Antibody Effector Functions Mediated by Fcγ-Receptors Are Compromised during Persistent Viral Infection

Highlights

- Antibody-mediated effector functions are compromised during chronic LCMV infection

- Immune complexes interfere with FcγR-mediated antibody effector functions

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In Brief

T cell dysfunction is well documented during chronic viral infections but little is known about functional abnormalities in humoral immunity. Ahmed and colleagues show that mice chronically infected with lymphocytic choriomeningitis virus (LCMV) exhibit a defect in FcγR-mediated antibody effector functions.

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Antibody Effector Functions Mediated by Fcγ-Receptors Are Compromised during Persistent Viral Infection

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SUMMARY

T cell dysfunction is well documented during chronic viral infections but little is known about functional abnormalities in humoral immunity. Here we report that mice persistently infected with lymphocytic choriomeningitis virus (LCMV) exhibit a severe defect in Fcγ-receptor (FcγR)-mediated antibody effector functions. Using transgenic mice expressing human CD20, we found that chronic LCMV infection impaired the depletion of B cells with rituximab, an anti-CD20 antibody widely used for the treatment of B cell lymphomas. In addition, FcγR-dependent activation of dendritic cells by agonistic anti-CD40 antibody was compromised in chronically infected mice. These defects were due to viral antigen-antibody complexes and not the chronic infection per se, because FcγR-mediated effector functions were normal in persistently infected mice that lacked LCMV-specific antibodies. Our findings have implications for the therapeutic use of antibodies and suggest that high levels of pre-existing immune complexes could limit the effectiveness of antibody therapy in humans.

INTRODUCTION

Antibodies are a key component of the immune system providing long-term protective immunity against many pathogens and regulating immune responses. Antibodies consist of two domains with distinct functions. Whereas the variable Fab domain mediates antigen specificity and binds its respective antigen, the Fc domain mediates diverse effector functions via recruitment of effector molecules such as complement and Fc receptors (FcRs). Although the Fc domain of immunoglobulin G (IgG) is considered to be an invariable region, it displays marked heterogeneity due to different subclasses with divergent amino acid sequences, as well as complex glycosylation patterns (Pincetic et al., 2014). This heterogeneity has been shown to modulate the effector function of IgG by altering the binding to activating and inhibitory FcγRs, thus triggering different pathways (Kaneko et al., 2006; Shields et al., 2002).

Besides complement, FcγRs, which are expressed by most hematopoietic cells, represent the main effector molecules recruited by IgG (Nimmerjahn and Ravetch, 2008). Upon antigen encounter, antibodies form immune complexes (ICs) with their cognate antigen and bind to FcγRs. The uptake of ICs by activating FcγRs on dendritic cells (DCs) has been shown to result in cell maturation and efficient presentation of antigen on MHC-I and MHC-II molecules (Kalergis and Ravetch, 2002; Regnault et al., 1999). FcγRs on follicular DCs in the germinal center can retain ICs and contribute to the affinity maturation of B cells (Barrington et al., 2002). Furthermore, Fc-FcγR interactions also play an important role in the protective capacity of neutralizing antibodies against various pathogens and toxins in vivo (Abboud et al., 2010; DiLillo et al., 2014; Halper-Stromberg et al., 2014; Hessell et al., 2007). Natural killer (NK) cells have been shown to contribute to the FcγR-dependent protective capacity of neutralizing antibodies against influenza and HIV by antibody-dependent cellular cytotoxicity (ADCC) (DiLillo et al., 2014; Hessell et al., 2007). In addition, it is well established that macrophages contribute to pathogen clearance by antibody-dependent phagocytosis in a number of infectious diseases (Kirimanjeswara et al., 2005; Zhang et al., 2005).

The mechanism of action of many therapeutic antibodies critically relies on Fc-FcγR interactions. Rituximab, a chimeric monoclonal antibody (mAb) directed against CD20, is widely used for treatment of non-Hodgkin's lymphoma and autoimmune diseases (Browning, 2006; Cheson and Leonard, 2008; Edwards et al., 2004). The engagement of activating FcγRs on effector cells, such as macrophages and NK cells, results in antibody-dependent phagocytosis or ADCC of opsonized B cells, which have been shown to be the major mechanisms of action of rituximab in vivo (Gong et al., 2005; Uchida et al., 2004). Trastuzumab, a mAb directed against the epidermal growth factor receptor HER2-neu on breast cancer cells, also depends on FcγR interactions as FcγR polymorphisms in human FcγRIIIa have

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shown to affect clinical efficacy (Musolino et al., 2008; Varchetta et al., 2007). Additional anticancer antibodies for which the engagement of activating FcγRs on effector cells has shown to mediate clinical efficacy comprise alemtuzumab, an anti-CD52 mAb used for treatment of B cell chronic lymphocytic leukemia, and cetuximab, an anti-HER1 mAb against metastatic colorectal cancer, metastatic non-small-cell lung cancer, and head and neck cancer (Hu et al., 2009; Yang et al., 2013). Bavituximab, a mAb directed against phosphatidylserine, which is translocated to the outer leaflet on the plasma membrane by malignant transformation or many viral infections, seems to mainly act via ADCC and is currently undergoing clinical trials (Soares et al., 2008). Furthermore, inhibitory FcγRs have been shown to play a crucial role for the in vivo activity of agonistic anti-CD40 antibodies to promote immune activation and anti-tumor immunity (Li and Ravetch, 2011, 2013).

In this study, we initially wanted to investigate the role of virus-specific CD4+ T cells during an established chronic lymphocytic choriomeningitis virus (LCMV) infection by depleting CD4+ T cells with a widely used anti-CD4 antibody. Surprisingly, CD4+ T cells in chronically LCMV infected mice were highly resistant to antibody-mediated depletion. While T cell dysfunction and the underlying molecular mechanisms during chronic infections such as LCMV have been extensively studied, little is known about defects in humoral immunity. We thus investigated the mechanisms underlying this unexpected resistance to antibody-mediated depletion. We show that chronic LCMV infection results in excessive IC formation and thereby interferes with antibody effector functions by competition for available FcγRs. Chronic LCMV infection severely impairs antibody-mediated depletion of various lymphocyte subsets as well as the activation of dendritic cells by a FcγR-dependent agonistic anti-CD40 antibody. These findings have important implications for the treatment of autoimmune diseases and cancer and infectious diseases with antibodies relying on the engagement of FcγRs. In addition, such defects in antibody-mediated effector functions could contribute to viral persistence.

RESULTS

Antibody-Mediated Depletion of CD4+ T Cells Is Impaired during Chronic LCMV Infection

To determine the role of virus-specific CD4+ T cells during an established chronic LCMV clone-13 infection, we treated LCMV clone-13-infected mice with a depleting anti-CD4 antibody (GK1.5), which has been extensively used in various studies (Jing et al., 2009; Matloubian et al., 1994). We injected C57BL/6 mice chronically infected with LCMV (4 weeks post infection) and age-matched naive controls on two consecutive days with 0.5 mg of anti-CD4 antibody intraperitoneally (i.p.). As expected, treatment of naive mice resulted in efficient depletion of CD4+ T cells from the peripheral blood (>98%) within 2 days, interestingly, CD4+ T cells of LCMV clone-13-infected mice seemed to be resistant to depletion showing a significantly reduced depletion efficiency (<10%) (Figures 1A and 1B). Similar results were obtained when we analyzed the total number of CD4+ T cells in the spleen of treated mice compared to untreated controls (Figures 1C and 1D). CD4+ T cells in the spleen of naive controls showed a high susceptibility to depletion (>92%) compared to

almost undetectable changes in the number of CD4+ T cells in LCMV clone-13 infected animals (<10%).

The observed resistance of CD4+ T cells in LCMV clone-13-infected mice to depletion was not due to reduced surface expression of CD4 as CD4+ T cells from chronically infected mice expressed comparable amounts as depletion-susceptible naive controls (Figure 1E). Increased expression of CD47, a ubiquitously expressed trans-membrane protein, on lymphomas was shown to facilitate evasion from phagocytosis by interacting with the inhibitory receptor SIRPα on macrophages (Jaiswal et al., 2009; Majeti et al., 2009). We thus examined whether the observed resistance of CD4+ T cells to antibody-mediated depletion during chronic LCMV clone-13 infection was caused by increased expression of CD47. However, we did not detect any significant differences in CD47 expression between CD4+ T cells of naive and chronically LCMV clone-13-infected mice (Figure 1F). These data thus suggest that the observed resistance of CD4+ T cells to antibody-mediated depletion is either CD4+ T cell-intrinsic due to unknown mechanisms or based on defects in the depleting effector population.

CD4+ T Cells of LCMV Infected Mice Are Not Intrinsically Resistant to Antibody-Mediated Depletion

To determine whether unknown target cell-intrinsic mechanisms or cell-extrinsic factors were responsible for the observed resistance to antibody-mediated depletion during LCMV clone-13 infection, we performed an in vivo depletion assay modified from our previous study (Barber et al., 2003). Splenic CD4+ T cells from C57BL/6 mice 4 weeks after infection with either LCMV clone-13 (chronic infection) or LCMV Arm (acute infection cleared by day 8), or from uninfected, age-matched controls, were differentially labeled with CFSE and CellTrace Violet and coated with anti-CD4 or left untreated. These cells were then co-transferred into uninfected age-matched, LCMV Arm immune, and chronically LCMV clone-13-infected mice (Figure 2A). Co-transfer of anti-CD4 coated and uncoated CD4+ T cells from donors differing in their infectious history allowed us to directly address the contribution of cell-intrinsic mechanisms to depletion resistance, whereas the transfer of cells into hosts differing in their infectious history allowed us to assess the role of cell-extrinsic factors. Anti-CD4 coated CD4+ T cells from naive, LCMV Arm immune and chronically LCMV clone-13-infected mice were efficiently depleted upon transfer into naïve, as well as LCMV Arm immune, mice. In contrast, chronically LCMV clone-13-infected mice failed to efficiently deplete antibody-coated CD4+ T cells irrespective of the donors' infectious history (Figure 2B). The depletion of antibody-coated CD4+ T cells in chronically LCMV clone-13-infected mice was reduced compared to naive or LCMV Arm immune mice, whereas no differences were observed between naive and LCMV Arm immune mice (Figure 2C). These data show that the striking resistance to antibody-mediated depletion is specific to chronic LCMV clone-13 infection. In addition, we did not observe differences in the depletion efficiency of target cells isolated from donor mice with different infectious history. Thus, the extent of antigen exposure and/or activation status of the target cells did not influence their susceptibility to depletion. In summary, these data demonstrate that cell-extrinsic but not cell-intrinsic factors are causing the observed
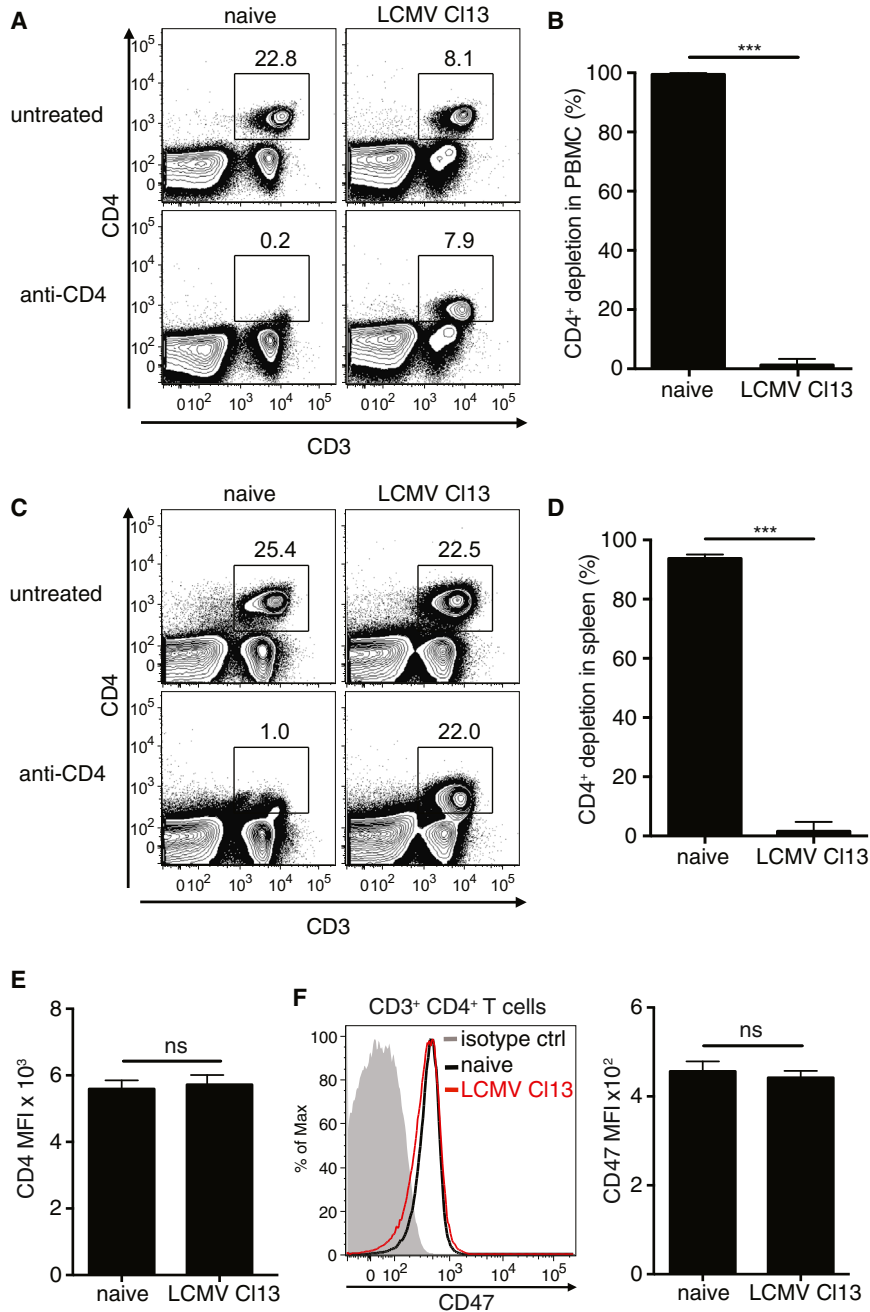
Figure 1. Antibody-Mediated Depletion of CD4⁺ T Cells Is Severely Impaired in Chronically LCMV Clone-13-Infected Mice

(A–F) Naive and LCMV clone-13-infected C57BL/6 mice (d28 p.i.) were injected twice with 0.5 mg anti-CD4 depleting antibody. CD4⁺ depletion in the peripheral blood and spleen was analyzed 2 days after treatment start. (A) Representative flow plots showing the frequency of CD4⁺ T cells as percentage of PBMC. (B) CD4⁺ depletion efficiency in PBMC. (C) Representative flow plots showing the frequency of CD4⁺ T cells as percentage of splenocytes. (D) CD4⁺ depletion efficiency in spleen. The surface expression of (E) CD4 and (F) CD47 on CD4⁺ T cells prior treatment start were analyzed by flow cytometry. Data (mean ± SEM) from one representative experiment of more than 5 (n = 3–4 mice per group) are shown. Unpaired two-tailed Student's t test was used. *p < 0.05, ***p ≤ 0.001. ns indicates not significant. See also Figure S1.

tion of NK cells using anti-NK1.1 antibody had no effect on the depletion activity of anti-CD4 (Figure 3A, Figures S1A and S1B). As LCMV clone-13 preferentially infects macrophages and DCs, resulting in virus-induced immunosuppression (Matloubian et al., 1993; Sevilla et al., 2000), we hypothesized that LCMV clone-13 infection might impair macrophage-mediated phagocytosis of antibody-coated target cells resulting in the observed resistance to depletion. Interestingly, binding/phagocytosis of fluorescent microspheres by macrophages and DCs in vivo was not impaired in LCMV clone-13-infected mice, suggesting a defect specific to FcγR-mediated phagocytosis (Figure S1C). Direct ex vivo analysis of various phagocytic subsets in the spleen showed slightly elevated expression of FcγRII/III on monocytes and neutrophils as well as of FcγRIV on monocytes of LCMV clone-13-infected mice compared to naive controls (Figure S2). We thus compared the phagocytic activity of monocytes/macro-

failure of antibody-mediated depletion of CD4⁺ T cells during chronic LCMV clone-13 infection.

Macrophages of Naive and Chronically LCMV-Infected Mice Show Comparable Phagocytic Activity In Vitro

Antibody-mediated depletion in vivo acts mainly through engagement of FcγRs and subsequent phagocytosis of antibody-coated target cells by macrophages (Gong et al., 2005; Uchida et al., 2004). We confirmed that phagocytosis is the main effector mechanism mediating the depletion of CD4⁺ T cells by the anti-CD4 antibody used in this study. Depletion of phagocytic cells using clodronate liposomes prevented antibody-mediated depletion of CD4⁺ T cells, whereas the deple-

phages from naive and chronically LCMV clone-13-infected mice in vitro using a previously described FcγR-dependent phagocytosis assay (Shashidharamurthy et al., 2008). We isolated CD11b⁺ splenocytes and thioglycollate-induced peritoneal exudate cells (PECs) from naive and chronically infected mice. CD11b⁺ splenocytes of naive and LCMV clone-13-infected mice showed comparable phagocytic activity in vitro, which was dependent on the engagement of FcγRs (Figure 3B). PECs obtained from chronically infected mice showed slightly enhanced FcγR-mediated phagocytic activity in vitro compared to naive controls (Figure 3C). Together, these experiments using phagocytic cells derived from two different anatomical sites demonstrate that chronic LCMV clone-13 infection does
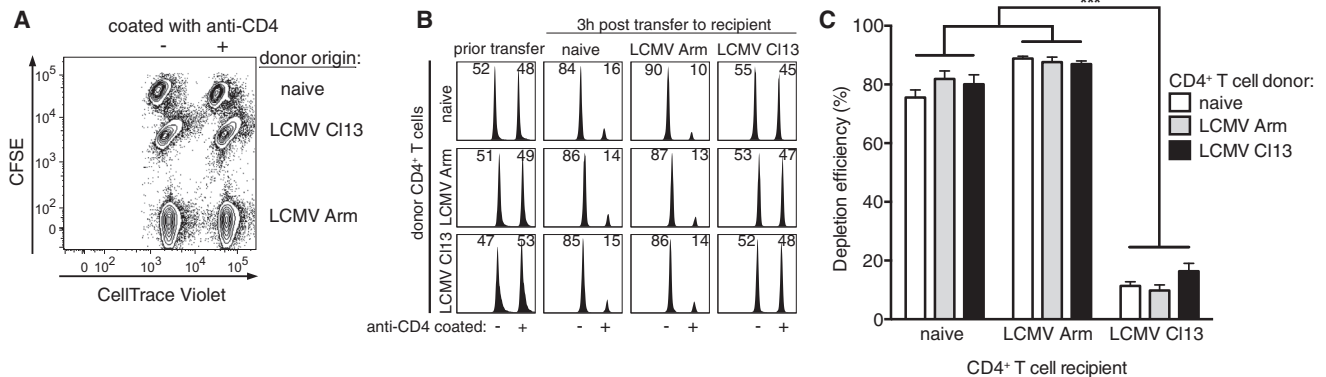
Figure 2. CD4+ T Cells of Chronically Infected Mice Are Not Intrinsically Resistant to Antibody-Mediated Depletion

(A–C) Anti-CD4 coated and uncoated CD4+ T cells of naive and LCMV clone-13/Arm-infected (d28 p.i.) mice were differentially fluorescently labeled, mixed to equal proportions and transferred to naive and LCMV clone-13/Arm infected (d28 p.i.) mice. (A) Representative flow plot of transferred cell populations. (B) Representative histograms are gated on fluorescently labeled CD3+ splenocytes of the indicated donor origin obtained 3 hr post transfer. Numbers indicate the percentages of anti-CD4 coated and uncoated cells. (C) Depletion efficiency of anti-CD4 coated CD4+ T cells in naive and LCMV clone-13/Arm infected (d28 p.i.) hosts within 3 hr post transfer. Data (mean ± SEM) from one representative experiment of two (n = 3–5 mice per group) are shown. A two-way ANOVA was used. ***p ≤ 0.001.

not impair FcγR-dependent phagocytosis in macrophages in vitro suggesting a target, as well as effector cell-extrinsic mechanism.

Excessive Immune Complex Formation Interferes with Antibody-Mediated Depletion

A hallmark of chronic LCMV infection is the induction of a vigorous B cell response resulting in hypergammaglobulinemia for a prolonged period of time (Hunziker et al., 2003). Thus, we sought out to determine whether this vigorous B cell response contributes to the observed resistance to antibody-mediated depletion. To address this issue, we took advantage of the IgHEL tg mouse model (MD4), in which B cells express a transgene encoding an immunoglobulin (Ig) specific for hen egg lysozyme (HEL) (Mason et al., 1992). Due to allelic exclusion, >98% of all peripheral B cells in IgHEL tg mice are specific for HEL (Silveira et al., 2002). This model allowed us to directly investigate the effect of a reduced anti-viral B cell response on antibody-mediated depletion. Wild-type (WT) and IgHEL tg mice showed comparable viral titers on day 28 post LCMV clone-13 infection (Figure 4A). As expected, LCMV clone-13-infected IgHEL tg mice showed reduced LCMV-specific antibody titers (~30-fold) compared to infected WT controls (Figures 4B and 4C). However, LCMV clone-13-infected IgHEL tg mice showed some heterogeneity regarding LCMV-specific antibody titers (Figure 4B). LCMV clone-13 infection of IgHEL tg mice resulted in hypergammaglobulinemia compared to naive controls with a 3- to 4-fold increase in total IgG (Figure 4D). However, total IgG titers in LCMV clone-13-infected IgHEL tg mice were lower compared to infected WT controls, which showed a 10-fold increase in total IgG.

Persistent LCMV infection has been shown to induce ICs containing the major structural LCMV proteins (Buchmeier and Oldstone, 1978; Oldstone et al., 1980). As we observed high viral load, as well as high antibody titers in LCMV clone-13 infected WT mice, we hypothesized that chronic LCMV infection results in excessive IC formation and thus impairs

FcγR-mediated clearance of opsonized target cells. We thus analyzed the serum for the presence of circulating immune complexes (CIC). In accordance with our hypothesis, we detected lower amounts of CIC in the serum of LCMV clone-13-infected IgHEL tg mice compared to infected WT controls (Figure 4E). CIC showed a comparable IgG subclass composition as total serum IgG (Figure S3). We thus compared the efficiency of depleting CD4+ T cells in WT and IgHEL tg mice in the presence or absence of a chronic LCMV clone-13 infection. Antibody-mediated depletion of CD4+ T cells was more efficient in chronically infected IgHEL tg mice compared to WT infected mice ranging from ~33%–98% versus ~10%, whereas no difference in the depletion efficiency of CD4+ T cells between naive WT and IgHEL tg mice was observed (Figures 4F and 4G). The majority of LCMV clone-13 infected IgHEL tg mice showed a CD4+ T cell depletion efficiency of >90%, whereas some IgHEL tg mice, which tended to have higher LCMV-specific antibody titers showed lower depletion efficiencies (Figure 4H). However, no correlation was observed between total IgG titers and CD4+ depletion (Figure S4). As PECs from chronically infected mice did not show intrinsic defects in FcγR-dependent phagocytosis (Figure 3C), we investigated whether pre-treatment of PECs with serum of LCMV clone-13-infected mice could inhibit FcγR-mediated phagocytosis in vitro and thus mimic the impaired depletion efficiency observed in vivo. Indeed, serum of LCMV clone-13-infected mice efficiently inhibited FcγR-mediated phagocytosis in vitro (Figure S5). Together, these data suggest that excessive formation of ICs during chronic LCMV clone-13 infection impairs the antibody-mediated clearance of opsonized target cells by competition for available FcγRs.

We next sought to confirm the results obtained in IgHEL tg mice using another chronic LCMV infection model lacking LCMV-specific antibodies, as well as virus-specific CD4+ T cells due to transient depletion of CD4+ T cells prior to infection (GK LCMV clone-13) (Matloubian et al., 1994; Zajac et al., 1998). We compared the CD4+ T cell depletion efficiency in
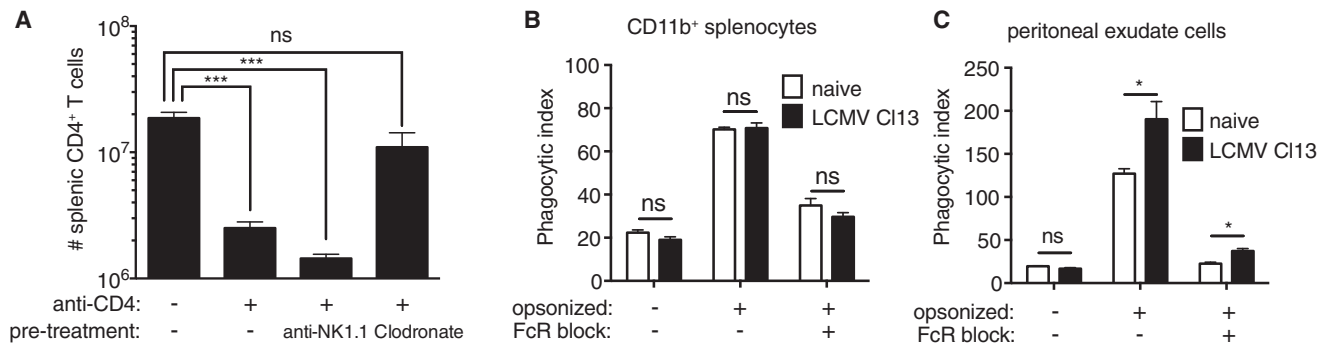
Figure 3. Phagocytes of Naive and LCMV Clone-13-Infected Mice Show Comparable Phagocytic Activity In Vitro

(A) On day 1, mice were injected i.p. with 100 μg anti-NK1.1, 200 μl clodronate liposomes or left untreated. Mice were then injected twice with 0.5 mg anti-CD4 and sacrificed on day 2. Number of splenic CD4$^+$ T cells on day 2 post anti-CD4 treatment start (n = 4–5 mice per group).

(B) Purified CD11b$^+$ splenocytes and (C) peritoneal exudate cells of naive and LCMV clone-13-infected mice (d35 p.i.) were incubated with fluorescently labeled unopsonized or opsonized erythrocytes in the presence or absence of FcγRII/III-blocking antibody 2.4G2. Phagocytic activity was analyzed by flow cytometry (n = 3 replicates per group). Data (mean ± SEM) from one representative experiment of two are shown. Unpaired two-tailed Student's t test was used. *p < 0.05, ***p < 0.0001. ns indicates not significant. See also Figure S2.

LCMV clone-13 and GK LCMV clone-13-infected mice 4 weeks post infection, as well as in age-matched, uninfected controls upon two intraperitoneal injections of anti-CD4. At this time point post infection, GK LCMV clone-13-infected mice already showed a reconstitution of their CD4$^+$ T cell compartment and high viral loads (Figure 5A). We observed high titers of LCMV-specific antibodies (Figures 5B and 5C) and a 10-fold increase in total IgG in the serum of LCMV clone-13-infected mice (Figure 5D). In contrast, GK LCMV clone-13-infected mice lacking virus-specific CD4$^+$ T cells did not mount a B cell response against LCMV and showed no increase in total serum IgG. We detected higher amounts of CIC in the serum of LCMV clone-13-infected mice compared to GK LCMV clone-13-infected mice and uninfected controls (Figure 5E). In accordance with the absence of LCMV-specific antibodies, GK LCMV clone-13-infected mice showed no increase in CIC compared to naive controls. Consistent with our hypothesis, the susceptibility of CD4$^+$ T cells in GK LCMV clone-13-infected mice to antibody-mediated depletion was strikingly higher compared to LCMV clone-13-infected mice (>98% versus <10%) and comparable to naive controls (>98%) (Figure 5F). These data further support our hypothesis that during chronic LCMV clone-13 infection a vigorous B cell response leads to excessive formation of ICs and thus impairs FcγR-mediated clearance of opsonized target cells.

Hypergammaglobulinemia Alone Does Not Interfere with FcγR-Mediated Clearance of Opsonized Cells

The above depletion experiments in IgHEL tg mice showed that reduced B cell responses during chronic LCMV clone-13 infection allowed efficient antibody-mediated depletion of CD4$^+$ T cells. However, these experiments did not address the question whether hypergammaglobulinemia in general could interfere with antibody-mediated depletion or antigen-specific antibodies and the presence of cognate antigen and the subsequent formation of ICs are required during chronic LCMV clone-13 infection. To test whether hypergammaglobulinemia in general interferes with antibody-mediated depletion, we transferred high amounts

of purified mouse IgG (polyclonal or monoclonal origin) intravenously (i.v.) to naive C57/BL6 mice. Compared to IgG of chronically infected mice, transferred polyclonal IgG showed only slight differences in its subclass composition but 2-fold lower amounts of sialylic acid in the total IgG glycan preparation, which includes Fc- and Fab-associated glycans (Figures S6A and S6B). Sialylation of the Fc domain has been shown to reduce the affinity to FcγRs, suggesting that the transferred polyclonal IgG might have higher affinity to FcγRs compared to IgG of chronically infected mice (Kaneko et al., 2006). High-dose monoclonal or polyclonal IgG transfer into naive mice resulted in an increase in serum IgG concentrations (~8 fold) comparable to chronically LCMV clone-13 infected controls (Figure 5G). We next performed an in vivo depletion assay as described above using anti-CD4 coated and uncoated purified CD4$^+$ T cells from naive mice. High-dose IgG recipients efficiently cleared anti-CD4 coated CD4$^+$ T cells despite high concentrations of serum IgG, whereas LCMV clone-13-infected mice failed to eliminate opsonized target cells (Figure 5H). Hypergammaglobulinemia induced by transfer of purified mouse IgG resulting in total IgG concentrations comparable to LCMV clone-13-infected mice did thus not interfere with antibody-mediated depletion, whereas serum of chronically infected mice containing ICs and only 4–5 mg IgG efficiently inhibited antibody-mediated depletion (Figure S7).

Chronic LCMV Infection Interferes with the Ability of Different Antibodies to Deplete Various Lymphocyte Subsets

We next sought out to determine whether the observed resistance to antibody-mediated depletion during chronic LCMV clone-13 infection is only restricted to CD4$^+$ T cells and the antibody used to deplete CD4$^+$ T cells or whether it represents a general defect in antibody-mediated depletion. Therefore, we investigated the depletion efficiency of additional antibodies directed against various surface molecules in naïve, as well as LCMV clone-13-infected, mice. We observed severely impaired depletion efficiency using antibodies targeting CD8α and CD25
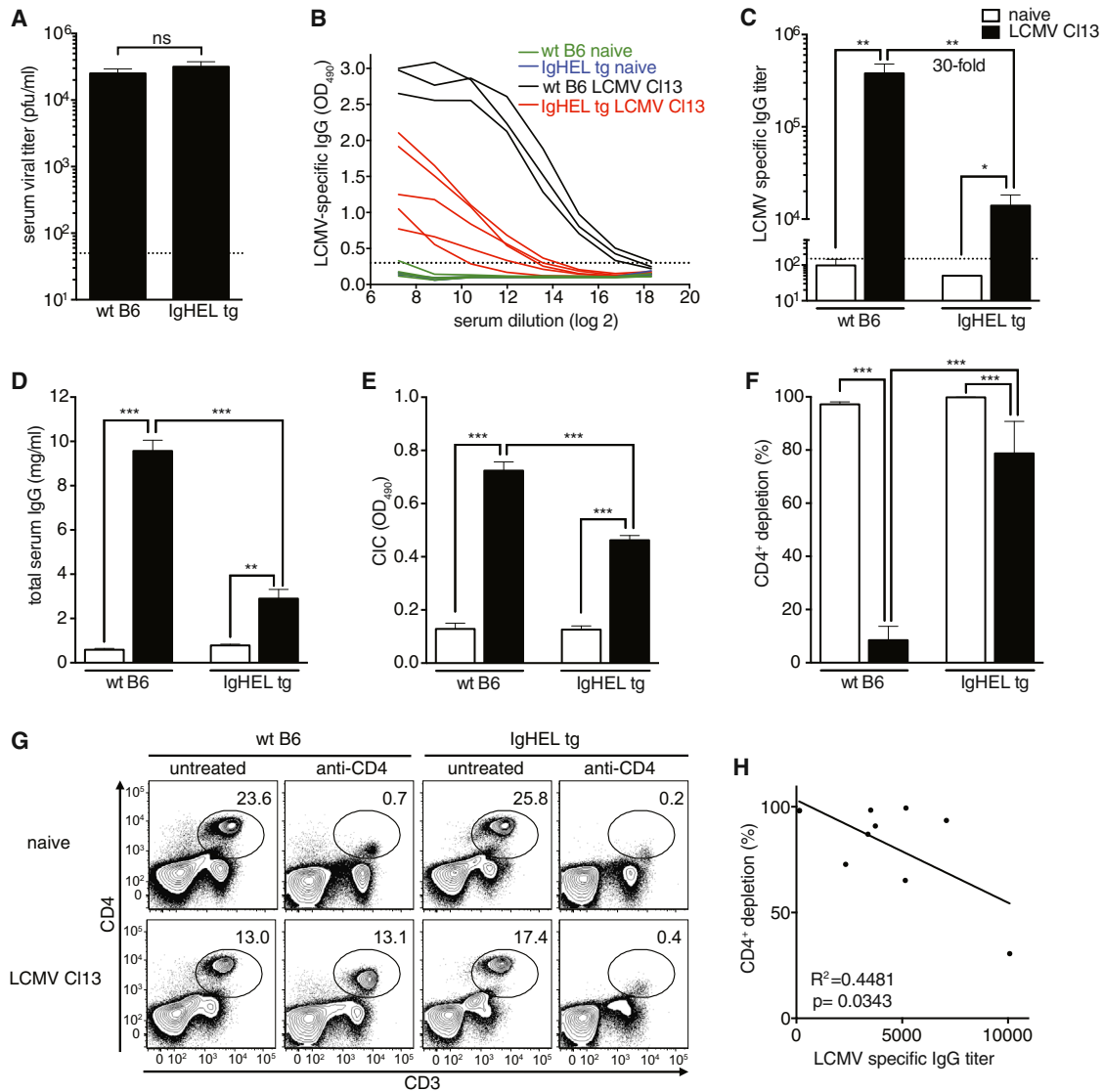
Figure 4. CD4+ T Cells Can Be Efficiently Depleted in Chronic LCMV Clone-13 Infected IgHEL tg Mice

(A–H) WT and IgHEL tg C57/BL6 mice were infected with LCMV clone-13. (A) Viral titers, (B and C) LCMV- specific IgG titers, (D) total IgG, and (E) circulating immune complexes (CIC) in serum were analyzed on day 28 p.i. The black dotted lines indicate the lower level of detection. (F and G) Mice were then injected twice with 0.5 mg anti-CD4 depleting antibody. CD4+ T cell depletion in the peripheral blood was analyzed 2 days after treatment start. (F) CD4+ depletion efficiency. (G) Representative flow plots of CD4+ T cells in the peripheral blood of WT B6 and IgHEL tg mice before and 2 days post treatment start. Numbers represent frequency of CD4+ T cells as percentage of PBMC. (H) Linear regression and Pearson's correlation were used to show the association between CD4+ depletion efficiency and LCMV-specific antibody titers in chronically infected IgHEL tg mice. R^2 and p value for Pearson's correlation are shown. Data (mean ± SEM) from one representative experiment of at least two (n = 3-10 mice per group) are shown. Unpaired two-tailed Student's t test was used. *p < 0.05, **p < 0.005, ***p ≤ 0.001. ns indicates not significant. See also Figures S3–S5.

during chronic LCMV clone-13 infection compared to naive controls (Table S1). These data further support our hypothesis that chronic LCMV clone-13 infection interferes with FcγR-mediated antibody effector functions by excessive formation of ICs and subsequent competition for available FcγRs. Interestingly, an antibody directed against CD90.2 (30H12) was able to deplete T cells in naive and LCMV clone-13-infected mice to a comparable extent demonstrating that the observed interference with FcγR-mediated antibody effector functions during chronic LCMV clone-13 infection can be overcome.

Rituximab-Mediated B Cell Depletion Is Severely Impaired in Chronically LCMV-Infected Mice

Rituximab, a humanized anti-hCD20 mAb, is widely used for treatment of non-Hodgkin's lymphoma and autoimmune diseases (Browning, 2006; Cheson and Leonard, 2008; Edwards et al., 2004). However, a number of patients fail to respond to treatment due to mostly unknown reasons (Alduaij and Illidge, 2011). To determine whether chronic LCMV infection also interferes with the depletion efficiency of rituximab, we assessed the effect of chronic LCMV clone-13 infection on the depletion
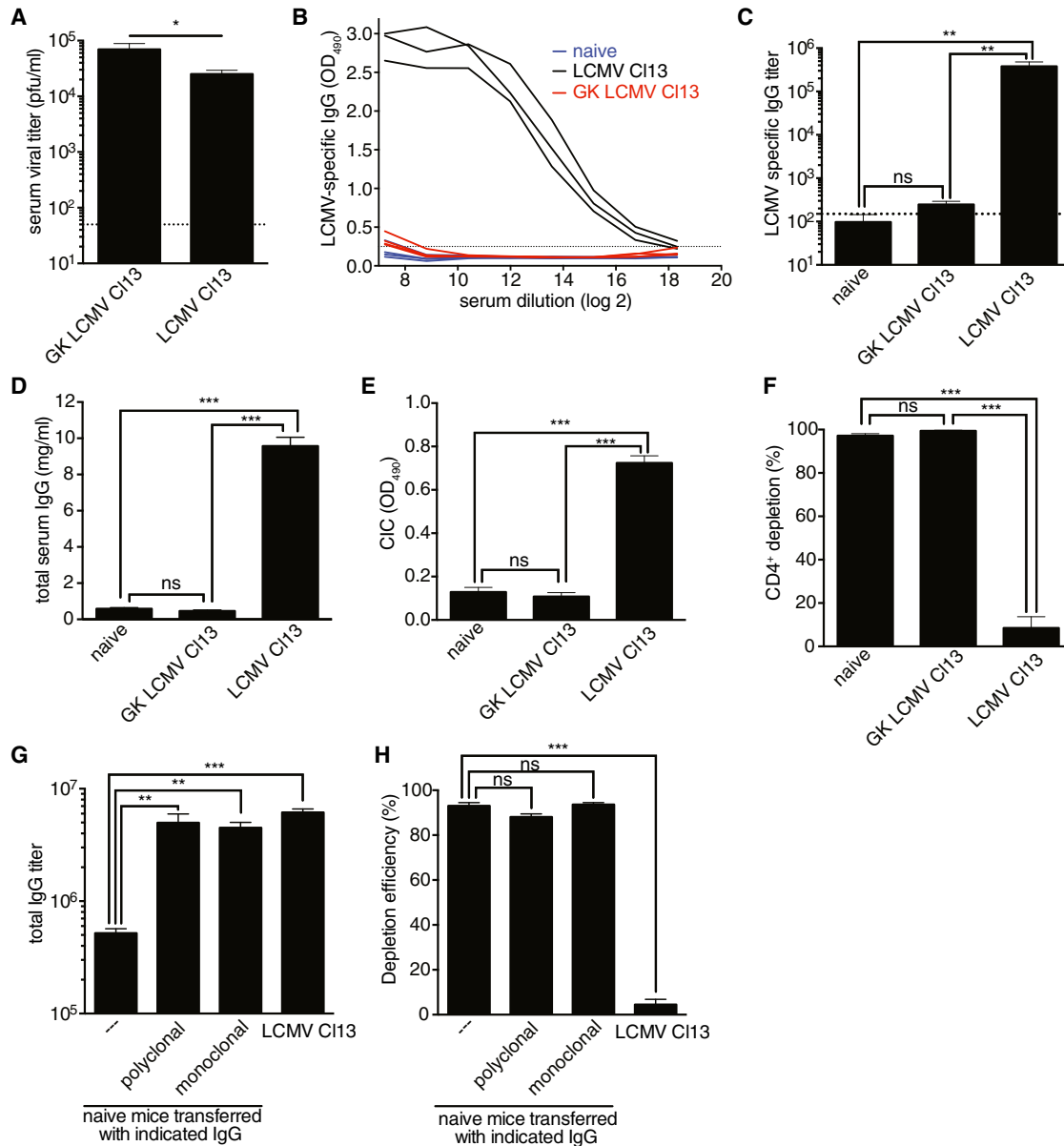
Figure 5. CD4+ T Cells Can Be Efficiently Depleted in LCMV Clone-13-Infected Mice Lacking LCMV-Specific Antibodies

(A–F) Mice were depleted of CD4+ T cells (GK) prior to LCMV clone-13 infection. (A) Viral titers, (B and C) LCMV specific IgG titers, (D) total IgG, and (E) circulating immune complexes (CIC) in serum were analyzed on day 28 p.i. The black dotted lines indicate the lower level of detection. (F) Mice were then injected twice with 0.5 mg anti-CD4 depleting antibody. CD4+ T cell depletion in the peripheral blood was analyzed 2 days after treatment start.

(G and H) Mice were infected with LCMV clone-13 28 days prior to analysis. Naive mice received 10 mg polyclonal IgG, monoclonal IgG, or PBS 18 hr prior analysis. Anti-CD4 coated and uncoated CD4+ T cells of naive mice were differentially fluorescently labeled, mixed to equal proportions and transferred to the indicated recipients. CD3+ splenocytes were analyzed 3 hr post transfer. (G) Total serum IgG concentrations were determined prior to target cell transfer. (H) Depletion efficiency of anti-CD4 coated T cells. Data (mean ± SEM) from one representative experiment of two (n = 3–5 mice per group) are shown. Unpaired two-tailed Student's t test was used. *p < 0.05, **p < 0.005, ***p ≤ 0.001. ns indicates not significant. See also Figures S6 and S7.

efficiency of rituximab in a hCD20 tg mouse model expressing hCD20 specifically on mature B cells (Ahuja et al., 2007). We treated chronically infected hCD20 tg mice (4 weeks post infection) and age-matched naive controls with 1 mg rituximab i.p. on days 0, 2, and 4. Treatment of naive hCD20 tg mice with rituximab resulted in efficient depletion of (CD19+ B220+) B cells in the circulation (>95%) within a week, whereas rituximab treat-

ment of LCMV clone-13 infected animals showed a lower depletion efficiency (Figures 6A and 6B). The B cell depletion efficiency in chronically infected mice varied between individual mice but did not exceed 15% on average. Similar results were obtained when hCD20 tg mice were treated with a murine anti-hCD20 antibody, 2H7, recognizing an epitope similar to that of rituximab (Table S1). To examine whether the observed resistance of B
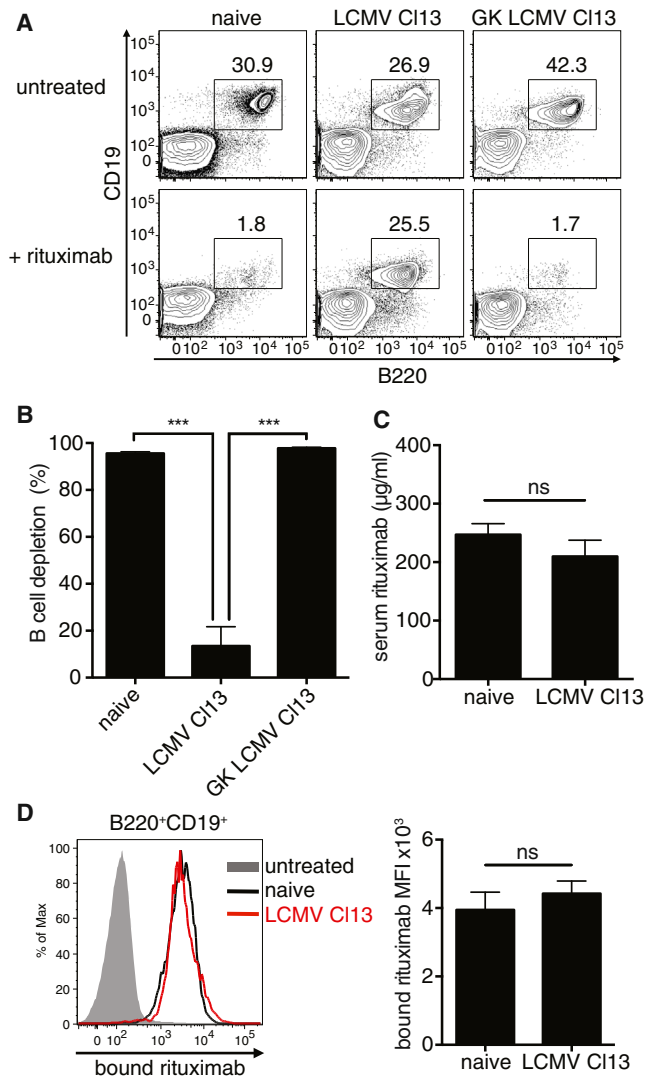
Figure 6. Rituximab-Mediated B Cell Depletion Is Severely Impaired in Chronically LCMV Clone-13-Infected Mice

(A–C) Naive and LCMV clone-13-infected hCD20 tg mice (d28 p.i.) were injected three times with 1 mg rituximab. (A) Representative flow plots of B cells in the peripheral blood of naive, LCMV clone-13-infected and GK LCMV clone-13-infected mice prior and 7 days post rituximab treatment. Numbers represent frequency of B cells as a percentage of PBMC. (B) B cell depletion and (C) rituximab concentrations in the peripheral blood on day 7 post treatment start. (D) Representative histogram (left panel) and statistics (right panel) of surface bound rituximab on B cells from naive and LCMV clone-13-infected mice 3 hr post treatment start. Data (mean ± SEM) from one representative experiment of three (n = 3–5 mice per group) are shown. Unpaired two-tailed Student's t test was used. ***p $\leq$ 0.001. ns indicates not significant. See also Table S1.

cells to depletion was due to reduced half-life of the injected rituximab in chronically infected mice, we measured the serum concentrations of intact, bio-active rituximab (able to bind hCD20) using an anti-idiotype ELISA. We detected no differences in serum concentrations of rituximab between naive mice showing efficient B cell depletion and chronically LCMV clone-13-infected mice with severely impaired B cell depletion (Figure 6C). In vitro activation of human B cells with agonistic

anti-CD40 has shown to result in rapid and dramatic downregulation of hCD20 (Anolik et al., 2003). In addition, previous studies showed rituximab-mediated downregulation of hCD20 on the surface of B cells from hCD20 tg mice, as well as patients with Chronic Lymphatic Leukemia or Mantle Cell Lymphoma (Jilani et al., 2003; Li et al., 2007). To examine whether B cells from naive and chronically infected hCD20 tg mice differentially downregulated surface expression of hCD20 in response to rituximab, we stained for rituximab on surface of B cells 3 hr post treatment start. We detected comparable amounts of rituximab bound to the surface of B cells from naive and chronically infected mice (Figure 6D), suggesting that the impaired depletion of B cells in chronically infected mice is likely not due to differences in binding of rituximab.

FcγR-Dependent Agonistic Anti-CD40 Fails to Activate DCs in LCMV Clone-13-Infected Mice

To further demonstrate that excessive IC formation during chronic LCMV infection can interfere with various FcγR-mediated antibody effector functions, we investigated the in vivo activity of an agonistic anti-CD40 antibody. Signaling through CD40, an important costimulatory member of the TNFR family, results in the activation and licensing of DCs allowing them to efficiently stimulate T cell responses (Bennett et al., 1998; Schoenberger et al., 1998). Agonistic anti-CD40 antibodies have been shown to require inhibitory FcγRs but not FcγR signaling components for their in vivo activity suggesting that inhibitory FcγRs due to their wide cellular distribution and high expression serve as a scaffold to allow cross-linking of CD40 (Li and Ravetch, 2011, 2013). We treated naive and LCMV clone-13-infected mice with 80 μg agonistic anti-CD40 antibody (FGK45) and analyzed the activation of DCs in the spleen. Lymphoid CD8α+ DCs in naive mice were efficiently activated by anti-CD40 and upregulated the costimulatory molecules CD80 and CD86 (Figures 7A and 7B). In contrast, lymphoid CD8α+ DCs in LCMV clone-13-infected mice did not upregulate CD80 or CD86 in response to anti-CD40 treatment. We obtained comparable results analyzing myeloid CD11b+ DCs (Figure 7C) demonstrating that the observed failure of the agonistic anti-CD40 to activate DCs is not specific to a distinct DC subset. However, consistent with the data from our depletion experiments, lymphoid and myeloid DCs could be efficiently activated in LCMV clone-13-infected mice lacking LCMV-specific antibodies due to CD4+ depletion prior to infection (Figures 7D and 7E). These data suggest that excessive IC formation during chronic LCMV infection can interfere with various FcγR-mediated processes by competition for available FcγRs.

DISCUSSION

In this report, we show for the first time that chronic LCMV clone-13 infection interferes with FcγR-mediated antibody effector functions. We observed a striking resistance to antibody-mediated depletion in mice chronically infected with LCMV clone-13, which was cell type independent as antibodies to different lymphocyte subsets failed to efficiently deplete their targets. We demonstrated that this resistance was not due to target cell intrinsic mechanisms but clearly linked to excessive IC formation as a result of a vigorous B cell response.
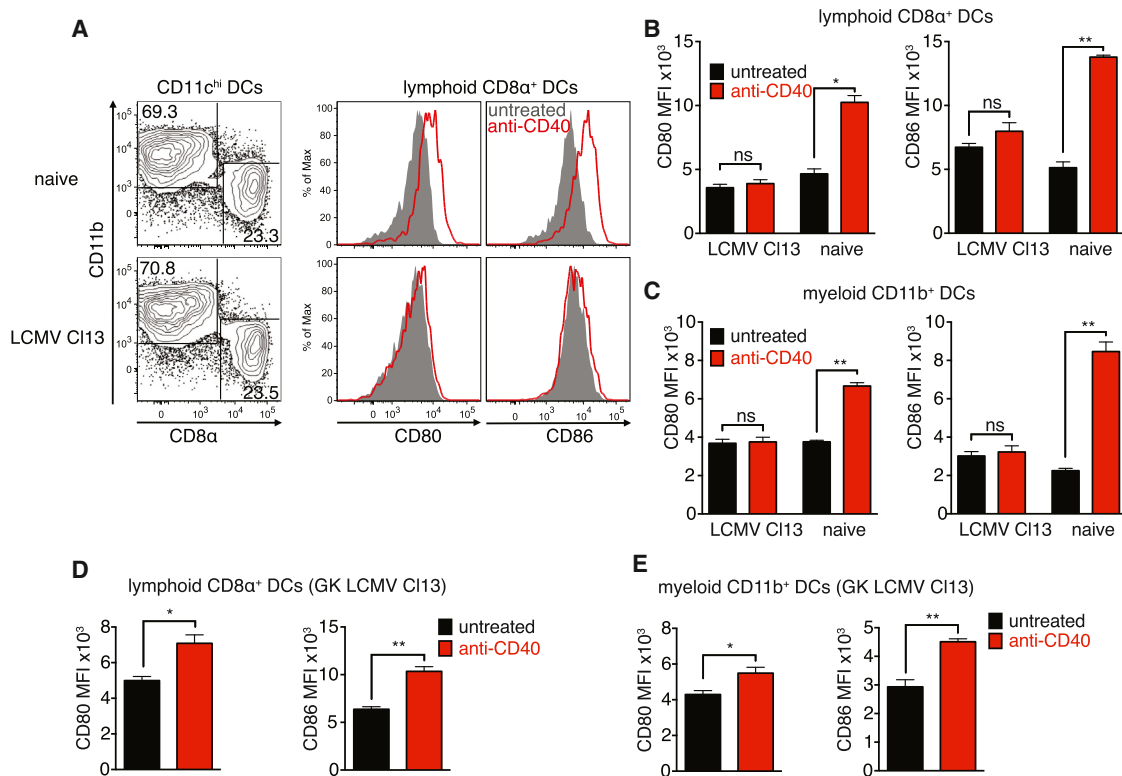
Figure 7. Agonistic Anti-CD40 Fails to Activate DCs in LCMV Clone-13-Infected Mice

(A–C) Naive and LCMV clone-13-infected mice (d21 p.i.) were injected i.p. with 80 μg agonistic anti-CD40 and splenic DC activation was analyzed after 24 hr. (A) Representative flow plots of splenic CD11c^{hi} DCs. Numbers represent frequency of lymphoid CD8α^{+} and myeloid CD11b^{+} DCs as percentage of CD11c^{+} DCs. Representative histograms of CD80 and CD86 expression on lymphoid CD8α^{+} DCs in untreated (gray) or anti-CD40 treated (red) mice. (B) Expression of CD80 and CD86 on lymphoid CD8α^{+} DCs. (C) Expression of CD80 and CD86 on myeloid CD11b^{+} DCs.

(D and E) Expression of CD80 and CD86 on (D) lymphoid DCs and (E) myeloid DCs upon anti-CD40 injection into mice depleted of CD4^{+} T cells prior to LCMV infection (GK LCMV Cl13). Data (mean ± SEM) from one representative experiment (n = 3 mice per group) are shown. Unpaired two-tailed Student's t test was used. *p < 0.05, **p < 0.005. ns indicates not significant.

As antibody-mediated depletion of cells in vivo is thought to be mainly FcγR-mediated (Gong et al., 2005; Uchida et al., 2004) and ICs are considered to be the main ligands for the relevant FcγRs (Nimmerjahn and Ravetch, 2008), we propose that during chronic LCMV clone-13 infection high amounts of ICs occupy FcγRs and thus restrict FcγR-mediated antibody effector functions. This hypothesis was further supported by the lack of DC activation after administration of a FcγR-dependent agonistic anti-CD40 antibody to mice chronically infected with LCMV.

Transfer of polyclonal mouse IgG showed that hypergamma-globulinemia alone at concentrations observed in chronically infected mice is not sufficient to impair the antibody-mediated depletion of cells. However, due to differences in the subclass composition and glycosylation profile of the transferred IgG and the IgG in chronically infected mice, we cannot exclude the possibility that monomeric IgG of LCMV clone-13-infected mice might partially contribute to the observed interference with FcγR-mediated antibody effector functions in vivo.

Rituximab, as well as other therapeutic antibodies, have improved survival rates of cancer patients; however, a number of patients fail to respond to treatment due to mostly unknown reasons. FcγR polymorphisms and increased expression of CD47 on cancer cells have been implicated to predict clinical outcome (Majeti et al., 2009; Musolino et al., 2008). Chronic viral infections, such as HBV, HCV, and HIV, have been linked to cancer development and are estimated to account for up to 18% of global cancer burden (Parkin, 2006). However, no study has yet investigated the effect of a chronic viral infection on ADCC in detail despite its suggested impact on viral control in several studies (DiLillo et al., 2014; Hessell et al., 2007). It is important to point out that we only observed impaired FcγR-mediated processes in our in vivo but not in vitro experiments as ICs probably occupy FcγRs in vivo and thus restrict antibody effector functions such as ADCC. Current assays to measure ADCC activity are routinely performed in vitro and might thus not be able to reflect the in vivo ADCC activity properly.

Reduced efficacy of B cell depletion was first reported in the MRL mouse model of systemic lupus erythematosus (Ahuja et al., 2007). Consistent with our data, a follow-up study demonstrated for the first time that the IgG fraction of lupus serum containing autoantibodies and presumably ICs interfere with antibody-mediated depletion in vivo (Ahuja et al., 2011). Like many chronic viral infections such as HIV, HBV, and HCV (Kawamoto et al., 1993; Zandman-Goddard and Shoenfeld, 2002), persistent LCMV infection has been shown to induce hypergammaglobulinemia for prolonged periods of time. In addition, chronic HBV

and HCV infections have also been reported to be accompanied by ICs (Tsai et al., 1995).

In addition, our results demonstrate that the in vivo activity of an agonistic anti-CD40 antibody, which has been shown to require inhibitory FcγRs, but not FcγR signaling components (Li and Ravetch, 2013), is severely compromised during chronic LCMV infection. This suggests that excessive IC formation during chronic infections and subsequent competition for FcγR-binding is not only impairing FcγR-mediated cellular depletion but also other FcγR-mediated processes in vivo, ranging from activation of innate cells to regulating immune responses and mediating ADCC (Nimmerjahn and Ravetch, 2008; Pincetic et al., 2014).

Uptake of ICs by DCs through activating FcγRs does not only result in efficient processing and presentation of antigens on MHC-I and MHC-II molecules but also influences the maturation of DCs, thereby shaping the cellular immune response (Kalergis and Ravetch, 2002; Regnault et al., 1999). Recent studies have shown that even broadly neutralizing antibodies against influenza virus and HIV require FcγR interactions to mediate their protective activity in vivo (Bournazos et al., 2014; DiLillo et al., 2014; Hessell et al., 2007). NK cells were implicated to play a crucial role for protection, because they are able to lyse virus-infected cells coated with antibodies by ADCC, an FcγR-dependent process likely to be also impaired by excessive IC formation, which could potentially contribute to viral persistence. In addition, because FcγR-mediated phagocytosis has been shown to contribute to clearance of multiple pathogens (Kirimanjeswara et al., 2005; Zhang et al., 2005), the reduced availability of FcγRs during chronic infections might also favor and/or prolong bacterial superinfections. Excessive IC formation might thus contribute in multiple ways to immune dysregulation observed during chronic infections.

Our results might also have important implications for therapeutic interventions aimed at improving immune responses during chronic viral infections and cancer, because multiple therapeutic strategies have shown to require engagement of FcγRs. The therapeutic efficacy of anti-CTLA-4, which has been successfully used to treat melanoma, appears to depend on FcγR interactions to deplete regulatory T cells and enhance antitumor T cell responses in mice (Simpson et al., 2013). In addition, a recent report showed that interleukin-7 (IL-7), a cytokine critical for immune development and homeostasis, applied as IL-7-anti-IL7 mAb complex, has increased potency by extending its half-life due to binding the neonatal FcγR (Martin et al., 2013).

Our results underline the need for more efficient antibody-mediated therapies to overcome the obstacle of high IC concentrations during chronic infections. Interestingly, depletion of T cells using an anti-CD90 antibody was not impaired during chronic LCMV clone-13 infection. One potential explanation might be the abundance of CD90 on the surface of T cells (Haeryfar and Hoskin, 2004). Upon treatment, the density of bound anti-CD90 antibody on the surface of T cells might be above the critical threshold set by competing ICs to allow efficient phagocytosis. However, the exact mechanism by which this antibody exerts its function in the presence of high IC is still under investigation.

A previous study showed that persistent administration of high doses of anti-hCD20 results in successful B cell depletion in MRL mice, demonstrating as well the general feasibility to deplete cells in the presence of high amounts of ICs (Ahuja et al., 2007). A more cost-effective and promising strategy might be the use of mAbs, designed to improve the delivered effector functions by enhancing their affinity for FcγRs (Alduaij and Illidge, 2011). Increased affinity for FcγRs might be especially beneficial in situations when therapeutic antibodies are competing with endogenous ICs for binding to FcγRs.

It has been recently shown that blocking CD47 synergizes with rituximab to promote phagocytosis of NHL (Chao et al., 2010; Jaiswal et al., 2009). In our study, we detected no differences regarding the expression of CD47 in naive and LCMV clone-13-infected mice. However, it is possible that in the presence of excessive ICs and thus reduced availability of FcγRs, signaling via basal CD47 expression is sufficient to outweigh the limited FcγR engagement and therefore prevent phagocytosis of target cells.

In summary, our study provides insight into a mechanism by which chronic viral infections can interfere with humoral immunity. While T cell dysfunction and its impact on viral control have been well studied, less is known about dysfunctions of the humoral immune system during chronic infections. Our study shows that chronic LCMV infection interferes with FcγR-mediated antibody effector functions by excessive IC formation. This might represent an additional mechanism besides T cell exhaustion contributing to viral persistence. Our study also has important implications for the treatment of autoimmune diseases and cancer with depleting antibodies relying on the engagement of FcγRs.

EXPERIMENTAL PROCEDURES

Mice and Viruses

The mouse and virus strains used and procedures are described in the Supplemental Experimental Procedures. Animals were housed at the Emory University School of Medicine animal facility, and experiments were performed in accordance with approved IACUC protocols.

Flow Cytometry and Antibodies

Lymphocytes were isolated from spleen and blood as described previously (Barber et al., 2006). Cells were stained with fluorochrome-conjugated antibodies (purchased from BioLegend). The polyclonal anti-human IgG Fcγ fragment-specific antibody used to detect bound rituximab on the surface of B cells was purchased from eBioscience. Dead cells were stained using Live/Dead Fixable Aqua Dead Cell Stain (Invitrogen). After staining, cells were fixed in 1% paraformaldeyde and acquired using FACS Calibur or Canto II flow cytometer (BD Biosciences). Data were analyzed using FlowJo (Treestar).

Depletion of Lymphocytes and Phagocytic Cells

Detailed procedures are described in the Supplemental Experimental Procedures.

DC Activation

Mice were injected i.p. with 80 μg of agonistic anti-CD40 antibody (FGK45; BioXCell) 24 hr prior to analysis.

ELISA

Detailed procedures are described in the Supplemental Experimental Procedures.

In Vitro Phagocytosis Assay

Peritoneal exudate cells (PECs) were obtained by peritoneal lavage of naive and LCMV clone-13 infected C57BL/6 mice 3 days after injection of 3% thioglycollate broth. Splenic macrophages/monocytes were purified from spleens

of naive and LCMV clone-13-infected mice using CD11b$^+$ magnetic beads (Miltenyi). The analysis of the phagocytic capacity of PEC and splenic macrophages/monocytes in vitro was carried out using flow cytometry as described earlier (Shashidharamurthy et al., 2008; Zhang et al., 2005). Briefly, sheep erythrocytes were coated with dinitrophenyl (DNP), opsonized with Alexa488-labeled rabbit anti-DNP antibodies, and incubated at a ratio of 1:50 (macrophages to erythrocytes) in the presence or absence of FcγRII/III-blocking antibody (2.4G2; BD PharMingen).

In Vivo Depletion Assay
This assay was performed similarly as a previously described in vivo cytotoxicity assay (Barber et al., 2003). CD4$^+$ T cells from spleens of infected and uninfected C57BL/6 mice were purified to >90% purity by depletion of non-CD4$^+$ T cells using magnetic beads (Miltenyi), followed by labeling with different concentrations of CellTrace Violet and CFSE (both from Invitrogen), and coating with anti-CD4 antibody (GK1.5; BioExpress). A mixture of cells containing equal proportions of anti-CD4 coated and uncoated cells from uninfected, LCMV Arm-immune and LCMV clone-13-infected mice was then transfer by i.v. injection to uninfected, LCMV Arm-immune and LCMV clone-13-infected C57/BL6 mice. Mice were sacrificed 3–4 hr post transfer and in vivo phagocytic activity was determined by flow cytometric analysis of fluorescently labeled CD3$^+$ CD8$^-$ splenocytes.

Immunoglobulin Transfer
Mice were injected i.v. with 10 mg mouse IgG in a total volume of 500 μl PBS. Polyclonal mouse IgG (Kunming strain) was purchased from Jackson ImmunoResearch. Monoclonal mouse IgG (isotype IgG2b) was purified from culture supernatants of the hybridoma 2H7, a kind gift of Edward Clark (University of Washington), as described previously (Ahuja et al., 2007).

Glycan Analysis
Procedures are described in the Supplemental Experimental Procedures.

Statistical Analysis
All data were analyzed using Prism 5.0 (GraphPad).

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2015.01.009.

REFERENCES

Abboud, N., Chow, S.K., Saylor, C., Janda, A., Ravetch, J.V., Scharff, M.D., and Casadevall, A. (2010). A requirement for FcγR in antibody-mediated bacterial toxin neutralization. J. Exp. Med. 207, 2395–2405.

Ahuja, A., Shupe, J., Dunn, R., Kashgarian, M., Kehry, M.R., and Shlomchik, M.J. (2007). Depletion of B cells in murine lupus: efficacy and resistance. J. Immunol. 179, 3351–3361.

Ahuja, A., Teichmann, L.L., Wang, H., Dunn, R., Kehry, M.R., and Shlomchik, M.J. (2011). An acquired defect in IgG-dependent phagocytosis explains the

impairment in antibody-mediated cellular depletion in Lupus. J. Immunol. 187, 3888–3894.

Alduaij, W., and Illidge, T.M. (2011). The future of anti-CD20 monoclonal antibodies: are we making progress? Blood 117, 2993–3001.

Anolik, J., Looney, R.J., Bottaro, A., Sanz, I., and Young, F. (2003). Down-regulation of CD20 on B cells upon CD40 activation. Eur. J. Immunol. 33, 2398–2409.

Barber, D.L., Wherry, E.J., and Ahmed, R. (2003). Cutting edge: rapid in vivo killing by memory CD8 T cells. J. Immunol. 171, 27–31.

Barber, D.L., Wherry, E.J., Masopust, D., Zhu, B., Allison, J.P., Sharpe, A.H., Freeman, G.J., and Ahmed, R. (2006). Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439, 682–687.

Barrington, R.A., Pozdnyakova, O., Zafari, M.R., Benjamin, C.D., and Carroll, M.C. (2002). B lymphocyte memory: role of stromal cell complement and FcgammaRIIB receptors. J. Exp. Med. 196, 1189–1199.

Bennett, S.R., Carbone, F.R., Karamalis, F., Flavell, R.A., Miller, J.F., and Heath, W.R. (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature 393, 478–480.

Bournazos, S., Klein, F., Pietzsch, J., Seaman, M.S., Nussenzweig, M.C., and Ravetch, J.V. (2014). Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. Cell 158, 1243–1253.

Browning, J.L. (2006). B cells move to centre stage: novel opportunities for autoimmune disease treatment. Nat. Rev. Drug Discov. 5, 564–576.

Buchmeier, M.J., and Oldstone, M.B. (1978). Virus-induced immune complex disease: identification of specific viral antigens and antibodies deposited in complexes during chronic lymphocytic choriomeningitis virus infection. J. Immunol. 120, 1297–1304.

Chao, M.P., Alizadeh, A.A., Tang, C., Myklebust, J.H., Varghese, B., Gill, S., Jan, M., Cha, A.C., Chan, C.K., Tan, B.T., et al. (2010). Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. Cell 142, 699–713.

Cheson, B.D., and Leonard, J.P. (2008). Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. N. Engl. J. Med. 359, 613–626.

DiLillo, D.J., Tan, G.S., Palese, P., and Ravetch, J.V. (2014). Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo. Nat. Med. 20, 143–151.

Edwards, J.C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P., Close, D.R., Stevens, R.M., and Shaw, T. (2004). Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N. Engl. J. Med. 350, 2572–2581.

Gong, Q., Ou, Q., Ye, S., Lee, W.P., Cornelius, J., Diehl, L., Lin, W.Y., Hu, Z., Lu, Y., Chen, Y., et al. (2005). Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. J. Immunol. 174, 817–826.

Haeryfar, S.M., and Hoskin, D.W. (2004). Thy-1: more than a mouse pan-T cell marker. J. Immunol. 173, 3581–3588.

Halper-Stromberg, A., Lu, C.L., Klein, F., Horwitz, J.A., Bournazos, S., Nogueira, L., Eisenreich, T.R., Liu, C., Gazumyan, A., Schaefer, U., et al. (2014). Broadly Neutralizing Antibodies and Viral Inducers Decrease Rebound from HIV-1 Latent Reservoirs in Humanized Mice. Cell 158, 989–999.

Hessell, A.J., Hangartner, L., Hunter, M., Havenith, C.E., Beurskens, F.J., Bakker, J.M., Lanigan, C.M., Landucci, G., Forthal, D.N., Parren, P.W., et al. (2007). Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449, 101–104.

Hu, Y., Turner, M.J., Shields, J., Gale, M.S., Hutto, E., Roberts, B.L., Siders, W.M., and Kaplan, J.M. (2009). Investigation of the mechanism of action of alemtuzumab in a human CD52 transgenic mouse model. Immunology 128, 260–270.

Hunziker, L., Recher, M., Macpherson, A.J., Ciurea, A., Freigang, S., Hengartner, H., and Zinkernagel, R.M. (2003). Hypergammaglobulinemia and autoantibody induction mechanisms in viral infections. Nat. Immunol. 4, 343–349.

Jaiswal, S., Jamieson, C.H., Pang, W.W., Park, C.Y., Chao, M.P., Majeti, R., Traver, D., van Rooijen, N., and Weissman, I.L. (2009). CD47 is upregulated

on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell 138, 271–285.

Jilani, I., O'Brien, S., Manshuri, T., Thomas, D.A., Thomazy, V.A., Imam, M., Naeem, S., Verstovsek, S., Kantarjian, H., Giles, F., et al. (2003). Transient down-modulation of CD20 by rituximab in patients with chronic lymphocytic leukemia. Blood 102, 3514–3520.

Jing, W., Gershan, J.A., and Johnson, B.D. (2009). Depletion of CD4 T cells enhances immunotherapy for neuroblastoma after syngeneic HSCT but compromises development of antitumor immune memory. Blood 113, 4449–4457.

Kalergis, A.M., and Ravetch, J.V. (2002). Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J. Exp. Med. 195, 1653–1659.

Kaneko, Y., Nimmerjahn, F., and Ravetch, J.V. (2006). Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313, 670–673.

Kawamoto, H., Sakaguchi, K., Takaki, A., Ogawa, S., and Tsuji, T. (1993). Autoimmune responses as assessed by hypergammaglobulinemia and the presence of autoantibodies in patients with chronic hepatitis C. Acta Med. Okayama 47, 305–310.

Kirimanjeswara, G.S., Mann, P.B., Pilione, M., Kennett, M.J., and Harvill, E.T. (2005). The complex mechanism of antibody-mediated clearance of Bordetella from the lungs requires TLR4. J. Immunol. 175, 7504–7511.

Li, F., and Ravetch, J.V. (2011). Inhibitory Fcγ receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. Science 333, 1030–1034.

Li, F., and Ravetch, J.V. (2013). Antitumor activities of agonistic anti-TNFR antibodies require differential FcγRIIB coengagement in vivo. Proc. Natl. Acad. Sci. USA 110, 19501–19506.

Li, Y., Williams, M.E., Cousar, J.B., Pawluczkowycz, A.W., Lindorfer, M.A., and Taylor, R.P. (2007). Rituximab-CD20 complexes are shaved from Z138 mantle cell lymphoma cells in intravenous and subcutaneous SCID mouse models. J. Immunol. 179, 4263–4271.

Majeti, R., Chao, M.P., Alizadeh, A.A., Pang, W.W., Jaiswal, S., Gibbs, K.D., Jr., van Rooijen, N., and Weissman, I.L. (2009). CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell 138, 286–299.

Martin, C.E., van Leeuwen, E.M., Im, S.J., Roopenian, D.C., Sung, Y.C., and Surh, C.D. (2013). 7/anti-IL-7 mAb complexes augment cytokine potency in mice through association with IgG-Fc and by competition with IL-7R. (IL: Blood).

Mason, D.Y., Jones, M., and Goodnow, C.C. (1992). Development and follicular localization of tolerant B lymphocytes in lysozyme/anti-lysozyme IgM/IgD transgenic mice. Int. Immunol. 4, 163–175.

Matloubian, M., Kolhekar, S.R., Somasundaram, T., and Ahmed, R. (1993). Molecular determinants of macrophage tropism and viral persistence: importance of single amino acid changes in the polymerase and glycoprotein of lymphocytic choriomeningitis virus. J. Virol. 67, 7340–7349.

Matloubian, M., Concepcion, R.J., and Ahmed, R. (1994). CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J. Virol. 68, 8056–8063.

Musolino, A., Naldi, N., Bortesi, B., Pezzuolo, D., Capelletti, M., Missale, G., Laccabue, D., Zerbini, A., Camisa, R., Bisagni, G., et al. (2008). Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J. Clin. Oncol. 26, 1789–1796.

Nimmerjahn, F., and Ravetch, J.V. (2008). Fcgamma receptors as regulators of immune responses. Nat. Rev. Immunol. 8, 34–47.

Oldstone, M.B., Buchmeier, M.J., Doyle, M.V., and Tishon, A. (1980). Virus-induced immune complex disease: specific anti-viral antibody and C1q binding material in the circulation during persistent lymphocytic choriomeningitis virus infection. J. Immunol. 124, 831–838.

Parkin, D.M. (2006). The global health burden of infection-associated cancers in the year 2002. Int. J. Cancer 118, 3030–3044.

Pincetic, A., Bournazos, S., DiLillo, D.J., Maamary, J., Wang, T.T., Dahan, R., Fiebiger, B.M., and Ravetch, J.V. (2014). Type I and type II Fc receptors regulate innate and adaptive immunity. Nat. Immunol. 15, 707–716.

Regnault, A., Lankar, D., Lacabanne, V., Rodriguez, A., Théry, C., Rescigno, M., Saito, T., Verbeek, S., Bonnerot, C., Ricciardi-Castagnoli, P., and Amigorena, S. (1999). Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J. Exp. Med. 189, 371–380.

Schoenberger, S.P., Toes, R.E., van der Voort, E.I., Offringa, R., and Melief, C.J. (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 393, 480–483.

Sevilla, N., Kunz, S., Holz, A., Lewicki, H., Homann, D., Yamada, H., Campbell, K.P., de La Torre, J.C., and Oldstone, M.B. (2000). Immunosuppression and resultant viral persistence by specific viral targeting of dendritic cells. J. Exp. Med. 192, 1249–1260.

Shashidharamurthy, R., Hennigar, R.A., Fuchs, S., Palaniswami, P., Sherman, M., and Selvaraj, P. (2008). Extravasations and emigration of neutrophils to the inflammatory site depend on the interaction of immune-complex with Fcgamma receptors and can be effectively blocked by decoy Fcgamma receptors. Blood 111, 894–904.

Shields, R.L., Lai, J., Keck, R., O'Connell, L.Y., Hong, K., Meng, Y.G., Weikert, S.H., and Presta, L.G. (2002). Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J. Biol. Chem. 277, 26733–26740.

Silveira, P.A., Johnson, E., Chapman, H.D., Bui, T., Tisch, R.M., and Serreze, D.V. (2002). The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. Eur. J. Immunol. 32, 3657–3666.

Simpson, T.R., Li, F., Montalvo-Ortiz, W., Sepulveda, M.A., Bergerhoff, K., Arce, F., Roddie, C., Henry, J.Y., Yagita, H., Wolchok, J.D., et al. (2013). Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J. Exp. Med. 210, 1695–1710.

Soares, M.M., King, S.W., and Thorpe, P.E. (2008). Targeting inside-out phosphatidylserine as a therapeutic strategy for viral diseases. Nat. Med. 14, 1357–1362.

Tsai, J.F., Margolis, H.S., Jeng, J.E., Ho, M.S., Chang, W.Y., Lin, Z.Y., and Tsai, J.H. (1995). Circulating immune complexes in chronic hepatitis related to hepatitis C and B viruses infection. Clin. Immunol. Immunopathol. 75, 39–44.

Uchida, J., Hamaguchi, Y., Oliver, J.A., Ravetch, J.V., Poe, J.C., Haas, K.M., and Tedder, T.F. (2004). The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J. Exp. Med. 199, 1659–1669.

Varchetta, S., Gibelli, N., Oliviero, B., Nardini, E., Gennari, R., Gatti, G., Silva, L.S., Villani, L., Tagliabue, E., Ménard, S., et al. (2007). Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer overexpressing Her2. Cancer Res. 67, 11991–11999.

Yang, X., Zhang, X., Mortenson, E.D., Radkevich-Brown, O., Wang, Y., and Fu, Y.X. (2013). Cetuximab-mediated tumor regression depends on innate and adaptive immune responses. Mol. Ther. 21, 91–100.

Zajac, A.J., Blattman, J.N., Murali-Krishna, K., Sourdive, D.J., Suresh, M., Altman, J.D., and Ahmed, R. (1998). Viral immune evasion due to persistence of activated T cells without effector function. J. Exp. Med. 188, 2205–2213.

Zandman-Goddard, G., and Shoenfeld, Y. (2002). HIV and autoimmunity. Autoimmun. Rev. 1, 329–337.

Zhang, J., Guo, J., Dzhagalov, I., and He, Y.W. (2005). An essential function for the calcium-promoted Ras inactivator in Fcgamma receptor-mediated phagocytosis. Nat. Immunol. 6, 911–919.