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gC1q Receptor Ligation Selectively Down-Regulates Human IL-12 Production through Activation of the Phosphoinositide 3-Kinase Pathway

Stephen N. Waggoner,*† Michael W. Cruise,*† Rachel Kassel,*† and Young S. Hahn2*†‡

gC1qR, a complement receptor for C1q, plays a pivotal role in the regulation of inflammatory and antiviral T cell responses. Several pathogens, including hepatitis C virus, exploit gC1qR-dependent regulatory pathways to manipulate host immunity. However, the molecular mechanism(s) of gC1qR signaling involved in regulating inflammatory responses remains unknown. We report the selective inhibition of TLR4-induced IL-12 production after cross-linking of gC1qR on the surface of macrophages and dendritic cells. Suppression of IL-12 did not result from increased IL-10 or TGF-β, but was dependent on PI3K activation. Activation of PI3K and subsequent phosphorylation of Akt define an intracellular pathway mediating gC1qR signaling and cross-talk with TLR4 signaling. This is the first report to identify signaling pathways used by gC1qR-mediated immune suppression, and it establishes a means of complement-mediated immune suppression to inhibit Th1 immunity crucial for clearing pathogenic infection. The Journal of Immunology, 2005, 175: 4706–4714.

The complement system is an important component of innate immunity, directing the induction and differentiation of adaptive immunity during infection (1, 2). Therefore, components of this system are attractive targets for microbial manipulation of host immunity (3). C1q is the recognition component of C1 and initiates the classical complement cascade upon activation (4, 5). The globular heads of C1q are recognized by a cell surface receptor, gC1qR, which has been implicated in numerous cellular processes ranging from homeostasis to phagocytosis (6). Although the physiologic role of gC1qR in the immune response remains to be established, natural C1q deficiency in humans (7, 8) as well as targeted deletion in mice (9) have identified a role for this ligand in immune regulation and prevention of autoimmune disease. Furthermore, C1q binding to cells abrogates mitogen-induced proliferation of T lymphocytes (10) as well as IL-12 expression by dendritic cells (DCs) (11). These data suggest that gC1qR may play a regulatory role in the initiation of innate immune responses and subsequent activation of adaptive immunity.

The development of an efficient immune response against infectious pathogens relies upon initial contact with cells of the innate immune system, triggering the release of proinflammatory cytokines that play a decisive role in shaping the subsequent adaptive immune response. Early and sustained production of IL-12 by APCs, such as macrophages and DCs, provides a crucial bridge between innate and adaptive immunities (12). Bioactive IL-12p70, a heterodimer of the p35 and p40 subunits of IL-12, is a critical inducer of IFN-γ production by NK cells and T lymphocytes, thereby driving the differentiation of a Th1-biased immune environment important to the proper development of cell-mediated immunity. However, IL-12 production is tightly regulated to prevent the detrimental effects of uncontrolled overexpression of this inflammatory mediator (13). Therefore, macrophages and DCs possess an elaborate series of mechanisms controlling the production of IL-12. Several infectious microbes, including measles virus (14), HIV (15), and Legionella pneumophila (16), have evolved immune escape strategies by using these host-mediated schemes of IL-12 regulation. Recently, hepatitis C virus (HCV) core protein has also been shown to inhibit IL-12 production in human monocytes and macrophages (17, 18).

HCV is remarkable in its ability to establish life-long, persistent infection in >80% of patients, suggesting that HCV exerts one or more means of immune dysregulation (19). In fact, chronically infected patients exhibit diminished virus-specific CTL responses (20, 21) and a predominance of Th2 cytokines in the periphery early after infection (22, 23). The use of HCV core-transgenic mice (24) or core-expressing recombinant vaccinia (25) and adenoviruses (26) has demonstrated a suppressive function for core protein, characterized by inhibition of T lymphocyte proliferation and Th1 cytokine (i.e., IL-2 and IFN-γ) production. The inhibition of lymphocyte function by HCV core was also observed in human macrophages and T cells, in which suppression was shown to be dependent upon interaction of the extracellular core with gC1qR on the surface of these cells (18, 27). The recognition that an association between HCV core protein and gC1qR is essential for core-mediated immune suppression adds to a growing list of microbial pathogens, including measles virus (CD46) (14) and EBV (complement receptor type 2 (CR2)) (28), that usurp the complement system for immune modulation. More importantly, the gC1qR has been reported to interact with numerous viral and bacterial proteins, including Listeria monocytogenes internalin B (29) and Staphylococcus aureus protein A (30), suggesting that gC1qR may facilitate a shared mechanism of pathogen-mediated immune suppression (6, 31–33). However, the mechanism of gC1qR-mediated immune suppression remains to be elucidated.

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1 Abbreviations used in this paper: DC, dendritic cell; CR2, complement receptor type 2; β-gal, β-galactosidase; HCV, hepatitis C virus; MFI, mean fluorescence intensity; rh, recombinant human; DHFR, dihydrofolate reductase.
Interestingly, recent studies have indicated a role for the PI3K pathway in negative feedback suppression of TLR4 signaling induced by LPS (34, 35). Treatment of monocytes and macrophages with pharmacological inhibitors of PI3K, LY294002 and wortmannin, augments LPS-induced signaling and results in increased production of proinflammatory genes. Furthermore, PI3K-deficient mice exhibit an exaggerated Th1 cytokine response after in vitro stimulation with TLR ligands or after in vivo infection with *Leishmania major*. Therefore, activation of the PI3K pathway, including downstream mediators such as Akt/protein kinase B, may mediate critical regulation of IL-12 production and prevent harmful exaggerated cell-mediated immune activation (reviewed in Ref. 36).

In this study we report a role for the PI3K pathway in gC1qR-mediated cross-talk with TLR4 and suppression of innate immune function. The specific inhibition of IL-12p70 production by gC1qR in LPS-stimulated monocytes/macrophages and DCs was not mediated by known regulators of IL-12, such as IL-10 and TGF-β, suggesting that gC1qR elicits a novel mechanism of suppression. Indeed, induction of the PI3K pathway upon gC1qR ligation was crucial for the abrogation of TLR4-induced IL-12p70 expression. To our knowledge, this is the first evidence of suppressive signaling initiated by gC1qR and the first demonstration of pathogen- or complement-induced PI3K-dependent cross-talk with TLR signaling pathways.

Materials and Methods

Reagents

Recombinant β-galactosidase (β-gal)-fused core protein was obtained from Virogen, and purified human C1q was purchased from Advanced Researching pathways. To our knowledge, this is the first evidence of suppressive signal-

Generation of gC1qR-specific Abs

Mouse mAbs specific for gC1qR were generated by immobilizing mice with recombinant full-length GST-gC1qR and were screened for specificity us-

RT-PCR

RT-PCR was used to determine the levels of IL-12p40, TNF-α, IL-6, IL-8, and β-actin mRNA as described previously (18). Briefly, IFN-γ–primed monocytes and THP-1 macrophages were treated for various times with 1 μg/ml LPS in the presence of 1 μg/ml anti-gC1qR IgM or IgG or isotype control (BD Pharmingen) as well as soluble recombinant His6-gC1qR and His6-dihydrofolate reductase (DHFR) (produced and purified in our laboratory) (27) were also added at the time of LPS addition. In the case of inhibitors of PI3K, various doses of LY294002 and wortmannin were added to the cells 30 min before the addition of LPS and other stimuli at 37°C.

Cell supernatants were harvested 24 h (48 h for DCs) after stimulation with LPS. IL-12p70 and TGF-β (BD Pharmingen) as well as IL-10 and TNF-α (eBioscience) were quantified by specific ELISA according to the manufacturer’s instructions. Identically prepared cell supernatants were subjected to multicytokine analysis using cytometric bead assay (BD Pharmingen) as described by manufacturer.

Flow cytometry

THP-1, human monocytes, and monocyte-derived DCs were incubated for 16 h in the presence or the absence of 100 ng/ml rh-IFN-γ. Washed, fixed, and nonpermeabilized cells were incubated with mouse anti-gC1qR IgG1 mAb (3A4) or control mouse IgG1 (eBioscience) at 4°C, followed by incubation with FITC-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). Cells were washed a final time and analyzed by flow cytometry. Primary human cells were gated on CD14+ (monocyte/ macrophage) or CD11c+ (DCs) using Abs and isotype controls from eBioscience.

In the case of TLR4 staining, THP-1 were incubated for 24 h in the presence or the absence of 1 μg/ml anti-gC1qR IgM or IgG or isotype control. Cells were washed, fixed, and stained at 4°C with PE-conjugated anti-TLR4 or PE-conjugated isotype control from eBioscience. After wash-
ing, cells were collected on FACS Calibur (BD Biosciences) and analyzed using Flowjo (TreeStar). For intracellular determination of protein phos-

Cells and culture conditions

Human PBMC were isolated from healthy blood donors (Virginia Blood Services) by Lymphocyte gradient centrifugation (Cedarlane Laboratories), and monocytes were purified by adherence to polysulone as described previously (18). Adherent cells, typically >90% CD14+ by FACS analysis, were washed and cultured in RPMI 1640 supplemented with 10% FBS (HyClone), penicillin/streptomycin (100 μg/ml), l-glutamine (2 mM), and 2-ME (55 μM) at 37°C with 7% CO2 in a humidified atmosphere. Monocyte-derived DCs were prepared by incubation of adherence-purified monocytes in medium containing 1000 IU/ml rhGM-CSF and 1000 IU/ml rH-4 for 5 days, with fresh medium containing cytokines added every second day. THP-1 cells (American Type Culture Collection) were cul-
tured in RPMI 1640 supplemented as recommended by American Type Culture Collection.

Determination of cytokine production

Adhesion-isolated monocytes were primed in the presence of 100 ng/ml rhIFN-γ for 16 h, followed by the addition of various concentrations of LPS for 24 h at 37°C. Differentiation of THP-1 monocytes into macrophages was driven by incubation in complete medium containing 1% DMSO for 24 h at 37°C. In fresh medium containing 100 ng/ml rhIFN-γ, macrophages were primed for 16 h at 37°C before stimulation with 1 μg/ml LPS for an additional 24 h.

At the time of LPS stimulation, cells were also treated with varying amounts of a mouse anti-gC1qR mAb IgM or an isotype control. In other experiments, various concentrations of recombinant β-gal-fused HCV core protein, β-gal control protein, or IgG were used in place of Abs against gC1qR. Neutralizing anti-IL-10 mAb and isotype control (BD Pharmingen) as well as soluble recombinant His6-gC1qR and His6-dihydrofolate reductase (DHFR) (produced and purified in our laboratory) (27) were also added at the time of LPS addition. In the case of inhibitors of PI3K, various doses of LY294002 and wortmannin were added to the cells 30 min before the addition of LPS and other stimuli at 37°C.

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gC1qR regulates TLR4-induced IL-12 through PI3K activation

To determine whether direct binding of gC1qR by 5F4 mAb is responsible for selective suppression of IL-12 production, we examined the specific binding of cell surface gC1qR by 5F4 mAb.

RESULTS

gC1qR mAb (5F4) inhibits LPS-induced IL-12p70 production by human monocytes and DCs

G1C1qR is ubiquitously expressed on most cell types, with different cellular localization of cytoplasm and cell surface (6). However, because few studies of gC1qR function focused on cells of the myeloid lineage, we initially sought to characterize the expression of this receptor on human monocytes and DCs. Western blot analysis of THP-1, a human monocytic cell line, verified the abundant expression of gC1qR by monocytes (Fig. 1a). The gC1qR rabbit polyclonal, but not prebleed sera from the same animal, detected a single protein of a size corresponding to gC1qR (33 kDa), whereas the slightly larger HisgC1qR migrated more slowly. In addition, although substantial evidence supports cell surface localization of gC1qR, the gene for this receptor does not encode a glycosylphosphatidylinositol anchorage or transmembrane motif (39). To confirm the specific localization of gC1qR at the cell surface, we analyzed receptor expression by flow cytometry. Primary human monocytes (Fig. 1c) and THP-1 mononuclear cells (Fig. 1b) express high levels of cell surface-bound gC1qR. Monocyte-derived DCs also express high levels of gC1qR on the cell surface (Fig. 1d). Furthermore, stimulation of monocytes with IFN-γ resulted in a 2-fold increase in cell surface localization of gC1qR on THP-1 (Fig. 1b) and a 9-fold increase on monocytes (Fig. 1c). These data suggest that the cell surface expression of gC1qR on monocytes may be up-regulated in the context of a proinflammatory IFN response, thereby enhancing the ability of pathogens to bind and modulate innate immunity.

Although gC1qR has been reported to play a role in regulation of inflammatory responses, and the binding of HCV core to gC1qR led to inhibition of the production of IL-12 (17, 18), the direct role of gC1qR in this inhibition has yet to be determined. To examine the downstream signaling mechanism responsible for gC1qR-induced regulation of the inflammatory response, we screened a set of gC1qR-specific mAbs for the ability to ligate this receptor and inhibit IL-12p70 production (40). Therefore, we identified another anti-gC1qR IgG1 mAb (3A4) specific for gC1qR that strongly induced suppression of IL-12p70 production (data not shown). However, we did not further characterize this effect of 3A4 mAb to avoid the additive effect of FcγR-mediated suppression of IL-12 production (40). Therefore, the core/gC1qR interaction results in inhibition of IL-12p70 production by activated human monocytes/macrophages and DCs.

Specific binding of cell surface gC1qR by 5F4 mAb is responsible for selective suppression of IL-12 production

To determine whether direct binding of gC1qR by 5F4 mAb is responsible for inhibition of IL-12 production, we examined the...
ability of soluble gC1qR to reverse the suppression of IL-12 production by anti-gC1qR mAb (5F4). As shown in Fig. 2d, soluble recombinant His6-gC1qR (sgC1qR), but not an irrelevant control protein, His6-DHFR (sDHFR), was able to inhibit anti-gC1qR mAb-mediated suppression of IL-12 expression in human monocytes. In addition, HCV core-mediated inhibition of IL-12 production was reversed by addition of sgC1qR (data not shown). These data suggest that suppression induced by this Ab or core protein is mediated by ligation or cross-linking of gC1qR on the cell surface.

We next determined the effect of gC1qR ligation on the cytokine milieu expressed by LPS-stimulated monocytes. Despite profound suppression of IL-12p70 production by gC1qR cross-linking, LPS-induced production of TNF-α was not reduced in any of the PBMC donors examined (Fig. 3a). In fact, levels of TNF-α were elevated slightly upon ligation of gC1qR in some donors, but this difference was not statistically significant when PBMC from multiple donors were collectively analyzed (Fig. 3b). In addition, gC1qR cross-linking did not affect levels of IL-6, IL-8, and TNF-α in LPS-stimulated monocytes (Fig. 3c). These results suggest that gC1qR selectively inhibits the production of IL-12 through an impairment of the signaling molecule(s) responsible for IL-12 synthesis.

**FIGURE 2.** gC1qR mAb inhibits LPS-induced IL-12p70 production by human monocytes. a, Dose-dependent suppression of IL-12p70 production by anti-gC1qR mAb ligation of gC1qR on human monocytes. IFN-γ-primed primary human monocytes were stimulated with 100 ng/ml LPS in the presence of varying concentrations of cross-linking anti-gC1qR mAb (5F4) or isotype IgM control mAb for 24 h at 37°C before analysis of IL-12p70 production by ELISA. Suppression by anti-gC1qR mAb is reproducible in >10 independent experiments. b, gC1qR-mediated inhibition of IL-12 occurs across a wide range (0.1, 1, 10, 100, and 1000 ng/ml) of LPS concentrations. c, Effect of gC1qR cross-linking on inhibition of IL-12 in different healthy PBMC donors tested (n = 13). The dash and adjacent value represent mean IL-12p70 production by the multitude of donors tested. d, Reversal effect of soluble gC1qR on anti-gC1qR mAb-mediated inhibition of IL-12. IFN-γ-primed human monocytes were stimulated with 100 ng/ml LPS and either 1 μg/ml anti-gC1qR mAb or isotype control in the presence of various concentrations of soluble recombinant His6-gC1qR or unrelated control protein, His6-DHFR. IL-12p70 production was determined by ELISA after 24-h culture. e, gC1qR ligation results in suppression of IL-12p70 production by monocyte-derived DCs. Monocyte-derived DCs were stimulated for 48 h at 37°C with various concentrations of LPS in the presence of various concentrations of anti-gC1qR mAb or isotype control IgM. These data were reproducible in two independent experiments with DCs derived from two different donors. All data are shown as the mean ± SD of triplicate samples. *p < 0.001; **p < 0.01.
possible that increased expression of these anti-inflammatory cytokines is one mechanism by which gC1qR may suppress IL-12p70 production. To test this possibility, we assessed the effect of gC1qR cross-linking mAb on IL-10 and TGF-β production. As shown in Fig. 4, the levels of IL-10 (Fig. 4a) and TGF-β (Fig. 4b) produced by monocytes in response to LPS did not differ significantly in the presence or the absence of anti-gC1qR mAb cross-linking of gC1qR despite profound suppression of IL-12p70 production by those same cells. Furthermore, neutralizing anti-IL-10 Ab was unable to reverse the suppression of IL-12p70 by either the anti-gC1qR agonist Ab (Fig. 4c) or the HCV core (18). Collectively, these data reveal that gC1qR ligation results in suppression of LPS-induced IL-12p70 expression by a novel mechanism distinct from elevation of the anti-inflammatory mediators IL-10 and TGF-β.

FIGURE 3. Selective suppression of IL-12 by cross-linking anti-gC1qR mAb. a and b, gC1qR ligation does not affect LPS-induced production of TNF-α. Human monocyte supernatants, prepared as described in Fig. 2a, were analyzed for TNF-α production by ELISA. Data are the mean ± SD of triplicate samples, and differences were not statistically significant (p > 0.05). b, TNF-α production by IFN-γ-primed human monocytes (n = 5 donors) in response to 100 ng/ml LPS and in the presence of 1 μg/ml anti-gC1qR mAb or isotype control IgM. Each donor was analyzed in triplicate, and data are the mean of five donors ± SEM. c, Suppression of IL-12p70 by anti-gC1qR mAb is not mediated by IL-10. IFN-γ-primed monocytes were stimulated with 100 ng/ml LPS and either 1 μg/ml anti-gC1qR mAb or isotype control in the presence or the absence of 10 μg/ml neutralizing anti-IL-10 mAb or isotype control. Culture supernatants were analyzed by ELISA for IL-12p70. Data are the mean ± SD of triplicate samples and were reproducible in two independent experiments using PBMC from three different donors.

FIGURE 4. gC1qR-mediated suppression of IL-12p70 production is independent of IL-10 and TGF-β. a and b, gC1qR ligation does not affect IL-10 or TGF-β production by LPS-stimulated IFN-γ-primed monocytes (n = 5 donors) prepared as described in Fig. 2a. Each donor was analyzed in triplicate, and data are the mean of five donors ± SEM. c, Suppression of IL-12p70 by anti-gC1qR mAb is not mediated by IL-10. IFN-γ-primed monocytes were stimulated with 100 ng/ml LPS and either 1 μg/ml anti-gC1qR mAb or isotype control in the presence or the absence of 10 μg/ml neutralizing anti-IL-10 mAb or isotype control. Culture supernatants were analyzed by ELISA for IL-12p70. Data are the mean ± SD of triplicate samples and were reproducible in two independent experiments using PBMC from three different donors. d, Inhibition of IL-12p40 mRNA synthesis by gC1qR cross-linking. Total RNA was isolated from LPS-stimulated IFN-γ-primed monocytes after treatment with anti-gC1qR mAb or control Ab for 3 h. Levels of IL-12p40, TNF-α, IL-6, IL-8, and β-actin mRNA were analyzed by RT-PCR.
Bioactive IL-12 is composed of a heterodimer of the p35 and p40 subunits. The IL-12p40 subunit is shared with another IL-12 family member, IL-23, and plays an important role in regulation of IL-12p70 production. To examine whether gC1qR ligation alters IL-12p40 synthesis, we determined mRNA levels for various cytokines (IL-12p40, TNF-α, IL-6, and IL-8) in LPS-stimulated monocytes by RT-PCR analysis. Although cross-linking of gC1qR led to a defect in transcription of IL-12p40 mRNA (Fig. 4d), mRNA levels of TNF-α, IL-6, and IL-8 were not affected by gC1qR ligation (Fig. 4d). This suggests that gC1qR cross-linking selectively inhibits the synthesis of the IL-12p40 subunit.

**TLR4 expression is not affected by gC1qR ligation**

The down-regulation of TLR4 has been reported to be a mechanism for LPS tolerance induction (42). Therefore, modulation of TLR4 expression and abrogation of the LPS response could account for gC1qR-induced suppression of IL-12p70 in response to LPS. To test this possibility, we analyzed the cell surface expression of TLR4 as well as TLR4 mRNA levels on human monocytes. As shown in Fig. 5, protein and mRNA levels of TLR4 were maintained after gC1qR ligation. Levels of mRNA were analyzed and were found to be unaffected 3, 6, and 24 h after stimulation (data not shown). Thus, inhibition of IL-12p70 expression is not mediated by changes in TLR4 expression, but could result from interference with downstream TLR signaling.

**gC1qR-mediated suppression of IL-12 production is PI3K dependent**

Recent evidence indicates a role for the PI3K pathway in negative feedback of TLR signaling (34, 35). PI3K-deficient mice produce excessive amounts of IL-12 after in vitro stimulation with TLR ligands and in vivo infection with *Leishmania major* (35). To determine the signaling mediator(s) involved in gC1qR ligation, we assessed the role of the PI3K pathway in gC1qR-mediated suppression of IL-12p70 using two specific pharmacological inhibitors of PI3K activation, LY294002 (43) and wortmannin (44). Addition of increasing concentrations of these inhibitors resulted in gradual abrogation of anti-gC1qR mAb-induced suppression of IL-12p70 production (Fig. 6, a–c). Similar results were obtained in macrophages treated with HCV core protein (data not shown). Low concentrations of LY294002 (5 μM) and wortmannin (10 nM) were sufficient to reverse anti-gC1qR agonist-mediated suppression of IL-12p70 to levels similar to those in the isotype control. Consistent with previous results (11), the natural ligand for gC1qR, C1q, also suppressed IL-12p70 production (Fig. 6d). Importantly, suppression of IL-12p70 by C1q was inhibited by the addition of His₆-gC1qR or wortmannin, suggesting that the mechanism of C1q-mediated suppression of IL-12p70 production is indeed gC1qR and PI3K dependent.

To demonstrate the direct role of gC1qR ligation in activating PI3K, we determined the activation of PI3K in THP-1 cells using an in vitro assay for PI3K activity (Fig. 6e). Importantly, cross-linking of gC1qR by anti-gC1qR mAb (5F4) or binding with HCV core increased the activity of PI3K over baseline levels 8- and 12-fold, respectively. In addition, the inhibitory effects of wortmannin and LY294002 on PI3K activation were confirmed in this assay, as evidenced by 81 and 96% reductions in gC1qR-induced PI3K activity, respectively. These data suggest that the PI3K pathway is responsible for gC1qR-mediated suppression of IL-12p70 production.

**Akt is phosphorylated upon gC1qR ligation in a PI3K-dependent manner**

To further delineate the downstream signaling mediator(s) after gC1qR-induced PI3K activation, we analyzed the phosphorylation of Akt/protein kinase B in response to gC1qR ligation. Akt has been reported to be involved in PI3K pathway signaling (45). Cross-linking of gC1qR led to the increased phosphorylation of Akt on Ser₄73 as early as 30 min to as late as 6 h after stimulation (Fig. 7a). Maximal phosphorylation of Akt was reached 1 h after gC1qR mAb treatment. Importantly, abrogation of this gC1qR-induced phosphorylation by LY294002 and wortmannin indicates that activation of Akt is PI3K dependent.

Furthermore, intracellular flow cytometry staining of THP-1 after gC1qR ligation confirmed the rapid phosphorylation of Akt in response to gC1qR stimuli (Fig. 7b). The mean fluorescence intensity (MFI) of phospho-AktS₄₇₃ staining increased ~2-fold over resting (MFI, 10.7) or isotype IgM-stimulated (MFI, 11.0) levels after gC1qR cross-linking (MFI, 21.6), compared with a 1.5-fold increase after LPS stimulation (MFI, 14.7). In addition, LY294002 reduced phospho-AktS₄₇₃ staining by 76% (MFI, 5.2) to levels of Akt phosphorylation below those seen in resting cells, confirming that gC1qR-induced activation of Akt is PI3K dependent. Thus, PI3K-dependent activation of Akt represents an important mediator of gC1qR signaling and may play a significant role in suppression of IL-12p70.
Discussion

Although an in vivo function for gC1qR has not been well established, several in vitro studies suggest that gC1qR participates in multiple biological functions (6). C1q, the ligand for gC1qR, reportedly plays an important role in regulation of inflammation, including the prevention of autoimmune disease and inhibition of IL-12 production in murine DCs (11). Although these C1q-mediated regulatory effects have not been attributed to any particular C1q receptor, gC1qR is strongly implicated in the modulation of host defense by the identification of pathogen-derived proteins, including HCV core (27), as gC1qR binding partners. In this study we report that cross-linking of gC1qR with specific mAb or core protein initiates inhibitory signals that interfere with TLR4-induced proinflammatory signaling and subsequent IL-12 production. To our knowledge, this is the first study to delineate the downstream signaling mediators of gC1qR and identify PI3K-dependent cross-talk between complement and TLR signaling.

To determine the role of gC1qR in delivering the signal for regulation of inflammatory responses, we first attempted to identify a gC1qR-specific agonist Ab capable of cross-linking gC1qR and inhibiting IL-12 production. Indeed, one gC1qR mAb was capable of selective suppression of IL-12 production in a dose-dependent manner. In contrast, the production of other cytokines, TNF-α, IL-6, IL-10, and IL-8, was not affected by gC1qR ligation. Furthermore, competition for cell surface receptor with soluble gC1qR revealed that suppression by anti-gC1qR mAb was receptor specific. Our results showed that ligation of cell surface gC1qR with mAbs, such as HCV core (17, 18), could abrogate the production of IL-12p70 by human monocytes, macrophages, and DCs stimulated with LPS. The reduction in IL-12 biosynthesis was observed at both the protein and mRNA levels.

The inhibition of IL-12 production at the level of IL-12p40 mRNA expression indicates that core/gC1qR suppression extends to IL-23 production, a cytokine that shares this IL-12p40 subunit (46). The similar outcome, but diverse targets of these cytokines (47), indicates that ligation of this complement receptor may affect multiple arms of T cell activation and differentiation by macrophages. Thus, the absence of a proper Th1-inducing cytokine environment may explain why HCV-specific T cells exist, but are deficient in their effector function (20, 21). It is likely that the inhibition of IL-12 and IL-23 may abrogate IFN-γ production and Th1 effector functions of both naive and memory T cells, respectively. Furthermore, core/gC1qR-mediated suppression of IL-12 production may represent an underlying cause of the diminished
cell-mediated immunity (20, 21) and Th2 cytokine dominance in the periphery of chronically infected patients (22, 23). Extracellular HCV core is present in the livers of chronically infected HCV patients at sufficient quantities to cross-link gC1qR on APCs (19), resulting in dysregulation of host immunity and the establishment of viral persistence.

The gC1qR-mediated abrogation of IL-12 production was independent of several known regulators of IL-12, indicating that it is the result of a novel inhibitory pathway. Suppression of IL-12 production induced by cross-linking gC1qR was not due to ligation of FcγR, because the Ab used was of the IgM isotype and as such does not bind significantly to FcγRs. In addition, the absence of increased IL-10 or TGF-β after gC1qR ligation as well as the failure of neutralizing anti-IL-10 Abs to reverse gC1qR-mediated suppression of IL-12 indicate that these anti-inflammatory cytokines do not mediate gC1qR-induced impairment of macrophage IL-12 production. These data strongly indicate that inhibition of IL-12 production after gC1qR engagement is mediated through a novel mechanism, possibly involving abrogation of one or more LPS-induced signals that regulate IL-12p40 subunit transcription. Importantly, we identify a critical role for PI3K-dependent activation of AKT in gC1qR-mediated signaling and IL-12p70 suppression. In addition, the recently described association of αs and β1 integrins with gC1qR provides candidate anchoring and signaling partners in the gC1qR complex and enables a link between gC1qR and PI3K activation (48). The PI3K-mediated activation of Akt is the first observation of a viral- or complement-induced role for this negative feedback pathway in cross-talk with TLR signaling and establishes an intracellular pathway activated after gC1qR activation. Activation of inhibitory signaling pathways by gC1qR implicates this receptor in the regulation of macrophage/DC function and the down-regulation of Th1 responses. However, the signaling intermediates suppressed in PI3K-mediated inhibition of IL-12 have yet to be identified, but provide strong evidence for cross-talk between complement and TLR signaling.

Interaction of various pathogens with gC1qR on the surface of host immune cells (6, 29–33), including macrophages, may result in a failure to establish proper Th1-mediated cell-mediated immunity and lead to pathogen persistence. Furthermore, the role of PI3K in the suppression of IL-12p70 indicates that gC1qR uses a novel mechanism to control innate immunity. The innate immune system plays an important role in the early control of viral infection and eventual activation of a specific adaptive response (2, 49). Thus, it is likely that gC1qR-mediated suppression of macrophage activation plays a significant role in delay and detrimental impairment of virus-specific T cell responses associated with persistent HCV infection. These results re-enforce the concept that innate immunity is linked to adaptive immunity, such that failure of the innate immune response results in defective generation of subsequent adaptive immunity.

Recently, the suppression of TLR-mediated IL-12 production by the complement component C5a (50, 51) was demonstrated to involve PI3K and ERK activation in mouse macrophages (52). Intriguingly, although pharmacological inhibition of the PI3K pathway activation abrogated C5a-induced suppression of IL-12p40 transcription, only inhibitors of ERK activation reversed C5a-mediated suppression of IL-12p70 production. This finding on C5a-mediated regulation of IL-12p70 production by activation of ERK in mouse macrophages contrasts sharply with our observation that gC1qR-mediated suppression of IL-12p70 in human macrophages is independent on PI3K activation. The use of several methods to specifically analyze and inhibit PI3K activation in our study strongly implicated this pathway in signaling by gC1qR and suppression of IL-12p70. Furthermore, activation of ERK is not observed in human macrophages after gC1qR-ligation (S. N. Waggner, unpublished observations), suggesting that although both gC1qR and C5aR suppress IL-12 production by macrophages, the mechanisms mediating cross-talk with TLR signaling vary significantly. In addition, the failure of C5a to inhibit IL-12 production in DCs (50, 51) suggests that gC1qR is distinct from C5a in the ability to impair DC function to prime naïve T cells (53). The discrepancy between C5a- and gC1qR-mediated suppression may derive from inherent differences in the regulation of IL-12p70 production in human and mouse cells, cell type differences in the expression of these CRs, or differences in dependence upon various signaling mediators. We are currently investigating whether gC1qR-mediated suppression of DCs/macrophages results in dysfunctional priming of Th1 responses.

In conclusion, gC1qR-mediated signaling via the PI3K pathway is responsible for the impairment of IL-12p70 expression induced by core protein and gC1qR cross-linking. The activation of PI3K and subsequent phosphorylation of Akt correlate with a failure in LPS-induced transcription of the IL-12p40 subunit and suggest that PI3K directs cross-talk between the gC1qR and TLR signaling

![Graphical representation of gC1qR signaling](http://www.jimmunol.org/)

**FIGURE 7.** gC1qR signaling involves PI3K-dependent Akt activation. 
*a,* Phosphorylation of Akt after gC1qR ligation. THP-1 were stimulated with 1 μg/ml anti-gC1qR mAb or isotype control as well as 5 μg/ml β-gal-HCV core or β-gal control for various times at 37°C. Lower panel, Cells were pretreated for 30 min at 37°C with 10 μM LY294002 or vehicle (DMSO). Phosphorylation of Akt (Ser473) was analyzed in cell lysates by Western blot. Blots were then stripped and reprobed for total Akt or total ERK to show equal loading. These results were reproducible in five independent experiments. 
*b,* Determination of phosphorylation of Akt by intracellular flow cytometry. THP-1 cells were pretreated with vehicle (DMSO) or LY294002 (dashed line), stimulated for 30 min at 37°C with 1 μg/ml anti-gC1qR mAb (heavy line) or isotype IgM control (solid line), and stained for phospho-Akt (Ser473). Secondary alone control (shaded histogram) and MFI of phospho-Akt staining are shown. The results were reproducible in two independent experiments.
pathway. HCV core-induced failure of macrophage- or DC-directed Th1 differentiation may play a significant role in the establishment of persistent infection and implicates gC1qR in complement-mediated regulation of adaptive immunity.

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