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C1q Modulates the Response to TLR7 Stimulation by Pristane-Primed Macrophages: Implications for Pristane-Induced Lupus

Francesco Carlucci,*,† Attia Ishaque,* Guang Sheng Ling,* Marta Szajna,* Ann Sandison,‡ Philippe Donatien,‡ H. Terence Cook,* and Marina Botto*

The complement component C1q is known to play a controversial role in the pathogenesis of systemic lupus erythematosus, but the underlying mechanisms remain poorly understood. Intraperitoneal injection of pristane induces a lupus-like syndrome whose pathogenesis implicates the secretion of type I IFN by CD11b^{+} Ly6C^{high} inflammatory monocytes in a TLR7-dependent fashion. C1q was also shown to influence the secretion of IFN-α. In this study, we explored whether C1q deficiency could affect pristane-induced lupus. Surprisingly, C1qa$^{-/-}$ mice developed lower titers of circulating Abs and milder arthritis compared with the controls. In keeping with the clinical scores, 2 wk after pristane injection the peritoneal recruitment of CD11b^{+} Ly6C^{high} inflammatory monocytes in C1qa$^{-/-}$ mice was impaired. Furthermore, C1q-deficient pristane-primed resident peritoneal macrophages secreted significantly less CCL3, CCL2, CXCL1, and IL-6 when stimulated in vitro with TLR7 ligand. Replenishing C1q in vivo during the pristane-priming phase rectified this defect. Conversely, pristane-primed macrophages from C3-deficient mice did not show impaired cytokine production. These findings demonstrate that C1q deficiency impairs the TLR7-dependent chemokine production by pristane-primed peritoneal macrophages and suggest that C1q, and not C3, is involved in the handling of pristane by phagocytic cells, which is required to trigger disease in this model. *The Journal of Immunology*, **2016, 196: 1488–1494.**

The role of complement in the pathogenesis of systemic lupus erythematosus (SLE) has been investigated for decades with controversial results (1). Paradoxically, lack of one of the early components of the classical pathway, especially C1q, is strongly associated with the development of SLE, yet complement activation supports tissue injury. Over the years, gene-targeted mice lacking C1q have helped to decipher the different roles of C1q in SLE and uncovered the consequences of impaired clearance of apoptotic cells in the disease pathogenesis (2). This observation, together with other studies showing that C1q can bind directly to dying cells (3, 4), provided support for C1q-dependent and complement activation–independent roles for this molecule. However, there is no consensus on the receptor(s) mediating these functions, and conflicting data in the literature would suggest that the C1q-mediated effects vary, according to which receptor/signaling pathway is coengaged (5). More recently, an association between C1q deficiency and a defective regulation of IFN-α was reported (6, 7). Although each of these elegant studies proposes a different mechanism by which C1q exerts its inhibitory effect on the release of IFN-α by plasmacytoid dendritic cells, this potential link is of great clinical relevance, particularly considering the growing evidence that most SLE patients have been exposed to type I IFN (IFN signature) and that type I IFN levels correlate with disease activity.

Pristane (2,6,10,14-tetramethylpentadecane) is a naturally occurring hydrocarbon oil derived from the metabolism of phytol. Intraperitoneal injection of pristane in BALB/c mice results in a chronic inflammatory state and in the formation of lipogranulomas in the peritoneum. Injected mice develop a type I IFN inflammatory response that is already evident at 2 wk after the administration; approximately 4–6 mo later, they produce high titers of autoantibodies, primarily anti-small nuclear ribonucleoprotein (snRNP). Other clinical manifestations include immune complex–mediated glomerulonephritis (GN), pulmonary vasculitis, and arthritis (8). The spectrum of disease manifestations is strongly dependent on the genetic background of the animals: for example, C57BL/6 mice are extremely susceptible to the pulmonary involvement, whereas BALB/c mice are resistant (9). Therefore, this experimental model, known as pristane-induced lupus (PIL), appears to recapitulate many key immunological features of human SLE. Resident peritoneal phagocytes are the first cells to encounter and engulf pristane (10); as a result of this interaction, cytokines/chemokines are released, and there is accumulation of inflammatory Ly6C^{high} monocytes in the peritoneum. This event is not limited to pristane, because treatment with mineral oil induces initially the same phenotype. However, only in the presence of pristane Ly6C^{high} monocytes persist, do not mature, and produce type I IFN before undergoing apoptosis. As a consequence, a unique accumulation of inflammatory monocytes is maintained and drives the autoimmune manifestations (8). Of note, studies by

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Abbreviations used in this article: BM, bone marrow; GN, glomerulonephritis; hC1q, human C1q; PB, peripheral blood; PEC, peritoneal exudate cell; PIL, pristane-induced lupus; PL, peritoneal lavage; poly(I:C), polyinosinic-polycytidylic acid; SLE, systemic lupus erythematosus; snRNP, small nuclear ribonucleoprotein.

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Reeves and colleagues (11) showed that pristane induces CCL2 expression and enhances TLR7 stimulation in vitro. Moreover, it was demonstrated that the TLR7-MyD88 pathway is the only pathway essential for the development of PIL (12).

In view of the strong association between C1q deficiency and SLE, as well as the proposed, direct or indirect, inhibitory effect of C1q on IFN-α secretion (6, 7), we hypothesized that C1q deficiency would exacerbate the type I IFN inflammatory response triggered by pristane and the subsequent lupus-like disease. Unexpectedly, we found that C1q-deficient mice on the BALB/c genetic background developed fewer autoantibodies and a milder arthritis than did the BALB/c controls. Consistent with this, we discovered a significantly impaired recruitment of Ly6C^{high} inflammatory monocytes to the peritoneum in the absence of C1q. A plausible explanation for this impaired recruitment is the defective cytokine response of the pristane-primed C1q-deficient peritoneal macrophages to TLR7 stimulation. Taken together, these observations demonstrate that C1q is involved in the pristane-mediated enhanced inflammatory response to TLR7 stimulation.

Materials and Methods

Reagents

Sterile pristane was obtained from Sigma-Aldrich (Dorset, U.K.). Ags used in the ELISA assays were 100 μg/ml herring sperm DNA (Promega) for anti-dsDNA Ab; 2 μg/ml RNP A (Arotec Diagnostic) for anti-snRNP Ab; 5 μg/ml chromatin for anti-chromatin Ab, and 5 μ/ml histone (Roche) for anti-histone Ab. Bound Abs were detected with alkaline phosphatase–conjugated goat anti-mouse IgG (γ-chain specific; Sigma-Aldrich). The TLR ligands LPS (serotype O111:B4), polyinosinic-polycytidylic acid [poly(I:C)], CpG ODN 1826, and gardiquimod were purchased from Source Bioscience (Nottingham, U.K.). LPS was from *Escherichia coli* 0111:B4 (Sigma-Aldrich).

Animals and pristane treatment

BALB/c.C1qa$^{-/-}$, C57BL/6.C1qa$^{-/-}$, and BALB/c.C3$^{-/-}$ mice were generated as previously reported (2, 13) and backcrossed onto the BALB/c or C57BL/6 genetic background for >10 generations. Only female mice were used, and sex/age-matched BALB/c and C57BL/6 mice were purchased from Harlan (Blackthorn, U.K.). Animals were kept under specific pathogen–free conditions, and all animal care and procedures were conducted according to institutional and national guidelines. At 2 mo of age, mice were injected once i.p. with 500 μl pristane and monitored for the following 7 mo. In a separate set of experiments, mice were humanely culled either 16 h or 2 wk after the pristane treatment. In the in vivo C1q-reconstitution experiments, mice were injected i.p. with 500 μl pristane and human C1q (hC1q; Hycult Biotech, 500 μg) and culled 16 h later. Pristane-elicited peritoneal exudate cells (PECs) were harvested by lavage with HBSS containing 5 mM EDTA (both from Life Technologies, Paisley, U.K.). Peripheral blood (PB) was diluted 1:1 with 5% EDTA and washed with cold PBS. After depletion of RBCs using RBC lysis buffer (Tris base plus NH_4Cl), cells were counted and analyzed by flow cytometry.

Abs and cytokines

Mice were bled at regular intervals before and after pristane injection, and autoantibody assays were conducted at the same time on stored serum samples. Anti-dsDNA, anti-snRNP, anti-chromatin, and anti-histone IgG Abs were measured by ELISA, as previously described (14). The results were expressed in arbitrary ELISA units relative to a standard positive sample derived from a serum pool from MRL/Mp.*lpr/lpr* mice. Cytokines were measured using a bead multiplex assay (eBioscience, San Diego, CA), according to the manufacturer's instructions. Data were acquired using a BD FACSVerse flow cytometer (BD Biosciences, San Jose, CA) and analyzed using BMS FlowCytomix Pro Software, Version 3.0 (eBioscience). CCL2, CCL3, CXCL1, and IL-6 levels were confirmed by ELISA using commercial kits (DuoSet from R&D Systems for CCL3 and CXCL1; eBioscience for CCL2 and IL-6), according to the protocol provided by the company.

Flow cytometry

Flow cytometry was performed using a three- or four-color staining protocol and analyzed with a BD FACSVerse (BD Biosciences, San Jose, CA). The following Abs were used: anti–Ly-6G (RB6-8C5), anti-CD115 (AFS98), anti–Ly-6C (HK1.4), anti–Ly-6A/E (D7), anti-CD11a (M17/4), anti-CD11b (M1-70), anti-F4/80 (BM8), anti–I-A (M5/114.15.2), anti-CXCR4 (2B11), anti-CCR2 (475301), anti-CD80 (B7-1), and anti-CD86 (B7-2). Abs were purchased from BD Biosciences Pharmingen (San Diego, CA), Abcam (Cambridge, U.K.), and Vector Laboratories (Peterborough, U.K.). Staining was performed in the presence of a saturating concentration of 2.4G2 mAb (anti-FcγRII/III). Data were analyzed using FlowJo software, version 6.4 (TreeStar, Ashland, OR), using gating strategies as previously described (11).

In vitro assays

Pristane-elicited PECs were harvested 16 h after the injection and seeded in 24-well plates at 10^6 cells/well. After 2 h of incubation, nonadherent cells were removed by washing, and a similar amount of adherent cells was assessed by CellTiter-Blue (Promega) (15). Adherent macrophages were cultured in DMEM supplemented with 100 U/ml penicillin/streptomycin (both from Life Technologies) and 10% (v/v) heat-inactivated FCS in the presence or absence of TLR ligands [poly(I:C) at 10 μg/ml, LPS at 1 μg/ml, gardiquimod at 1 μg/ml, CpG ODN 1826 at 100 nM]. In the in vitro reconstitution experiments, hC1q was added at different doses (ranging from 250 to 50 μg/ml) concomitantly with gardiquimod (1 μg/ml). Supernatants were collected after 24 h and stored at −80°C until analysis.

Histology

Kidneys were fixed in Bouin's solution and processed to be embedded in paraffin; sections were stained with periodic acid-Schiff. GN was scored blindly as previously described (14). Right and left hind paws, collected at the time of culling, were fixed in 10% buffered formalin, decalcified in formic acid, processed, and embedded in paraffin wax. Serial sections (3.5 μm) were cut and stained with H&E for conventional histology. Both tarsal and interphalangeal joints were analyzed where present. Joints were scored based on the presence or absence of inflammatory cell infiltrates (defined as aggregates of lymphocytes with or without polymorph neutrophils, score 0–3), as well as on the presence of degenerative changes in articular cartilage, including erosion (defined as irregularity of the cartilage outline with or without reactive changes in subchondral bone evidenced by active bone remodeling, score 0–3) (16). Histological analyses were performed by experienced histopathologists who were blinded to the experimental detail.

Statistical analysis

The unpaired *t* test or the Mann–Whitney *U* test was applied, as indicated, with differences considered significant for p values < 0.05. Outliers were identified and excluded from analysis using the Grubb's test at $\alpha = 0.05$. Survival was compared using the log-rank (Mantel–Cox) test. Graphs were constructed and statistical analyses were performed using Prism software (version 6.0; GraphPad, San Diego, CA).

Results

C1q deficiency delays and dampens the autoimmune effects triggered by pristane

To investigate the role of C1q in the pathogenesis of PIL, 2-mo-old BALB/c ($n = 8$) and BALB/c.C1qa$^{-/-}$ ($n = 7$) female mice were injected i.p. with pristane and monitored for the next 7 mo. As previously described (17), wild-type animals developed anti-snRNP IgG starting from 3 mo posttreatment. Surprisingly, BALB/c.C1qa$^{-/-}$ mice displayed a delayed production of this particular class of autoantibodies, with significantly lower titers at 4 mo posttreatment. However, at later time points, this difference became less pronounced, and the titers of anti-snRNP IgG were similar to wild-type animals. Conversely, anti-dsDNA, anti-chromatin, and anti-histone Ab titers were comparable between the two groups at all time points (Fig. 1A). The slightly milder serological abnormalities of the BALB/c.C1qa$^{-/-}$ mice did not affect the survival rate of these animals compared with BALB/c mice ($p = 0.270$) (Fig. 1B). Renal histology assessed at the endpoint revealed GN scores that were not significantly different between the two experimental groups (Fig. 1C).

Pristane also triggers joint inflammation that tends to be less aggressive compared with other murine models of rheumatoid

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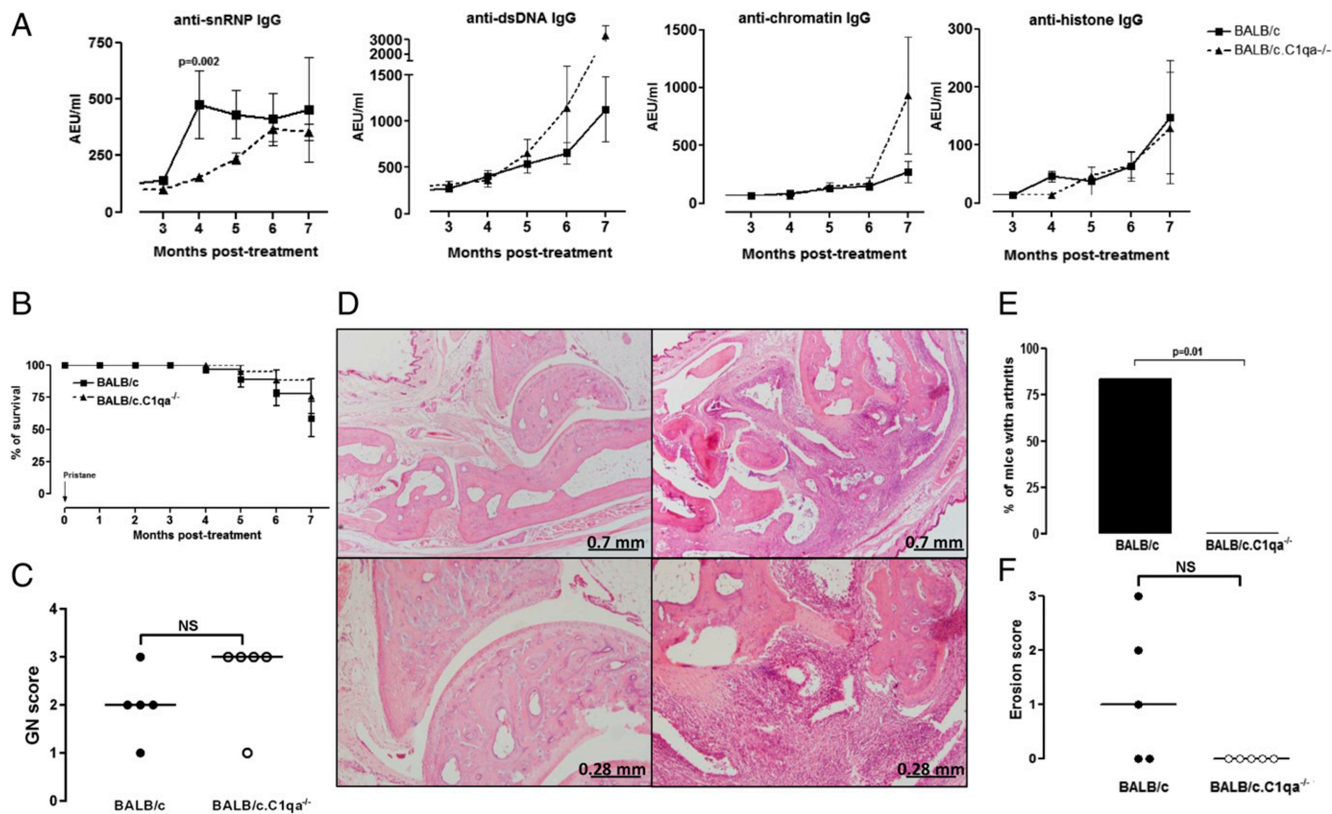

FIGURE 1. PIL in C1q-deficient mice. (**A**) Titers of anti-snRNP, anti-dsDNA, anti-chromatin, and anti-histone IgG at different time points after pristane treatment. Results are expressed as arbitrary ELISA units. All samples were tested at the same time. Data are mean ± SEM. The unpaired t test was calculated at each time point. Only significant p values are indicated. BALB/c (n = 8), BALB/c.C1qa$^{-/-}$ (n = 7) at time 0. (**B**) Survival rate after pristane injection. p = 0.27, log-rank test. (**C**) GN score 7 mo after pristane injection. Horizontal line represents the median. Statistical analysis by Mann–Whitney U test. (**D**) Representative histological sections of tarsal hind paw joints showing normal appearance (*left panels*, C1qa$^{-/-}$ sample) and severe inflammatory infiltrate and bone loss (*right panels*, BALB/c sample). (**E**) Assessment of arthritis at 6 mo postpristane. Mice were classified as positive or negative for arthritis, according to the presence of swelling and redness of at least one hind paw joint. BALB/c (n = 6), BALB/c.C1qa$^{-/-}$ (n = 6). The Fisher exact test was used to calculate the p value. (**F**) Histology scores of pristane-induced arthritis of the tarsal hind paw joints. Erosion score (0–3) as described in Inglis et al. (16). Horizontal line represents the median. Statistical analysis by Mann–Whitney U test.

arthritis. We observed obvious signs of swelling and redness of the hind paw joints 6 mo after pristane injection, a relatively late time point compared with other literature reports (18). Mice were classified blindly in a binomial fashion as positive or negative for arthritis on the basis of at least one hind paw showing swelling and redness of the toes or ankles. Based on this clinical assessment, none of the BALB/c.C1qa$^{-/-}$ mice had noticeable signs of joint inflammation, whereas 75% of the controls had clinical evidence of arthritis (Fig. 1E). Histological assessment at the time of culling showed normal interphalangeal joints but inflammatory infiltrates and bone erosion in the tarsal joints (Fig. 1D, *right panels*). Although histological scores were not statistically different between the two experimental groups, none of the C1qa$^{-/-}$ mice showed signs of bone erosion (Fig. 1F). Collectively, our observations showed that the lack of C1q had an unexpected ameliorating effect on the autoimmune clinical manifestations triggered by pristane, indicating that C1q might contribute to the immunological events mediated by this hydrocarbon oil.

C1q deficiency alters the composition of the pristane-induced peritoneal exudate

Previous reports showed that i.p. injection of pristane maintains the influx of inflammatory monocytes, defined as CD11b$^+$Ly6C^{high}, which drives autoantibody production by secreting type I IFNs in a TLR7-dependent fashion (11). To explore the mechanisms by which C1q deficiency dampens the PIL model, we assessed the cellular composition of bone marrow (BM), PB, and peritoneal lavage (PL), by flow cytometry, 16 h and 2 wk after the i.p. administration of pristane. We first confirmed that the absence of C1q had no effect on the number of CD11b$^+$Ly6C^{high} monocytes present in the BM and PB of untreated mice. As previously reported (11), we also found no CD11b$^+$Ly6C^{high} monocytes in the PL (data not shown). Sixteen hours after the i.p. injection of pristane, the total number of cells and the percentage of CD11b$^+$Ly6C^{high} monocytes in the BM, PB, and PL remained comparable between the two experimental groups. Of note, at this time point posttreatment, the percentage of peritoneal CD11b$^+$Ly6C^{high} monocytes increased to ~7% of PECs in both experimental groups (Fig. 2A). Furthermore, the expression of CCR2 and CXCR4, chemokine receptors crucial for the egress of CD11b$^+$Ly6C^{high} monocytes from the BM and their migration into the inflamed peritoneum (19), was not affected by the lack of C1q (Supplemental Fig. 1A). Similarly, the surface expression of the adhesion molecule LFA-1 on the PB monocytes did not differ between C1q-deficient and wild-type mice (Supplemental Fig. 1A). In contrast, 2 wk after the treatment, the proportions of CD11b$^+$Ly6C^{high} monocytes in the PB and PL of C1q-deficient animals were distinctly different from those in wild-type animals. In the mice lacking C1q, the percentage of circulating CD11b$^+$Ly6C^{high} monocytes was significantly higher, whereas the PL was hypocellular as the result of a marked decrease in the proportion of inflammatory CD11b$^+$Ly6C^{high} monocytes (Fig. 2B). Impaired

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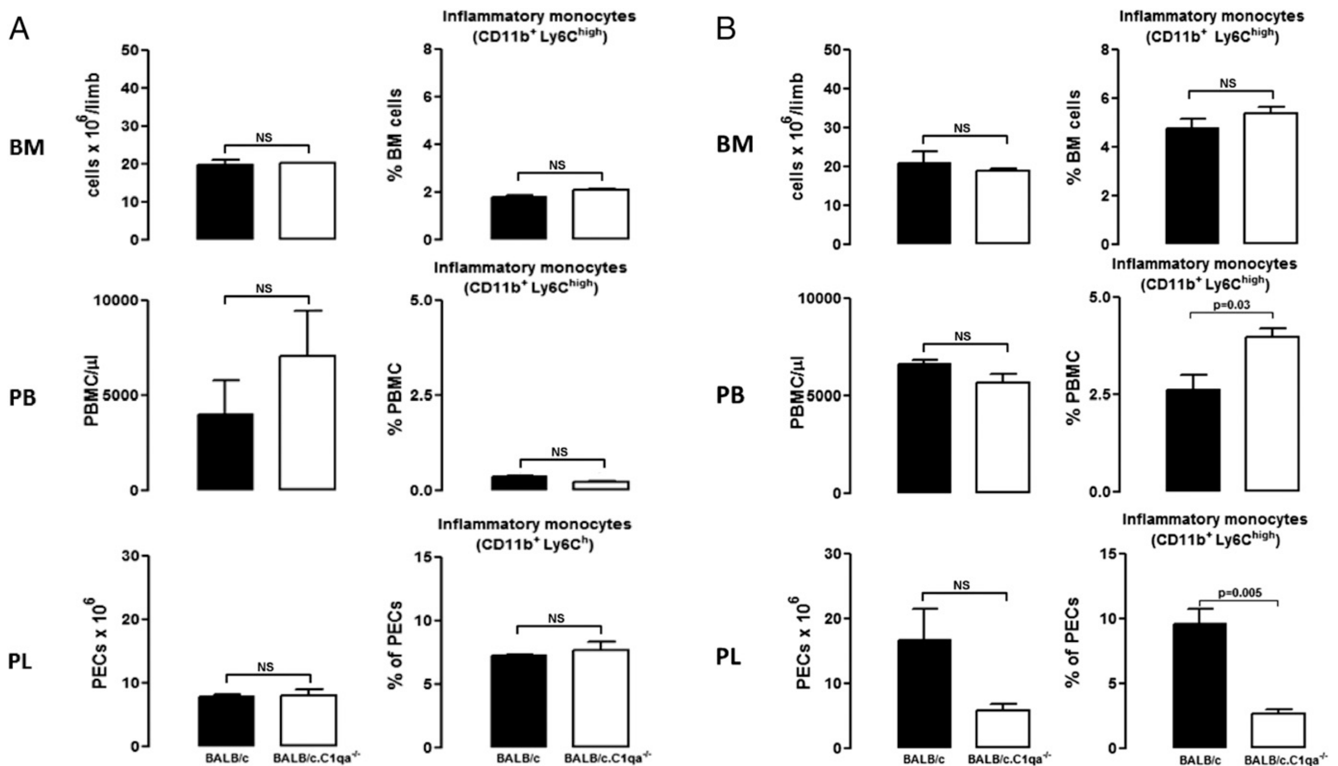

FIGURE 2. Distribution of CD11b$^+$Ly6C^{high} monocytes in BM, PB, and PL after pristane injection. BALB/c and BALB/c.C1qa$^{-/-}$ mice were culled 16 h (**A**) and 2 wk (**B**) after treatment. Cellular composition of BM, PB, and PL was analyzed by flow cytometry. The data are representative of three independent experiments (n = 3 mice/group). Data are mean ± SEM. The unpaired t test was used to calculate p values (significant at $p < 0.05$).

peritoneal recruitment was also observed using C1q-deficient mice on the C57BL/6 background, indicating that the defect was unrelated to the genetic background of the strain (Supplemental Fig. 2A). These findings suggest that peritoneal cell recruitment triggered by pristane is impaired in the absence of C1q. The difference was evident only at the later time point, indicating that C1q may influence the influx of CD11b$^+$Ly6C^{high} monocytes induced by the resident peritoneal macrophages as result of their interaction with pristane.

Impaired cytokine secretion by pristane-primed peritoneal macrophages in the absence of C1q but not C3

Resident peritoneal macrophages are the first cells to sense pristane after the i.p. injection (10). To dissect the mechanism(s) underlying the impaired ingress of the CD11b$^+$Ly6C^{high} monocytes into the peritoneum of C1q-deficient mice, we investigated the response of peritoneal macrophages primed in vivo with pristane to TLR stimulation in vitro. Because some models of sterile peritonitis depend on complement activation (20), we initially analyzed, in detail, the PL harvested 16 h postpristane treatment. This analysis failed to reveal any significant difference in the myeloid cell composition of the lavage fluid or the percentage of F4/80$^+$ CD11b$^+$ macrophages recovered from the pristane-treated BALB/c.C1qa$^{-/-}$ mice (Supplemental Fig. 1B). Furthermore, equivalent levels of MHC class II, CD86, and Sca-1 were expressed by C1qa$^{-/-}$ macrophages compared with their wild-type counterparts, indicating a similar status of activation (Supplemental Fig. 1C). Similar amounts of pristane-elicited peritoneal macrophages were then stimulated in vitro for 24 h with the TLR7 ligand gardiquimod, a costimulation that was shown to be required for cytokine/chemokine secretion and the development of PIL (12). Under these experimental conditions, we found that pristane-elicited BALB/c.C1qa$^{-/-}$ macrophages produced significantly less CCL3, CCL2, CXCL1, and IL-6 in response to gardiquimod compared with the wild-type macrophages (Fig. 3A). Reduced CCL2 production was also observed using pristane-primed peritoneal macrophage from C57BL/6.C1qa$^{-/-}$ animals (Supplemental Fig. 2B). Furthermore, the cytokine response of pristane-primed BALB/c.C1qa$^{-/-}$ macrophages to other TLR ligands, including TLR4, TLR9, and TLR3, was not impaired, indicating a specific defect in the TLR7 pathway following pristane stimulation (Fig. 4). Of note, these differences were detectable only with pristane-primed macrophages, because untreated resident peritoneal macrophages from BALB/c.C1qa$^{-/-}$ and BALB/c animals secreted equivalent amounts of cytokines/chemokines after stimulation with TLR ligands, including gardiquimod (Supplemental Fig. 3). Furthermore, the mRNA expression of *Ifn-α*, *Ifn-β*, and the IFN-induced gene *Mx1* in the pristane-primed macrophages was not affected by C1q deficiency. When we checked for TLR7 expression in the cells used in these experiments, we also found that the lack of C1q was not affecting receptor expression in itself (Supplemental Fig. 4).

The finding that C1q was required for the cytokine/chemokine secretion by pristane-elicited macrophages raised the question of whether this effect was mediated by C1q in a complement-independent or complement-dependent manner. To address this point, we tested the cytokine/chemokine response of pristane-primed BALB/c.C3$^{-/-}$ macrophages to gardiquimod. In contrast to observations with pristane-primed BALB/c.C1qa$^{-/-}$ macrophages, we found that, in the absence of C3, the production of cytokines/chemokines by pristane-elicited macrophages after TLR7 stimulation was not impaired (data not shown). We then attempted to rectify the impaired cytokine secretion from pristane-elicited BALB/c.C1qa$^{-/-}$ macrophages by reconstituting C1q with hC1q in vivo concomitantly with pristane or in vitro after the macrophages had been primed. Interestingly, the addition of hC1q in vivo restored the

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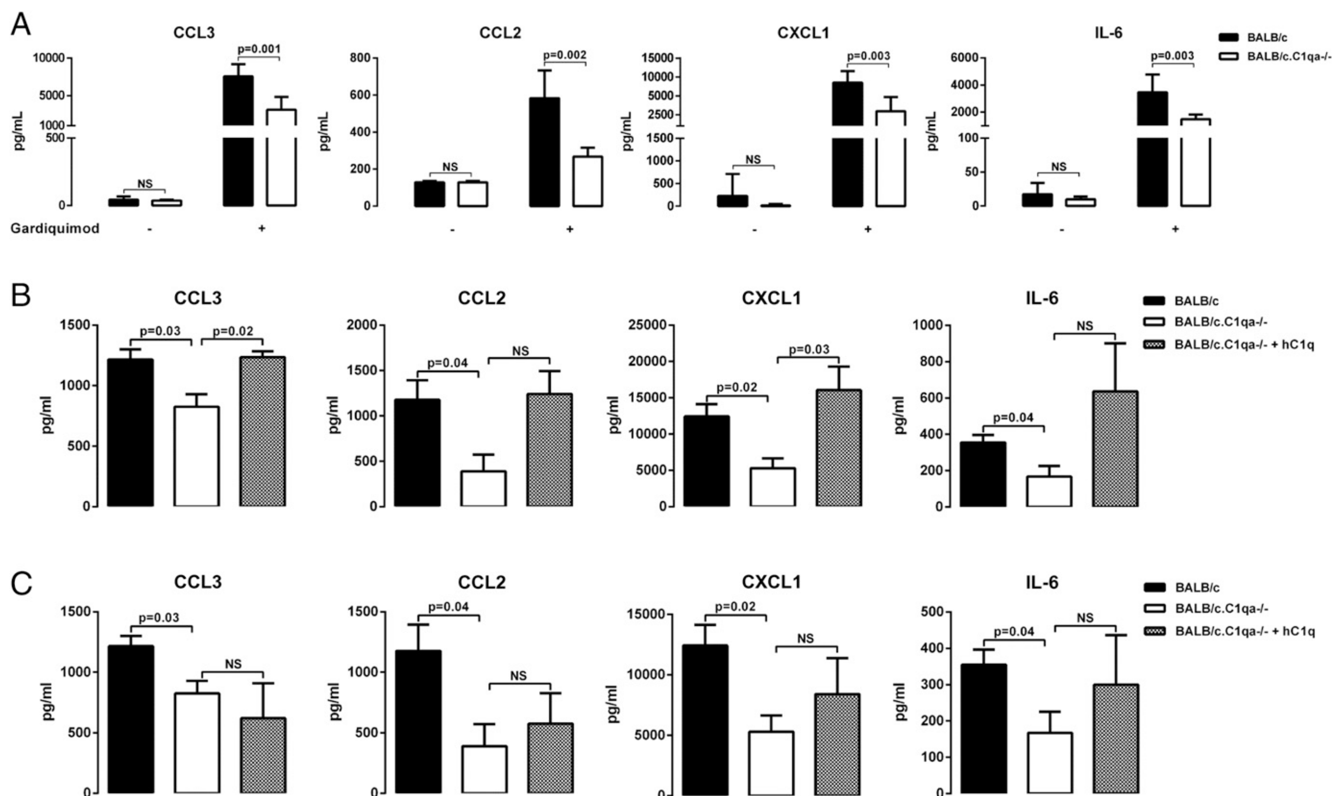

FIGURE 3. Chemokine/cytokine production by pristane-primed macrophages stimulated with gardiquimod. (**A**) Pristane-primed macrophages from BALB/c and BALB/c.C1qa$^{-/-}$ mice were treated or not with TLR7 ligand (gardiquimod, 1 μg/ml) for 24 h, and the production of chemokines/cytokines was measured by ELISA. Data shown are pooled from three independent experiments (total *n* = 9/group). (**B**) BALB/c.C1qa$^{-/-}$ mice (*n* = 3/group) were given 500 μg of hC1q or PBS i.p.; they received pristane 5 min later. Control BALB/c mice were treated with pristane only. Production of chemokines/cytokines by pristane-primed macrophages stimulated in vitro with gardiquimod, as described in (A). (**C**) Pristane-primed macrophages from BALB/c.C1qa$^{-/-}$ mice (*n* = 3/group) were treated in vitro for 24 h with gardiquimod in the presence or absence of 250 μg/ml of hC1q. Production of chemokines/cytokines was measured 24 h later. Data are mean ± SEM. The unpaired *t* test was used to calculate *p* values (significant at $p < 0.05$).

production of CCL3 and CXCL1 (Fig. 3B), whereas the addition in vitro to pristane-primed cells failed to change the cytokine/chemokine levels (Fig. 3C). Taken together, these findings demonstrate that C1q modulates the pristane-induced peritoneal recruitment of circulating CD11b^{+}Ly6C^{high} monocytes by altering the TLR7 signaling pathway in the resident macrophages, and this effect is independent of complement activation beyond C1, C4, or C2.

Discussion

PIL is a widely used experimental model of SLE that recapitulates key immunological and clinical features of the disease in humans, including the type I IFN signature. We investigated the role of complement in this model of autoimmunity and demonstrated that the lack of C1q, but not C3, decreases the cytokine/chemokine response to TLR7 stimulation in pristane-primed peritoneal macrophages. This appeared to be independent from the mouse genetic background, because it was reproducible using Th1 and Th2 mouse strains. As a result, in the absence of C1q, the peritoneal recruitment of CD11b^{+}Ly6C^{high} inflammatory monocytes, the main source of IFN-α (11), was impaired, and the subsequent development of PIL was delayed and mildly ameliorated. These findings support the notion of an in vivo role for C1q in mediating the interaction of pristane with the resident peritoneal macrophages and the TLR7 signaling pathway. Of note, inhibition of TLR7 signaling in macrophages was shown to attenuate kidney and lung tissue injury in experimental lupus (21).

The observations that C1q opsonizes apoptotic debris to prevent SLE (3, 4), as well as suppresses IFN-α production by plasmacytoid dendritic cells (7, 22), led us to hypothesize that, in the absence of this molecule, the autoimmune response induced by pristane, which is a powerful inducer of both processes, would be augmented. However, contrary to our expectations, C1q-deficient mice displayed a milder phenotype, with a delayed onset in the production of autoantibodies and a trend toward a less aggressive arthritis. Because CD11b^{+}Ly6C^{high} inflammatory monocytes were shown to be the predominant source of IFN-α in pristane-treated mice (11), we investigated whether C1q could affect the migration or differentiation of these cells, as described in other experimental conditions (23, 24). Interestingly, in C1q-deficient animals, we found that the Ly6C^{high} monocyte population remained significantly higher in circulation 2 wk after treatment, whereas the accumulation of these cells in the peritoneal cavity was reduced. These findings strongly indicated an impaired recruitment of Ly6C^{high} monocytes from the PB into the peritoneum and not a defect in the egression of these cells from the BM, as reported in transcription factor IFN regulatory factor 5–deficient mice (25). Consistent with this, the surface expression of CCR2, CXCR4, and LFA-1 on the inflammatory monocytes of pristane-treated C1q-deficient mice was equivalent to that in C1q-sufficient animals, making a defect in this pathway unlikely (Supplemental Fig. 1A). This raised the possibility of a defect in the production of CCL2, a chemokine elicited by pristane treatment and responsible for driving the recruitment of Ly6C^{high} monocytes (11). As previously documented (12), we found that overnight exposure to pristane enhanced the response of macrophages to subsequent stimulation with a TLR7 ligand (gardiquimod). More

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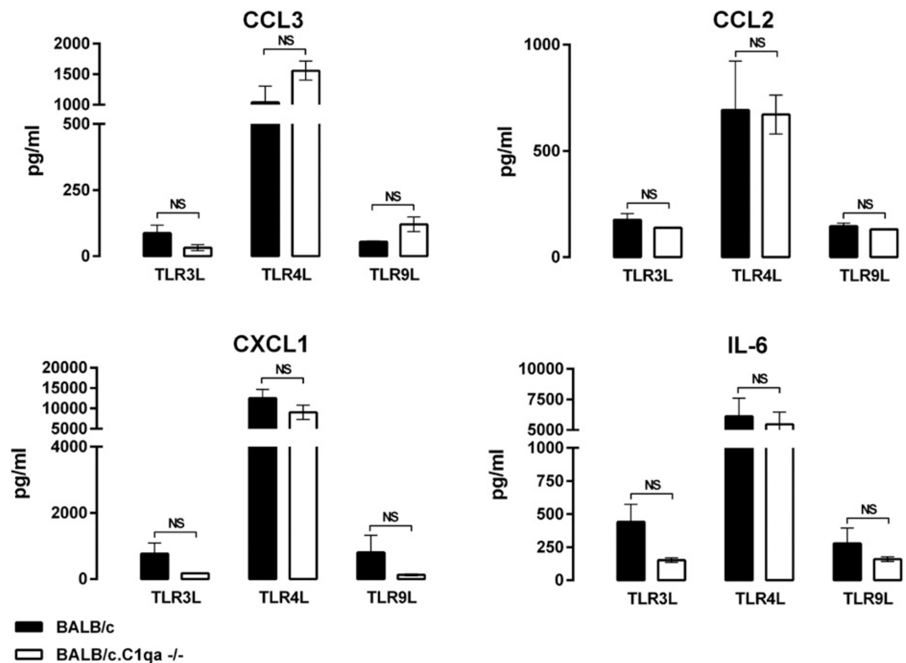

FIGURE 4. Chemokine/cytokine production by pristane-primed macrophages stimulated with TLR ligands. Chemokine/cytokine production by pristane-elicited macrophages after stimulation with TLR3, TLR4, or TLR9 ligand (TLR3L, TLR4L, TLR9L). Data are mean ± SEM pooled from three independent experiments (total n = 9/group). The unpaired t test was used to calculate p values (significant at $p < 0.05$). TLR3L, poly(I:C) (10 μg/ml); TLR4L, LPS (1 μg/ml); TLR9L, CPG ODN 1826 (100 nM).

importantly, pristane-primed C1q-deficient peritoneal macrophages were significantly less responsive to TLR7 stimulation and produced markedly less CCL2, CCL3, CXCL1, and IL-6, despite similar TLR7 expression. The defective secretion of these chemokines/cytokine provided a plausible explanation for the impaired recruitment of Ly6C^{high} monocytes observed in mice lacking C1q. Interestingly, the mRNA expression of type I IFNs and the IFN-inducible gene *Mx1* in pristane-primed macrophages was not affected by the lack of C1q, indicating that C1q does not affect the IFN-α pathway in these cells. Our findings were specific to TLR7-mediated signaling, because treatment of the pristane-primed macrophages with other TLR ligands induced equivalent cytokine/chemokine production. This is not surprising if one considers that pristane can induce apoptosis and the release of U1 snRNP complexes that are known to be TLR7 ligands (8). Thus, the TLR7 signaling axis is already primed in pristane-elicited macrophages, and the subsequent stimulation in vitro with gardiquimod may just help to boost this pathway. In addition, we failed to uncover any abnormality in the response of resident C1q-deficient peritoneal macrophages to a wide range of TLR ligands, including gardiquimod, thus excluding an intrinsic defect in the complement-deficient cells. Intriguingly, the lack of C3 did not have any impact on the TLR7-mediated inflammatory response by pristane-primed macrophages, arguing against a potential involvement of complement receptors like CD11b/CD18 (CR3) in the interaction between pristane and phagocytic cells. Rather, our results support the notion of a complement-independent function/role for C1q, similar to the findings of other investigators using different experimental models (23). However, C1q appears to promote the inflammatory response in the PIL model, an effect that is very different from the anti-inflammatory response observed when C1q is bound to apoptotic cells (26).

Alkanes similar to pristane are membrane-active compounds that can interact with phospholipid bilayers, such as cell membranes; indeed, magnetic resonance studies showed that pristane is present in lipid bilayers (27). Nevertheless, in in vivo situations, the interaction of pristane with cell membranes, as with other hydrophobic materials, is likely to be mediated by plasma protein(s) coating the material. In this regard, C1q is known to remain bound to apoptotic cells during phagocytosis, to direct macrophage polarization (5), and to regulate the subsequent T cell response (26). In view of our findings, it is tempting to speculate that C1q may be one of those proteins that absorbs to foreign bodies and acts as an endogenous carrier molecule, facilitating pristane removal by phagocytic cells. Consistent with this notion, only the addition of hC1q in vivo, during the initial interaction with pristane, was able to reverse the impaired chemokine/cytokine secretion by pristane-elicited C1q-deficient macrophages, whereas its supplementation in vitro to pristane-primed macrophages failed to do so. Thus, further studies assessing the incorporation of pristane into lipid bilayers and its membrane distribution in the absence of C1q will be required to understand how this molecule may be involved in the cell response to hydrocarbons and endogenous ligands.

Pristane-induced arthritis is characterized by inflammatory cell infiltrates with marginal erosions and pannus formation. Under our experimental conditions, only wild-type mice developed erosions and bone remodeling of the tarsal joint; C1q-deficient animals showed a milder joint involvement. Although the histological score was not statistically different, most likely because the number of samples was very small, the clinical assessment supported the notion that C1q deficiency attenuated the tissue damage. Notably, in a study, 81% of pristane-injected BALB/c mice developed paw swelling, but only 58% presented clear histological evidence of arthritis (18), consistent with our findings. Because we observed reduced production of CCL2 and CCL3 by pristane-primed peritoneal macrophages lacking C1q, and these chemokines are known to be involved in the pathogenesis of inflammatory arthritis (28, 29), it is tempting to speculate that this may have had an indirect effect on inflammation in the joints of C1q-deficient animals. Nevertheless, because the arthritis in PIL is likely to be mediated by immune complexes, we cannot exclude that the milder phenotype observed in mice lacking C1q could also be due to decreased complement activation.

In summary, our report describes for the first time, to our knowledge, the consequence of C1q deficiency in the PIL model and demonstrates that C1q, and not C3, is required for the enhanced response to TLR7 stimulation in pristane-primed peritoneal macrophages. By hampering the inflammatory events

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mediated by the peritoneal macrophages, upstream of IFN-α production by inflammatory monocytes, C1q deficiency led to amelioration of the clinical features triggered by pristane. Taken together, our findings unveiled another complement-independent function of C1q and support the notion of a multifaceted role for this molecule in the regulation of autoimmunity and macrophage activation.

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Disclosures

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