C1q-LAL and Mϕ + LAL). Error bars are SD; $*P < 0.03$, referring to the difference between the mean fold change value of each subset and a theoretical mean of 1.0 by one sample *t*-test (two-tailed).

Similar to the allogeneic MLR, in the absence of extrinsic TLR ligand, C1q did not instigate a statistically significant effect on Mϕ-mediated autologous T cell subset proliferation for any subset (data not shown), consistent with earlier studies, demonstrating that C1q acts as a "check" on low-level stimulation of inflammatory cytokines and surface-marker expression [13, 17, 42, 43]. Finally, there was no consistent effect on $Foxp3^+$ subset proliferation, probably in part as a result of the limited presence of these subpopulations in the T cells cultured for 7 days in IL-7, whereas the Mϕ differentiation was induced.

To determine whether this in vitro culturing of T cells with IL-7 might influence the generality of our findings, we differentiated monocytes to Mϕ and added freshly isolated T cells from blood of the same donor, drawn 1 week later than that for the monocytes. In 3 donors, we found that Mϕ ingesting C1q-LALs suppress Th17 and Th1 proliferation relative to Mϕ ingesting LAL by an average of 25% and 34%, respectively. Similar to the autologous MLR containing T cells that had been maintained in IL-7, there was no consistent C1q effect on $CD4^+$ or $CD8^+$ Treg proliferation.

C1q-polarized Mϕ suppress inflammatory T cell subsets in an autologous MLR when TLR7/8 agonist R848 is added to the Mϕ before the MLR

A number of studies have demonstrated a clear role for TLR7 in SLE pathogenesis [47, 48]. To evaluate whether the presence of another TLR ligand would affect the influence of C1q-polarized Mϕ on autologous T cell subset induction and proliferation in the same manner as the TLR4 ligand LPS, we added R848 (TLR7/8 ligand) instead of LPS to Mϕ after ingesting LAL or C1q-bound LAL. C1q-polarized Mϕ, treated with R848, decreased Th17 proliferation by an average of 51% ($P < 0.02$), similar to LPS-treated Mϕ in parallel wells (Fig. 4D–F, O, and Q). Additionally, C1q-polarized Mϕ consistently suppressed Th1 proliferation by an average of 46% in the presence of R848,

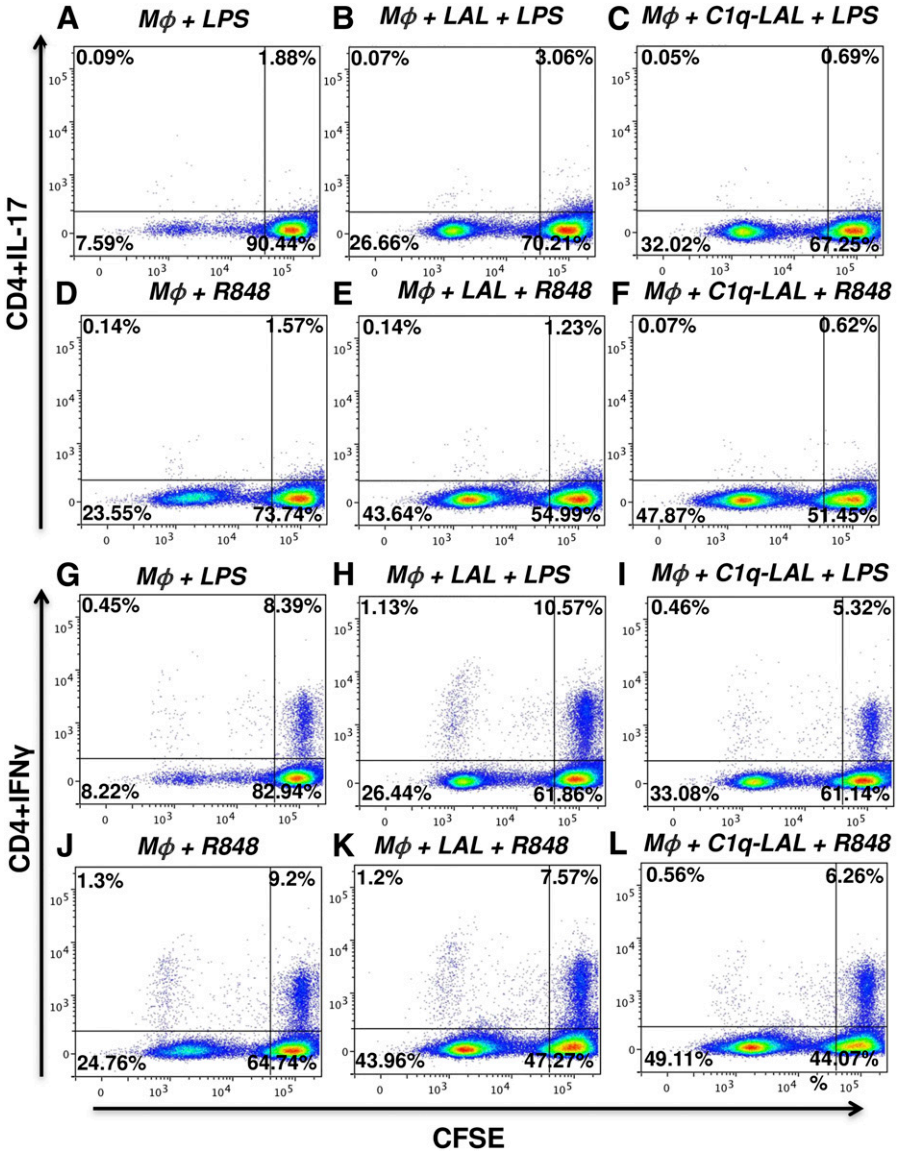

Figure 4. C1q suppresses Mϕ-mediated autologous T cell proliferation of inflammatory T cell subsets in the presence of exogenous TLR4 and TLR7/8 ligands. Autologous primary human T cells (95% $CD3^+$), cultured in IL-7 for 8 days, were prelabeled with CFSE and added 24 h after 10 ng/ml LPS (TLR4 agonist; A–C and G–I) or 5 μM R848 (TLR7/8 agonist; D–F and J–L) treatment of Mϕ that had ingested LAL (B and H or E and K) or C1q-bound LAL (C and I or F and L) or without ACs (A, D, G, and J), as described in Fig. 1. After 6 days, the nonadherent cells were removed, treated as described in Fig. 3, and assessed by flow cytometry on a BD LSRII. With the use of FlowLogic, cells were gated on α-CD4-PE, and MFI of IL-17-PE-Cy7 (A–F), IFN-γ-APC-Cy7 (G–L), and CFSE dilution (percent proliferating and nonproliferating cells) are depicted. Percent of total events is indicated in each quadrant. (M) The ratio of Mϕ + C1q-LAL versus Mϕ + LAL for percent proliferating Th17, Th1, and $CD8^+IFN\text{-}\gamma^+$ cells for 13 independent experiments. Darkened line at 1.0 equals no difference for Mϕ + C1q-LAL/Mϕ + LAL for the percent proliferating subsets. (N) Average ratio of the percent proliferating subsets in the presence of Mϕ + C1q-LAL relative to Mϕ + LAL for the 13 donors. (O and P) The ratio of the percent proliferating Th17 (O) or Th1 (P) cells in the presence of Mϕ + C1q-LAL relative to Mϕ + LAL is shown for 5 donors stimulated with 10 ng/ml LPS (blue) or 5 μM R848 (red). Darkened line at 1.0 equals no difference between Mϕ + C1q-LAL and Mϕ + LAL for the percent proliferating subsets. (Q) Average ratio of Mϕ + C1q-LAL versus Mϕ + LAL for 5 donors for the percent proliferating subsets in the presence of LPS (blue) or R848 (red) added to cultures. Error bars are SD; $^{***}P < 0.0001$; $^{**}P < 0.01$; $^{*}P < 0.03$, by two-tailed *t*-test for the difference between the proliferating subset average value and 1.0 (1 indicates no difference between Mϕ + C1q-LAL and Mϕ + LAL) for each subset.

(*continued on next page*)

similar to LPS-treated Mϕ from the same donor (Fig. 4J–L, P, and Q). In contrast, C1q bound to LAL did not consistently affect $CD8^+IFN\text{-}\gamma^+$ T cell proliferation when R848 was added to the Mϕ, similar to the lack of effect of C1q on this subset in the presence of the low-level TLR4 signal (data not shown).

C1q-polarized Mϕ conditioned media suppresses inflammatory T cell subsets in an autologous MLR

To evaluate whether the effect of C1q-polarized Mϕ on autologous T cell subset proliferationand induction is, at least in part attributed to soluble factors (such as increased IL-27 and IL-10) versus surface-bound factors (such as increased PD-L1 and PD-L2 or reduced CD40), conditioned media (supernatants) from 24 h LPS-treated, C1q-polarized Mϕ were added to untreated, M-CSF-derived Mϕ plus autologous T cells. Similar to the effect observed with C1q-polarized Mϕ in parallel wells from the same donor, conditioned media from C1q-polarized Mϕ reduced the percentage of proliferating Th17 cells relative to Mϕ ingesting LAL alone in all cases by 43% ($n = 5$; $P = 0.05$; **Fig. 5A–F** and **M–O**). Additionally, relative to conditioned media from Mϕ ingesting LAL alone, conditioned media from C1q-polarized Mϕ reduced the percentage of proliferating Th1 cells by an average of 38% ($n = 5$; $P < 0.04$), although not to the same extent as the polarized Mϕ themselves.

In the previous report from this laboratory, it was demonstrated that IL-27 and other regulatory cytokines were expressed at a higher level in C1q-polarized Mϕ relative to Mϕ ingesting LAL [17]. To validate under the conditions used here for the MLR (X-VIVO 15 vs. HL-1 media) that IL-27 was increased, IL-27 mRNA was assessed 4–7 h after ingestion of LALs. In three different donors, IL-27 expression was increased 3- to 6-fold in C1q-polarized Mϕ (Supplemental Fig. 1A) relative to Mϕ ingesting LAL. Taken together, these data suggest that soluble factors released by Mϕ that ingested C1q-coated LAL contribute to the suppression of Th17 and Th1 cells.

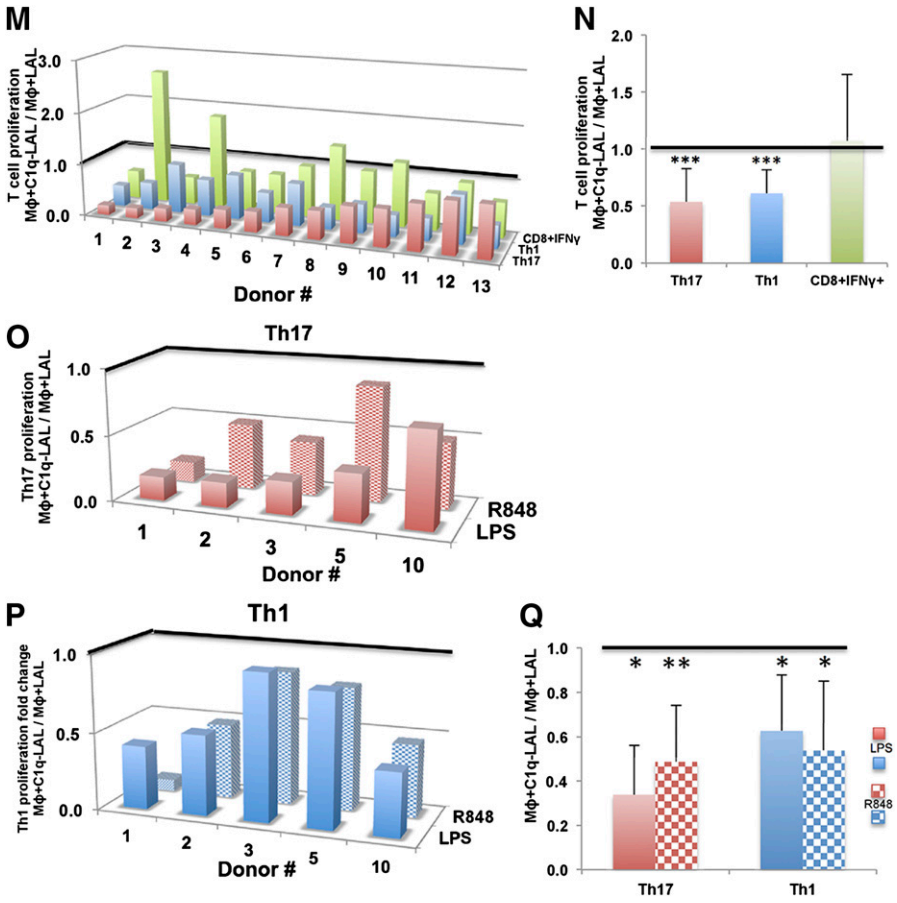

Figure 4. (*continued*)

C1q-polarized DCs suppress proliferation of autologous inflammatory T cell proliferation

To elucidate whether C1q-polarized DCs functionally modulate the adaptive immune responses, human autologous T cells were incubated with DCs that had ingested LAL only or DCs that had ingested C1q-bound LAL (C1q-polarized DC), and the proliferation of $CD4^+$ and $CD8^+$ T cell subsets was assessed. Relative to DC ingesting LAL in the absence of C1q, C1q-polarized DCs decrease autologous Th17 proliferation by an average of 53% ($n = 5$; $P < 0.01$; **Fig. 6A–C, G,** and **H)** and Th1 proliferation by an average of 35% ($n = 5$; $P < 0.01$; Fig. 6D–H). As with the C1q-polarized Mϕ, DCs that had ingested C1q-LAL expressed higher levels of IL-27 mRNA (2.9- to 3.6-fold), as determined by quantitative PCR (Supplemental Fig. 1B). Similar to the MLR experiments done with Mϕ and fresh T cells, DCs ingesting C1q-LAL suppress freshly isolated Th17 and Th1 proliferation relative to DC ingesting LAL by an average of 58% and 10%, respectively, in two donors. Again, C1q-polarized DCs did not have a consistent effect on the proliferation of $CD8^+$IFN-γ^+ T cell proliferation or $CD4^+$Foxp3^+ or $CD8^+$Foxp3^+ subsets (data not shown).

DISCUSSION

In this study, human Mϕ and DCs ingesting late ACs to which C1q was bound suppressed the proliferation of allogeneic and autologous inflammatory Th17 and Th1 T cell subsets known to be elevated in autoimmunity [49–51]. C1q attenuation of autologous Mϕ-mediated Th17 and Th1 cell proliferation was similar, whether TLR4 ligand LPS or TLR7/8 ligand R848 was added before the MLR (Fig. 4). This is notable, given that TLR7 and -8 appear to play a significant role in autoimmunity [52], and suggests that C1q is broadly suppressive of the Mϕ-mediated proliferation of these subsets. A persistent reduction in these populations over time, during continuous autoantigen clearance, could have biologic consequences, particularly in individuals in which there is a propensity for deficient or inefficient clearance or excessive tissue damage that exceeds the capacity for "silent" clearance of dying cells and debris. Additionally, C1q-polarized Mϕ had elevated surface expression of PD-L1 and PD-L2 and significantly suppressed induction of the surface costimulatory CD40R relative to Mϕ ingesting LAL alone. Furthermore, C1q-polarized DCs exhibited elevated surface expression of PD-L2 and CD39 and significantly suppressed induction of the surface costimulatory CD86R. These observations establish a functional consequence of the C1q-induced changes in APCs on elements of the adaptive immune response in ex vivo conditions, extending previous work, demonstrating that primary human Mϕ and DCs ingesting C1q-bound LAL up-regulate and secrete significantly more anti-inflammatory cytokines, such as IL-27 and IL-10 [13, 17]; produce significantly less proinflammatory cytokines, such as IL-1β, TNF-α, and IL-12 [13, 16, 43, 53]; and in the case of Mϕ, have reduced inflammasome activation relative to Mϕ ingesting LAL alone [17] (**Fig. 7**).

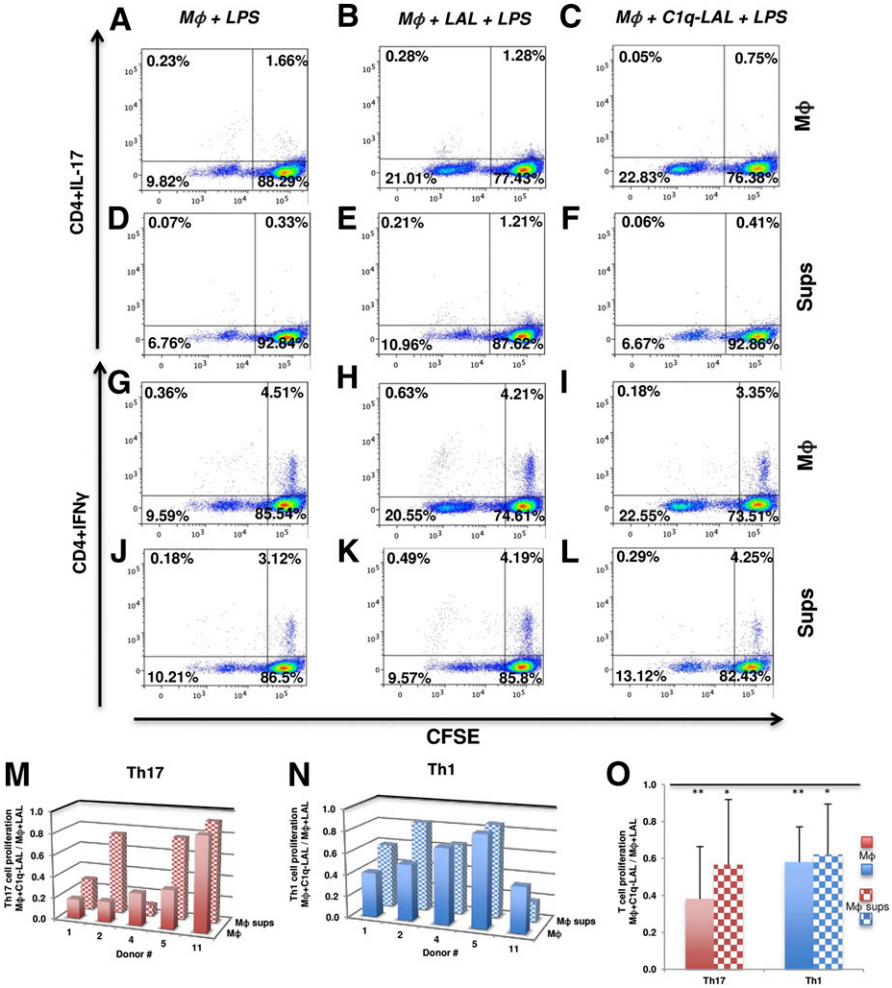

Figure 5. Culture supernatants from C1q-polarized Mϕ suppress autologous T cell proliferation of inflammatory T cell subsets. Mϕ were treated as described in Fig. 4A–C and G–I) or with 24 h conditioned media (supernatants, or "sups") from LPS-treated, C1q-polarized Mϕ (F and L), Mϕ ingesting LAL without C1q (E and K), or Mϕ alone (D and J). CFSE-stained autologous primary human T cells (95% $CD3^+$) were added (3:1). After 6 days, the nonadherent cells were removed, restimulated, and stained and fluorescence assessed by flow cytometry on a BD LSRII, as described in Fig. 4. With the use of FlowLogic, cells were gated on α-CD4-PE, and MFI of IL-17-PE-Cy7 (A–F) and IFN-γ-APC-Cy7 (G–L) and CFSE dilution (percent proliferating and nonproliferating cells) is depicted. Data are representative of 5 independent experiments for each subset. Percent of total events is indicated in each quadrant. (M and N) The ratio of the percent proliferating Th17 (M) or Th1 (N) cells for 5 donors is shown for Mϕ in the presence of Mϕ + C1q-LAL conditioned media relative to Mϕ + LAL conditioned media (Mϕ sups) or Mϕ + C1q-LAL relative to Mϕ + LAL (Mϕ). Darkened line at 1.0 equals no difference for Mϕ + C1q-LAL/Mϕ + LAL for the percent proliferating subsets. (O) Average ratio of Mϕ + C1q-LAL conditioned media relative to Mϕ + LAL conditioned media or Mϕ + C1q-LAL relative to Mϕ + LAL for 5 donors for the percent proliferating subsets. Error bars are SD; $*P \leq 0.05$ $**P < 0.01$ by two-tailed *t*-test for the difference between the proliferating subset average value and 1.0 (no difference for Mϕ + C1q-LAL/Mϕ + LAL).

PD-L1 and PD-L2 bind the T cell inhibitory receptor PD-1 to reduce Teff activation [32], and CD39 decreases inflammasome activation (by reducing extracellular ATP as a secondary signal for the inflammasome) and limits Th17 and Th1 generation [24]. Given that IL-27 is known to inhibit the Th17 subset [24, 25, 54, 55], possibly through up-regulation of PD-L1 [56] and/or CD39 [24], the increased IL-27 observed in the case of C1q-polarized Mϕ (Supplemental Fig. 1A) may contribute to the reduced Th17 proliferation, in part, via regulation of these cell-surface markers. The C1q-mediated suppression of CD40 induction relative to Mϕ that have ingested LAL without C1q may be attributed to the decrease in TNF-α and IL-1β, observed previously in C1q-polarized Mϕ [13, 17]. CD40 is known to interact with CD40L on T cells to enhance Th1 responses [41, 57], and thus, suppression of CD40 may partially contribute to the decreased stimulation of Teff subsets by C1q-polarized Mϕ. Benoit et al. [17] also found a C1q-mediated enhancement of expression of IL-13, a typical M2-driven cytokine; IL-33, a member of the IL-1 family that can amplify M2 (alternative) polarization of Mϕ; and IL-37 (IL-1F7), a natural suppressor of innate inflammatory responses [58]. Because C1q-LAL clearly enhances IL-27 expression in both HL-1 and X-VIVO 15 media (Supplemental Fig. 1A and B), it is likely that, in this media, C1q-LAL promotes the expression of these other negative modulators as well and is consistent with the negative regulatory effect of the supernatants from C1q-polarized Mϕ on the Th17 and Th1 subsets. Alternatively, C1q bound to LAL may suppress expression of LAL-induced Th1- and Th17-stimulating mediator(s).

Previously, we have shown that C1q suppresses DC production of IL-1β, TNF-α, and IL-12 and enhances DC production of IL-10 [13]. This C1q-induced cytokine milieu may contribute to the effect of C1q-polarized DCs on diminished Th17 and Th1 proliferation, given that IL-12 induces Th1 cells [59] and that IL-1β is required for Th17 cell induction [60]. The enhanced C1q-mediated PD-L2 expression and/or suppressed CD86 expression [61] and/or enhanced IL-27 expression (Supplemental Fig. 1B) may also be contributing to the suppression of Th1 and Th17 subsets [33].

The inhibition of Th1 proliferation by C1q-polarized Mϕ and DC is consistent with the up-regulation of IL-10 by C1q-polarized Mϕ and DC [13, 17] and the known ability of IL-10 to suppress the Th1 subset in certain contexts [62–64]. Indeed, our data demonstrate that conditioned media from C1q-polarized Mϕ, added to untreated M-CSF-derived Mϕ, resulted in a reduction in Th17 and Th1 proliferation at MLR day 6 relative to that seen with conditioned media from Mϕ that ingested LAL without C1q. The effect of the conditioned media from C1q-polarized Mϕ was similar to, although smaller in magnitude, the effect of the

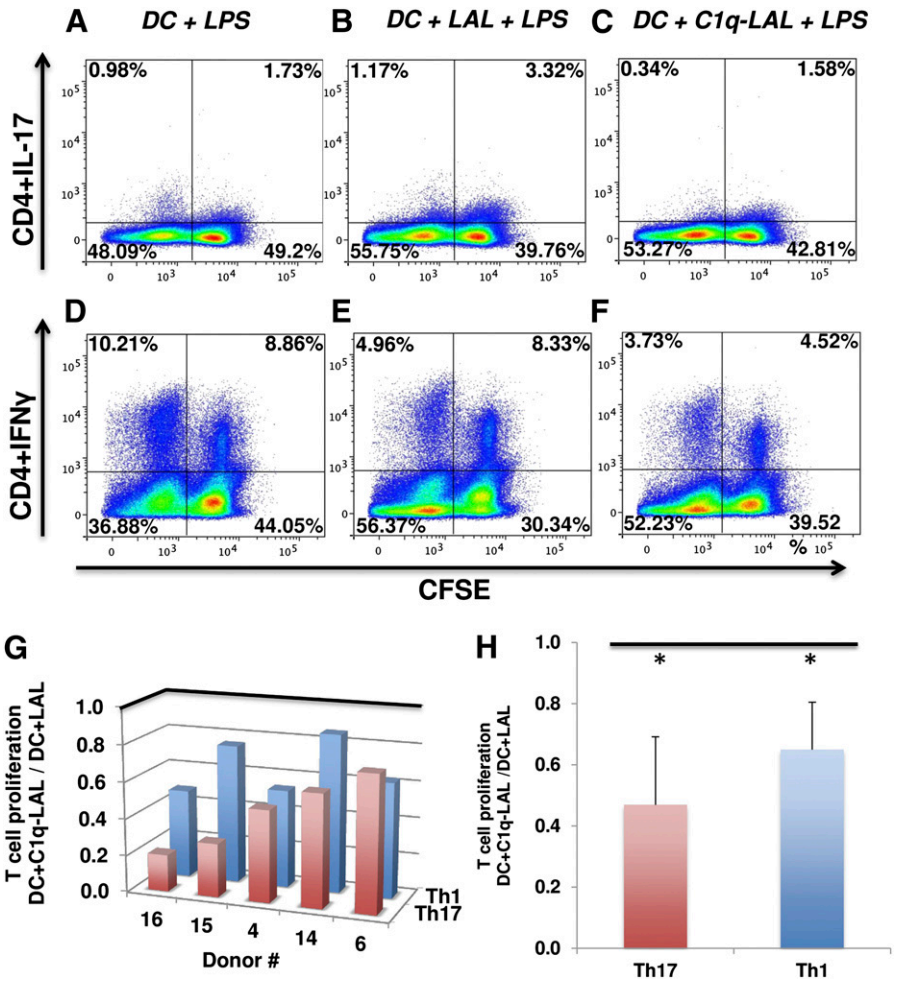

Figure 6. C1q-polarized DC suppress autologous T cell proliferation of inflammatory T cell subsets. CFSE-stained autologous primary human T cells (95% $CD3^+$, maintained in IL-7 for 1 week) were added (3:1), 24 h after LPS treatment of DCs that had ingested LAL (B and E) or C1q-bound LAL (C and F). After 5 days, the nonadherent cells were removed, restimulated, processed, and assessed, as in Fig. 4. With the use of FlowLogic, cells were gated on α-CD4-PE and IL-17-PE-Cy7 (A–C) and IFN-γ-APC-Cy7 (D–F). Data are representative of 5 independent experiments. Percent of total events is indicated in each quadrant. (G) The ratio of DC + C1q-LAL/DC + LAL for the percent proliferating Th17 and Th1 cells for each of 5 donors. Darkened line at 1.0 demarks no difference for DC + C1q-LAL/DC + LAL for the percent proliferating subsets. (H) Average value of 5 donors for fold change DC + C1q-LAL/DC + LAL for the percent proliferating subsets. Error bars are SD; $*P < 0.01$ by two-tailed *t*-test for the difference between the proliferating subset average value and 1.0 (no difference for DC + C1q-LAL/DC + LAL).

C1q-polarized Mϕ themselves (Fig. 5), and elevated mRNA levels for IL-27 were seen in both C1q-polarized Mϕ and DCs (Supplemental Fig. 1A and B), suggesting that these soluble factors and others are critically contributing to the suppression of Th17 and Th1 lineage proliferation. Thus, the effect of C1q may be to modulate Mϕ and DC responses to low levels of DAMPs accumulating during cell death/tissue repair [65] to suppress expansion of potentially damaging Th17 and Th1 cells in the tissue in the absence of significant infection.

The allogeneic MLR used here may be recapitulating some of the conditions occurring during allograft transplantation, and in this way, C1q may play an anti-inflammatory role in suppressing T cell-mediated allograft rejection, consistent with previous in vivo mouse data [66]. However, coculture of C1q-polarized Mϕ and DCs with autologous T cells is a situation that more closely mimics the in vivo clearance of ACs. The T cells that were maintained in IL-7 were nearly 100% $CD45RA^+$. Thus, whereas they may not be strictly naïve [67, 68, 69], freshly isolated autologous T cells responded similarly. Given that C1q-polarized Mϕ and DC consistently suppressed Th17 and Th1 proliferation, even in the absence of substantial $Foxp3^+$ T cells, it is likely that the APC themselves (via cell interaction or their secreted products) are contributing substantially to suppression of the Th17 and Th1 subsets rather than an indirect C1q-mediated decrease in these lineages by way of $CD4^+Foxp3^+$ or $CD8^+Foxp3^+$-mediated suppression.

It remains to be determined what molecular mechanisms are involved in the synergistic effect of C1q-LAL and TLR agonists. Our earlier studies showed that some C1q-induced signaling events are independent of TLR ligation, such as the induction of NF-κB p50p50, a negative regulator of proinflammatory activation, which is induced in the presence and absence of LPS [16]. Thus, C1q-induced p50p50 complexes may "compete" for TLR-triggered proinflammatory NF-κB complexes (p65p65 or p65p50), thereby limiting proinflammatory cytokine production, and thus, the suppressive effects are seen only in the presence of a TLR agonist. In addition, C1q-LAL may alter "cargo" trafficking, processing, and/or antigen presentation (which would then have downstream effects on T cell subset activation/proliferation). Such alterations have been reported recently for C3 in the murine system, although the end effect of this was enhanced antigen presentation, as may be expected, to facilitate an immune response to a complement-activating invader [70].

Although at first it was surprising that the addition of LAL to the Mϕ or DC increased overall levels of proliferation compared with the Mϕ or DC + LPS in the absence of LAL, APC ingestion of ACs has been shown previously to promote T cell proliferation in a primary human autologous MLR [71, 72]. Because Mϕ and DCs

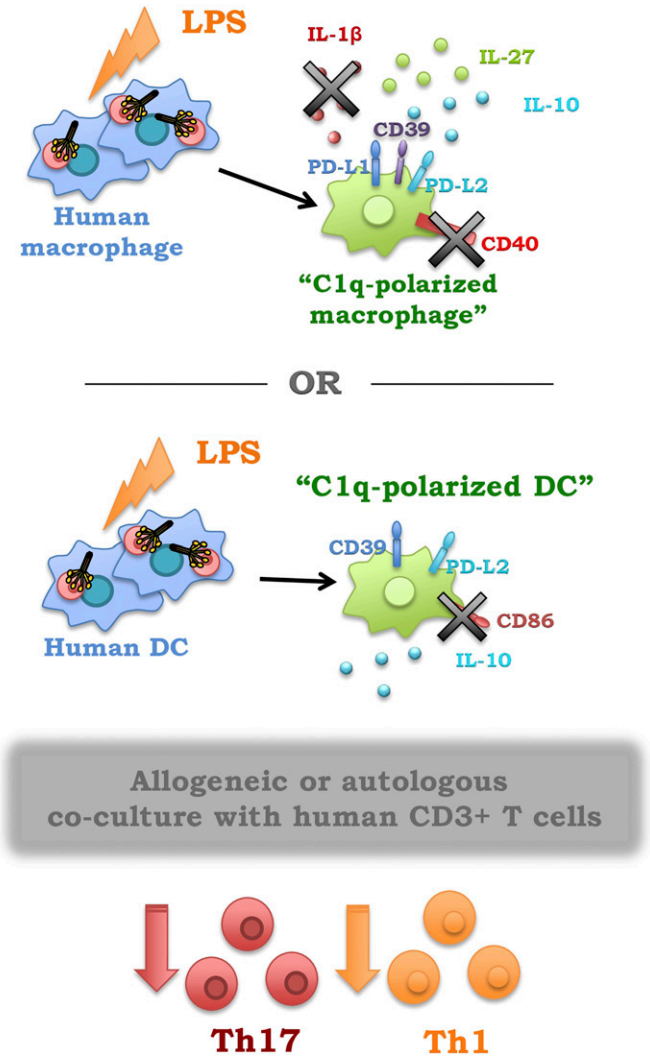

Figure 7. Summary of complement protein C1q effects on human Mϕ and DC polarization and Teff proliferation. Human monocyte-derived Mϕ or DCs ingest C1q-bound apoptotic lymphocytes, after which, they were stimulated with a low level of the TLR agonist. The "C1q-polarized macrophage" exhibits elevated PD-L1 and PD-L2 and reduced CD40 surface expression and produces greater IL-27 and IL-10 and less IL-1β than Mϕ that had ingested LAL alone. "C1q-polarized DC" exhibits elevated PD-L2 and CD39 and reduced CD86 surface expression and produces more IL-10 than DCs that had ingested LAL alone. The proliferation of allogeneic and autologous Th17 and Th1 T cell subsets was diminished significantly in an MLR when cultured with C1q-polarized APC versus APC ingesting LAL without C1q.

are maintained in RPMI 1640 media + 10% FBS (containing alloantigens) and are likely sampling that environment during their 7 day differentiation from freshly isolated peripheral blood monocytes, it has been suggested that they are presenting these alloantigens to the T cells, which then proliferate in response [73].

Intriguingly, the C1qR responsible for these effects remains to be identified. C1q has been shown to interact with several different myeloid cell-surface molecules, including LAIR-1, resulting in suppression of human monocyte-to-DC differentiation and activation [74]. Additionally, SCARF1 [75] and calreticulin [76] have been implicated in the C1q-mediated enhancement of clearance of ACs. We found that SCARF1 is expressed on the primary human Mϕ and DC used in our experiments and that both LAIR-1 and SCARF1 are expressed on the primary human Mϕ (data not shown); thus, future experiments should be able to determine the C1qR(s) responsible for the effect of C1q-bound ACs on Mϕ and DC cytokine production, surface molecule expression, and Mϕ- and DC-mediated T cell activation that we report here.

This study adds to a growing body of literature, demonstrating a role for complement proteins in modulating T cell function during disease and under homeostatic conditions (reviewed in ref. [77]). Indeed, it is becoming increasingly clear that complement proteins, traditionally thought to regulate only innate immune cells, are also capable of influencing the adaptive immune system. However, to our knowledge, this is the first instance demonstrating the immunoregulatory role of C1q on Mϕ- and DC-mediated T cell activation in an entirely human system with the more physiologic stimulus of autologous dying cells. Only a couple of studies to date have investigated whether C1q plays a role in modulating T cell activation. Cutler et al. [78] demonstrated that in C1q knockout mice, antigen-specific T cells exhibited a significant reduction in IFN-γ production compared with control mice. Likewise, a more recent study from the same group [57] used DCs from C1q knockout mice and found that in this context, exogenously added C1q augments the production of IL-12 by DCs and increases the number of $CD4^+IFN\text{-}\gamma^+$ (Th1) and $CD8^+IFN\text{-}\gamma^+$ T cells in response to DC CD40 ligation, although no difference was seen in response to TLR ligands. However, these experiments were conducted in mice, with complete genetic knockout (lifetime) of C1q. Indeed, although routinely used in studies of inflammation, the mouse model fails to recapitulate some human inflammatory diseases [79–81]. Importantly, Teh et al. [15] used C1q-treated primary human DCs and found that C1q decreased IL-12 and IL-23 production and reduced Th1 and Th17 induction from allogeneic $CD4^+$ T cells in a MLR. These data are consistent with the effect of C1q bound to ACs that we report here and suggest that C1q influences human DCs to skew T cells toward a more anti-inflammatory function, consistent with a role of C1q in promoting tolerance, while avoiding autoimmunity [67, 82].

However, unique to the studies presented here, human Mϕ- and DC-mediated CD4 and CD8 T cell responses were examined specifically as the functional consequence of the ingestion of C1q-coated human autologous ACs (as opposed to C1q immobilized on a plate [15] or by use of transformed cell lines as the source of ACs). Finally, our novel, entirely primary human cell system provides a better approximation of the effects of C1q on T cells found in tissue (e.g., skin, liver, spleen, kidneys, etc.) where C1q can be secreted by myeloid cells in the absence of the enzymatic C1r and C1s subcomponents of C1 and thus in the absence of activation of the complement cascade [13, 83]. Therefore, C1q would be present during the phagocytosis of ACs by these cells. As the tissue can become the site of inflammation in autoimmune scenarios, the understanding of what regulates Mϕ and DC responses in tissues that ultimately influence T cell activation is critical.

In summary, the data presented here identify the ability of C1q alone to control the Mϕ- and DC-mediated T cell immune response in primary human cells to ACs, a physiologically relevant

self cargo. It is noteworthy that, whereas it has been long known that SLE may be initiated/propagated in the absence of C1q or in the case of reduced C1q function [84] in humans, our studies provide a pathway by which C1q may critically contribute to the regulation of the adaptive immune response and prevention of lupus-like autoimmunity in normal individuals. The novel combination of human peripheral blood-derived T cells, autologous APCs, and autologous ACs, with or without the addition of purified human C1q, is unique among studies approaching this topic and avoids the caveats in previously published studies performed in mice, with transformed/foreign cell lines and with nonphysiologic presentation of the C1q molecule. These new findings demonstrate that C1q bound to dying cells influences Mϕ and DC cytokine production, surface molecule expression, and subsequent T cell subset proliferation, sculpting the adaptive immune system to avoid autoimmunity and promote tolerance. It is noteworthy that these studies may lead to the identification of novel target pathways for therapeutic and/or preventative intervention in SLE and other autoimmune diseases.

AUTHORSHIP

E.V.C. designed and performed the study, collected and analyzed data, and wrote the paper. B.M.W. and C.M.W. designed the study and analyzed data. A.J.T. designed the study, analyzed data, and wrote the paper.

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DISCLOSURES

The authors declare no conflict of interest. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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