# Title: C1q restrains autoimmunity and viral infection by regulating CD8$^+$ T-cell metabolism

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**One Sentence Summary:** C1q regulates the development of balanced effector CD8$^+$ T-cell responses by acting as a rheostat of mitochondrial metabolism.

**Abstract**: Deficiency of C1q, the initiator of the complement classical pathway, is associated with the development of systemic lupus erythematosus (SLE). Explaining this association in terms of abnormalities in the classical pathway alone remains problematic because C3 deficiency does not predispose to SLE. Here, using a mouse model of SLE, we demonstrate that C1q, but not C3, restrains the response to self-antigens by modulating the mitochondrial metabolism of CD8$^+$ T-cells, which can themselves propagate autoimmunity. C1q deficiency also triggers an exuberant effector CD8$^+$ T-cell response to chronic viral infection leading to lethal immunopathology. These data establish a link between C1q and CD8$^+$ T-cell metabolism and explain how C1q protects against lupus, with implications for the role of viral infections in the perpetuation of autoimmunity.

**Main Text:** Systemic lupus erythematosus (SLE) is an autoimmune condition that develops as a result of complex genetic and environmental interactions. B- and $CD4^+$ T-cell abnormalities are well known features of SLE (*1*), but the role of $CD8^+$ T lymphocytes remains poorly understood. Transcriptomic data suggest that a $CD8^+$ T-cell signature can predict disease outcome (*2, 3*).

There is evidence for a strong association between SLE and complement C1q deficiency (*4*). Previous work has shown that C1q deficiency leads to the ineffective clearance of apoptotic cells and consequently enhanced exposure to self-antigens, which facilitate autoimmunity (*5*). However, there are multiple pathways, including those mediated by C3, through which apoptotic cell clearance occurs (*6*). This suggests that the contribution of C1q is redundant and the "waste disposal" hypothesis (*5*) is inadequate to fully explain why C1q deficiency, and not C3 deficiency, is associated with autoimmunity. Alternative, but not mutually exclusive, hypotheses have been proposed (*7*). Further, an explanation for this strong association in terms of classical complement pathway abnormalities alone remains unsatisfactory. Given that there is evidence that C1q has multiple roles that are independent of complement activation (*8*), we searched for an alternative function that could explain why C1q is so critical for maintaining self-tolerance.

Chronic graft-versus-host-disease (cGvHD) is a well established inducible model of SLE. We used the bm12-cGvHD model (*9*) and injected splenocytes from B6.C-*H2-Ab1*$^{bm12}$/KhEg (bm12) mice into co-isogenic C57BL/6 (B6) recipients lacking C1q (*C1qa*$^{-/-}$) or C3 (*C3*$^{-/-}$). Lupus autoantibody levels were similar at disease onset (weeks 0-4), but increased at later time points only in the *C1qa*$^{-/-}$ mice **(Fig. 1A)**. At week 10, *C1qa*$^{-/-}$ mice displayed more severe glomerulonephritis and increased glomerular deposition of IgG and C3 **(Fig. 1B)**. They also presented with splenomegaly and higher percentages of germinal center B cells, follicular helper T-cells ($T_{FH}$), activated $CD4^+$ and $CD8^+$ T-cells than the WT and *C3*$^{-/-}$ counterparts **(Fig. S1)**.

During the course of the disease there were no differences in blood B- and $CD4^+$ T-cell activation between experimental groups, but the proportion of activated $CD44^{hi}CD62L^-CD8^+$ T-cells in the *C1qa*$^{-/-}$ mice was increased with a relative expansion of $KLRG1^+IL\text{-}7R^-$ short-lived effector cells (SLECs) and a corresponding reduction in $KLRG1^-IL\text{-}7R^+$ memory precursor effector cells (MPECs) **(Fig. 1C)** (*10*). Consistent with the alterations in blood, cGvH-treated *C1qa*$^{-/-}$ mice had early (from week 1) splenic $CD8^+$ T-cell abnormalities, whereas the initial B- and $CD4^+$ T-cell responses were similar to WT and *C3*$^{-/-}$ animals **(Fig. S1)**. Furthermore, the in vitro restimulation of *C1qa*$^{-/-}$$CD8^+$ T-cells resulted in increased IFN-γ and granzyme B expression and fewer $IL\text{-}2^+$ cells **(Fig. S2)**, indicating that the lack of C1q, but not of C3, resulted in $CD8^+$ T-cell responses skewed towards an effector phenotype. To determine whether bystander inflammation or self-antigen stimulation promoted $CD8^+$ T-cell activation during bm12-cGvH induction, naïve $CD8^+$ T-cells from B6.$CD45.1^+$ and OVA-specific TCR transgenic (OT-I) mice were co-transferred into B6.$CD45.2^+$ animals, which were challenged with bm12 splenocytes. Donor $CD45.1^+CD8^+$ T-cells expanded and activated like the host $CD45.2^+CD8^+$ T-cells, whereas $pentamer^+$OT-I cells remained quiescent **(Fig. S3)**, suggesting that TCR engagement by self-antigen was required.

$CD8\alpha^+$ DCs cross-present apoptotic cell-associated antigens to $CD8^+$ T-cells (*11*). However, the cross-priming by $CD8\alpha^+$ DCs in *C1qa*$^{-/-}$ animals was not impaired **(Fig. S4A-S4C).** Furthermore, after cGvH induction the number and phenotype of $CD8\alpha^+$ DCs was unaffected by C1q deficiency **(Fig. S4D, S4E)**. We then depleted $CD8^+$ T-cells to demonstrate their direct contribution to the autoimmune response in cGvHD. Although similar autoantibody levels were initially detected in all groups **(Fig. 1D)**, from week 4 onwards, $CD8^+$ T-cell-depleted *C1qa*$^{-/-}$ animals displayed a progressive decline in lupus-associated autoantibodies, whereas total IgG

levels remained unaffected **(Fig. 1D)**. $CD8^+$ T-cell-depleted *C1qa*$^{-/-}$ mice also showed reduced glomerular deposition of IgG and C3 compared with non-depleted mice **(Fig. 1E)**. Thus these data suggest that $CD8^+$ T-cells are responsible for perpetuating the lupus-like disease observed in cGvH-treated *C1qa*$^{-/-}$ mice.

To explore whether C1q also modulates $CD8^+$ T-cell immunity during infections we used lymphocytic choriomeningitis virus (LCMV) models. *C1qa*$^{-/-}$ mice, subjected to acute LCVM-Armstrong (Arm) infection, had an aberrant effector $CD8^+$ T-cell response at day 8 **(Fig. 2A** and **Fig. S5A)**, but did not show markedly different memory and recall responses (**Fig. S5B** to **Fig S5D**). We next used the chronic LCMV-clone 13 (Cl13) model where an exaggerated effector immune response can cause lethal lung immunopathology (*12*). When compared to WT mice, Cl13-infected *C1qa*$^{-/-}$ mice experienced greater body-weight loss and had to be culled at day 11 **(Fig. 2B).** Examination of *C1qa*$^{-/-}$ lung tissue showed edema that was absent in the controls **(Fig. 2C** and **FigS6A)**. Consistent with a pronounced $CD8^+$ T-cell response, *C1qa*$^{-/-}$ mice showed increased LCMV-specific $gp33^+$ and $gp276^+CD8^+$ T-cell populations **(Fig. 2D** and **Fig. S6B)**. On day 8, when *C1qa*$^{-/-}$ mice still had comparable numbers of LCMV-specific $CD8^+$ T-cells to WT animals, virus-specific *C1qa*$^{-/-}$$CD8^+$ T-cells were functionally over-reactive, with enhanced degranulation and cytokine production **(Fig. 2E, 2F)**. Consistent with an enhanced $CD8^+$ T-cell response, serum viral loads were lower in Cl13-infected *C1qa*$^{-/-}$ mice compared to WT mice (**Fig. 2G)**. Moreover, the upregulation of PD-1 expression was similar in WT and *C1qa*$^{-/-}$LCMV-specific $CD8^+$ T-cells, indicating that C1q deficiency did not impair the PD-1 signaling pathway **(Fig. 2H)**. These findings demonstrate that C1q plays a pivotal role in regulating effector $CD8^+$ T-cell responses in both autoimmunity and viral infection.

Complement can mediate its cellular effects via both extracellular and intracellular pathways (*13*). To explore how C1q affect $CD8^+$ T-cells, we co-transferred naïve $CD8^+$ T-cells, isolated from B6.CD45.$1^+$ and *C1qa*$^{-/-}$. CD45.$2^+$ mice, into B6.CD45.$1^+$.CD45.$2^+$ mice, which were challenged with bm12 $CD4^+$ T-cells one day later. C1q-sufficient and C1q-deficient donor $CD8^+$ T-cells showed similar expansion and activation, suggesting a cell-extrinsic effect of C1q **(Fig. S7)**. We corroborated this using the lymphopenia-induced proliferation model by co-transferring CFSE-labelled $CD8^+$ and $CD4^+$ T-cells from B6.CD45.$1^+$ mice into irradiated B6.CD45.$2^+$ and *C1qa*$^{-/-}$.CD45.$2^+$ mice. Fourteen days later, donor B6.CD45.$1^+CD8^+$ T-cells showed greater proliferation and activation in *C1qa*$^{-/-}$. CD45.$2^+$ recipients, whereas the co-transferred B6.CD45.$1^+CD4^+$ T-cells were unaffected **(Fig. 3A** and **Fig. S8A)**. As in the bm12-cGvHD model, C1q operated independently of C3 (**Fig. S8B)**. Lymphopenia-induced T-cell expansion is triggered by low-affinity interactions (*14*). Analysis of OT-I proliferation with OVA peptides of different affinities showed that C1q had an inhibitory effect only in response to partial (T4) and weak (G4) agonists, but not to a strong (N4) ligand **(Fig. S8C).** Similarly, C1q inhibited human $CD8^+$ T-cell activation, proliferation, and cytotoxic functions under suboptimal stimulation **(Fig. S9)**. C1q was detected mainly on activated $CD8^+$ T-cells (mouse and human) **(Fig. 3B** and **Fig. S10A)** and almost exclusively on MPECs **(Fig. 3C)**. Pre-incubation with the globular C1q region, but not with the collagen tail, inhibited C1q binding in a dose-dependent manner (**Fig. S10B** and **S10C)**, indicating that C1q recognizes activated $CD8^+$ T-cells via its globular domain. Correspondingly, expression of the globular C1q receptor (p32/gC1qR) (*15*), a mitochondrial molecule present on the surface of several immune cells (**Fig. S11A)**, was increased on activated mouse and human $CD8^+$ T-cells (**Fig. S11B, S11C).** Consistent with the preferential binding of C1q to MPECs **(Fig. 3C)**, cGvH-treated *C1qa*$^{-/-}$MPECs expressed lower levels of the anti-

apoptotic factor Bcl-2 and higher levels of Blimp-1, a repressor that promotes cytotoxic T functions (*16*) **(Fig. 3D, Fig. S12A** and **S12B)**. Furthermore, the proportion of *C1qa*$^{-/-}$MPECs, but not SLECs, secreting granzyme B was higher compared to WT cells **(Fig. S12C)**. Abnormal Bcl-2 expression in *C1qa*$^{-/-}$ mice suggests that C1q may influence MPEC viability. In cGvH-treated *C1qa*$^{-/-}$ mice, MPECs, but not SLECs, displayed a higher rate of BrdU decay compared to WT animals **(Fig. 3E)**, indicating a more rapid turnover of this subpopulation. Moreover, the percentage of *C1qa*$^{-/-}$MPECs expressing active caspase 3/7 was higher **(Fig. 3F** and **Fig. S12D)**. Altogether these findings suggest that C1q controls the programming and survival of MPECs through its globular domain.

CD8$^{+}$ T-cells undergo major metabolic changes upon activation (*17*). CD44$^{+}$CD62L$^{+}$CD8$^{+}$ (MPECs) and CD44$^{+}$CD62L$^{-}$CD8$^{+}$ (SLECs) T-cells from cGvH-treated *C1qa*$^{-/-}$ animals exhibited similar extracellular acidification rate (ECAR) and basal oxygen consumption rate (OCR) when compared to WT cells. However, *C1qa*$^{-/-}$ MPECs, but not SLECs, had impaired mitochondrial spare respiratory capacity (SRC) **(Fig. 4A, 4B, 4C)**. SRC has been shown to correlate with mitochondrial mass (*17*) and MitoTracker staining showed reduced mitochondrial content in *C1qa*$^{-/-}$MPECs compared to WT MPECs **(Fig. 4D)**. The addition of C1q to *C1qa*$^{-/-}$MPECs increased the MitoTracker staining **(Fig. 4E)** and upregulated the expression of mitochondrial biogenesis genes such as *Tfam* and *Pgc1*□ in IL-15-differentiated memory-committed OT-I cells, but not in IL-2-differentiated effector-like cells **(Fig. 4F)**. Consistent with a C1q-dependent pathway regulating MPEC mitochondrial biogenesis, in vitro metabolic conditions favoring a MPEC molecular profile (*18*) promoted p32/gC1qR surface expression on activated CD8$^{+}$ T-cells **(Fig. 4G** and **S13).** The internalization of surface-bound C1q occurred via an endocytic pathway **(Fig. S14)** and C1q co-localised with p32/gC1qR in the mitochondria **(Fig. 4H)**.

Altogether these data link C1q to the metabolic reprogramming and regulation of activated $CD8^+$ T-cells and lead us to propose a new paradigm for the protective role of C1q in SLE: C1q limits tissue damage and autoimmunity by acting as a "metabolic rheostat" for effector $CD8^+$ T-cells that are capable of propagating autoimmunity via the generation of unique autoantigen fragments by granzyme B (*19, 20*) (**Fig. S15)**. The role of $CD8^+$ T-cells in SLE has been largely overlooked and remains poorly characterized, with conflicting findings in human and animal studies (*20-24*), perhaps reflecting a changing role of these cells at different stages of the disease. By uncovering the role of effector $CD8^+$ T-cells in a lupus-like disease associated with C1q deficiency, our data demonstrate that an aberrant effector $CD8^+$ T-cell response to viral infection may auto-amplify the breakdown of self-tolerance. This is in addition to molecular mimicry and the bystander activation of autoreactive T-cells (*25*). Furthermore, very little is known about the metabolic profile of $CD8^+$ T-cells in SLE. As a $CD8^+$ T-cell transcriptional signature can predict the clinical outcome (*2, 3*), it is conceivable that metabolic abnormalities in these cells play a key role. Our study showing that C1q, a key lupus susceptibility gene in humans, can influence the mitochondrial metabolism of $CD8^+$ T-cells demonstrates this link. As p32/gC1qR is ubiquitously present in mitochondria and is indispensable for mitochondrial function (*26*) one hypothesizes that the surface expression of p32/gC1qR coupled with another receptor may determine the specificity of the cellular effect(s) mediated by C1q. Thus, our findings describe a new paradigm to explain how C1q may prevent lupus flares and highlight the importance of the interplay between complement and immunometabolism in autoimmunity.

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Authors declare no competing interests.

All data are available in the main text or the supplementary materials

## Figure legends

**Fig. 1. Autoimmune features and CD8$^+$ T-cell response in *C1qa*$^{-/-}$ mice after bm12-cGvH induction. (A)** Autoantibody levels after bm12 injection (arrows) (n = 5 mice/group). **(B)** IgG, C3, and PAS staining of kidney sections at week 10. Quantification of glomerular IgG and C3 deposition expressed as arbitrary fluorescence units (AFU). Glomerulonephritis score: 0-4, bars indicate the median. ND: not detectable. **(C)** Flow cytometric analysis of blood cells after cGvH induction (arrows) (n = 8-10 mice/group). **(D** to **E)** *C1qa*$^{-/-}$ mice were administrated PBS, anti-CD8α, or isotype-matched IgG2b antibody (n = 4-7 mice/group). **(D)** Autoantibody and IgG levels after cGvH induction (arrows). **(E)** Images and quantification of glomerular IgG and C3 deposition at week 10. **p*<0.05, ***p*<0.01, *****p*<0.001; One-way ANOVA (**B** and **E**), Kruskal–Wallis test **(B)**; **p*<0.05, ***p*<0.01, ****p*<0.005, *****p*<0.0001 (WT *vs* *C1qa*$^{-/-}$), $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.005, $^{\#\#\#\#}p$<0.0001 (*C1qa*$^{-/-}$ *vs* *C3*$^{-/-}$) two-way ANOVA (**A** and **C**); **p*<0.05, ***p*<0.01, ****p*<0.005 (isotype *vs* anti-CD8), $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.005, $^{\#\#\#\#}p$<0.0001 (PBS *vs* anti-CD8) two-way ANOVA (**D**). Data are mean ± SEM unless indicated otherwise; pooled results of two experiments (**C**); representative of two (**D** to **E**) or three (**A** and **B**) experiments. Scale bars, 100 μm (**B** and **E**).

**Fig. 2. Essential role for C1q in chronic LCMV infection. (A)** Numbers of splenic np396$^+$, gp33$^+$, and gp276$^+$ CD8$^+$ T cells and the proportion of SLECs and MPECs among LCMV-specific CD8$^+$ T cells in LCMV-Arm-infected WT and *C1qa*$^{-/-}$ mice at day 8 (n = 5-8 mice/group). **(B** to **G)** Analysis of WT and *C1qa*$^{-/-}$ mice infected with LCMV-Cl13. **(B)** Percentage of body weight loss (n = 5 mice/group). **(C)** Representative lung histology on day 11. Scale bars, 100 μm. **(D)** Numbers of splenic LCMV-specific CD8$^+$ T cells at day 11 (n = 5 mice/group). **(E** to **F)** Percentages of CD8$^+$ T cells positive for CD107a and the proportion of LCMV-specific CD8$^+$ T cells producing IFN-γ, TNF-α, and IL-2 after incubation with LCMV gp33 peptide (**E**) or gp276 peptide (**F**) at day 8 (n = 6 mice/group). **(G)** Serum viral load measured using quantitative PCR. **(H)** PD-1 expression on LCMV-specific CD8$^+$ T cells at day 8 after LCMV-Cl13 and LCMV-Arm. NS: not significant; *$p$<0.05, **$p$<0.01, ****$p$<0.0001; unpaired Student's $t$-test (**A, D** to **F**); two-way ANOVA (**B**). Data are mean ± SEM and representative of two experiments.

**Fig. 3. C1q selectively regulates MPEC programming and survival. (A)** Analysis of $CFSE^+CD45.1^+CD8^+$ and $CD45.1^+CD4^+$T cells co-transferred in sub-lethally irradiated $CD45.2^+$ WT or *C1qa*$^{-/-}$ hosts (n = 5 mice/group). Percentages of activated and fast proliferating donor T-cell subsets in spleen on day 14. **(B)** Flow cytometric gating of C1q staining on blood $CD8^+$ T cells at day 14 after cGvHD. **(C)** KLRG1 and IL-7R expression in WT $CD44^+CD8^+$ T cells (left); histogram of C1q staining (middle) and quantification (right) on MPECs and SLECs. Dotted line indicates MFI of isotype control. Each symbol represents a mouse. **(D)** Expression of transcription factors in splenic SLECs and MPECs from WT and *C1qa*$^{-/-}$ mice two weeks after cGvH induction (n = 3-5 mice/group). **(E)** Decay of $Brdu^+$SLECs and $Brdu^+$MPECs over 6 days (from day 11 after cGvH induction) in WT and *C1qa*$^{-/-}$ mice. Half-life times ($t_{1/2}$) of the decay and the R-squared value ($r^2$) of the linear regressions are indicated (n = 6 mice/group). **(F)** Fractions of splenic SLECs and MPECs Caspase $3/7^+$ at week 3 after cGvHD (n = 6 mice/group). NS: not significant; **p*<0.05, ***p*<0.01; unpaired Student's *t*-test **(A,C,D,F)** or two-way ANOVA **(E)**. Data are mean ± SEM and representative of three experiments.

**Fig. 4. C1q regulates mitochondrial metabolism in MPECs. (A** to **B)** ECAR and OCR under basal conditions and after mitochondrial inhibitors in sorted splenic $CD8^+$ T cells from WT and *C1qa*$^{-/-}$ mice two weeks after cGvHD. Curve showing mean ± SEM of four technical replicates of pooled samples from four animals. Data representative of three experiments. **(C)** SRC of the $CD8^+$ T-cell subsets as in **A** and **B.** Each symbol represents a biological replicate. **(D)** MTDR staining in SLECs and MPECs two weeks after cGvHD (n = 3 mice/group). **(E)** MTDR staining of in vitro IL-2- ($T_E$) and IL-15- ($T_M$) differentiated OT-I cells with and without C1q (n = 6 mice/group). **(F)** Mitochondrial gene expression in $T_E$ and $T_M$ cells with and without C1q (n = 5 mice/group). **(G)** Percentages of activated $CD8^+$ T cells expressing p32/gC1qR under different metabolic conditions (n = 4 mice/group). **(H)** Confocal images of $T_M$ cells cultured with hC1q, stained with MitoTracker (red), anti-C1q (green), anti-p32/gC1qR (cyan) and DAPI (blue). Scale bar: 5 μm. NS: not significant, **p*<0.05, ***p*<0.01, ****p*<0.005, *****p*<0.0001; two-way ANOVA **(A** and **B)**, unpaired Student's *t*-test **(C** to **G)**. Data are mean ± SEM and representative of three experiments **(D** to **G)**. FCCP: fluorocarbonylcyanide phenylhydrazone; ΔMFI = MFI−FMO; MTDR: MitoTracker deep red; 2DG: 2-deoxyglucose.

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317 **Supplementary Materials:**

318 Materials and Methods

319 Fig. S1 – S15

320 References (*27-37*)
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