Macrophages prevent the differentiation of autoreactive B cells by secreting CD40 ligand and interleukin-6

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Activation of the innate immune system promotes polyclonal antibody secretion to eliminate invading pathogens. Inherent in this process is the potential to activate autoreactive B cells and induce autoimmunity. We showed previously that TLR-stimulated dendritic cells and macrophages regulate B cell tolerance to Smith antigen, in part through the secretion of interleukin-6 (IL-6). In this manuscript, we show that neutralization of IL-6 fails to abrogate macrophage-mediated repression and identify soluble CD40 ligand (CD40L) as a second repressive factor secreted by macrophages. CD40L selectively repressed Ig secretion by chronically antigen-experienced (anergic) immunoglobulin transgenic and nontransgenic B cells but not by transiently stimulated B cells. The importance of macrophages in maintaining B cell tolerance was apparent in lupus-prone MRL/lpr mice. Compared with C57BL/6 mice, macrophages from MRL/lpr mice were significantly less efficient at repressing immunoglobulin secretion coincident with diminished IL-6 and CD40 ligand production. These data indicate that macrophages regulate autoreactive B cells by secreting repressive factors that prohibit terminal differentiation of B cells. The regulation of autoreactive B cells by macrophages is diminished in lupus-prone mice suggesting a role in autoimmunity. (Blood. 2007;110:1595-1602) © 2007 by The American Society of Hematology

Introduction

Recognition of microorganisms by Toll-like receptors (TLRs) promotes inflammation and stimulates the innate immune system to produce antibody, responses that are beneficial in clearing infections. However, TLR ligation of autoreactive B cells can lead to transient or persistent autoimmunity.1 Studies of rheumatoid factor-specific B cells show that immune complexes containing TLR and B-cell receptor (BCR) ligands induce proliferation of autoreactive B cells.2-4 Likewise, anti–double-stranded DNA (dsDNA)–specific B cells proliferate in response to BCR-mediated internalization of chromatin.5 Because most nuclear self-antigens contain BCR and TLR ligands, these findings suggest that stimulation of autoreactive B cells through the BCR and/or TLR activates some autoreactive B cells.

During T-dependent immune responses, CD40 stimulation induces B-cell proliferation, increases the expression of costimulatory molecules, and promotes germinal center formation leading to high affinity, class-switched antibodies. Continuous exposure to CD40 ligand (CD40L) promotes the formation of memory cells by blocking B lymphocyte–induced maturation protein-1 (Blimp-1) expression and arresting plasma cell differentiation.6 CD40/CD40L interactions also regulate autoreactive B cells that encounter activated CD4+ T cell.7 Hen egg lysozyme (HEL)–specific B cells that have been continuously exposed to self-antigen up-regulate Fas in response to CD40L stimulation. Subsequent encounter with an activated HEL–specific T cell induces Fas-dependent B-cell apoptosis, thereby preventing autoimmunity. Thus, persistent exposure to self-antigen modulates Fas and CD40 to induce apoptosis or prevent terminal differentiation.

We recently identified a mechanism of tolerance that regulates autoreactive B cells during innate immune responses. In response to lipopolysaccharide (LPS), dendritic cells (DCs) and macrophages (MФs) regulate HEL-, p-azophenylarsonate (Ars)- and low-affinity (2-12H/V) Smith antigen (Sm)-specific B cells through the secretion of interleukin-6 (IL-6).8 Regulation of immunoglobulin (Ig) secretion is selective in that chronically antigen-experienced (anergic) B cells are repressed, whereas transiently stimulated naive B cells are not. This indicates that tolerance within the B-cell compartment extends beyond antigen-induced receptor desensitization and that persistent BCR ligation affects other receptors. Herein, we report that in addition to IL-6, MФs secrete soluble CD40L (sCD40L), which selectively represses Ig secretion by chronically antigen-experienced B cells. Regulation was also apparent in nontransgenic (non-Tg) B cells where anti-nucleosome responses were repressed by sCD40L. sCD40L-mediated repression did not reflect changes in proliferation; rather, it revealed a reduction in the number of B cells that differentiated into antibody secreting cells (ASCs). Finally, we show that MФs derived from autoimmune-prone MRL/lpr mice failed to repress Sm-specific B cells coincident with diminished production of IL-6 and sCD40L, suggesting that MФ-mediated tolerance may play a role in regulating autoimmunity. Collectively, the data show that the history of antigen binding determines whether LPS-induced Ig secretion is repressed or enhanced by IL-6.
Materials and methods

Mice

2-12H/Vsx8 (80% follicular [FO], 1% marginal zone [MZ]), 2-12H10 (70% FO, 12% MZ),12 and Ars/A1 (78% FO, 0.9% MZ)13 (K.A., L.J.W., unpublished observations, November 2002) Ig transgenic (IgTg) mice have been described. HEL-Ig (MD4; 70% FO, 7% MZ) and HEL-Ig (encoded by 2-12H/Vsx8 or Ars/A1) was measured as described previously.9 A standard curve was generated using mouse IgMa/HEL complexes (mHEL-2) and recombinant sCD40L (rsCD40L) was from R&D Systems (Minneapolis, MN). The trimeric form of CD40L has the most biologic activity; however, the manufacturer reports no trimeric protein by Silver stain. 183; Sigma). IgM (C57BL/6) was detected using anti-mouse IgM (clone HB101, 33-60 (anti-IgM), B7.6 (anti-IgM), MR1 (anti-CD40L), and 54.1 (3-83 (anti-CD40L), B7.6 (anti-IgM), and revealed how naive and autoreactive B cells are differentially regulated to ensure immunity in the absence of autoimmunity during innate immune responses.

Antibodies and other reagents

Neutralizing anti-CD40L, hamster IgG (isotype control for anti-CD40L), neutralizing anti-IL-6, and recombinant IL-6 (rIL-6) were from BD Biosciences (San Jose, CA). Recombinant sCD40L (rsCD40L) was from R&D Systems (Minneapolis, MN). The trimeric form of CD40L has the most biologic activity; however, the manufacturer reports no trimeric protein by Silver stain. 183; Sigma). IgM (C57BL/6) was detected using anti-mouse IgM (clone HB101, 33-60 (anti-IgM), B7.6 (anti-IgM), MR1 (anti-CD40L), and 54.1 (3-83 (anti-CD40L), B7.6 (anti-IgM), and revealed how naive and autoreactive B cells are differentially regulated to ensure immunity in the absence of autoimmunity during innate immune responses.

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5'-TGTGGATCTTCTGAGGAAA-3'  and  5'-GGTTGCGCTTTGGAGTCC-3' and 5'-GGTGAAGCTGCTTTGGAG-3'. Data were analyzed using the 2^(-ΔΔCT) method: relative expression = 2^(-ΔΔCT) where ΔΔCT = (cycle threshold [CT] gene of interest) – (CT 18s rRNA) in experimental sample – (CT gene of interest) – (CT 18s rRNA) time zero.20

**Quantum dot staining of CD40L**

C57BL/6 and MRL/lpr BMMΦs were plated on poly-d-Lysine-coated coverslips for 2 hours then LPS-stimulated (15 μg/mL; List Biological Laboratories Inc, Campbell, CA) for 3 hours. Cells were fixed with 1.5% paraformaldehyde, permeabilized with ice-cold 100% methanol, and stained with biotinylated anti-mouse CD40L (clone MR1) followed by secondary staining with Quantum dot (Qdot) 655 streptavidin (Invitrogen). Samples were mounted using 60%/1.4 numerical aperture oil plan apochromat objective lens and the Olympus Fluoview with Time acquisition software. Data analysis was done using ImageJ 1.37c software (http://rsb.info.nih.gov/ij/).

**Results**

B cells expressing the 2-12H Ig light chain transgene bind the small nuclear ribonucleoprotein, Sm, with low affinity.21 Sm-specific B cells fail to secrete Ig upon LPS stimulation unless the splenic B-cell preparations lack DCs and MΦs.8,9 We previously described a novel mechanism of tolerance in which DCs and MΦs regulate autoreactive B cells during innate immune responses.8 IL-6, secreted by DCs and MΦs, represses Ig secretion by chronically antigen-experienced B cells but has no affect on Ig secretion by naive B cells.8 We were surprised, however, that repression of autoantibody secretion by CM prepared from MΦs was not alleviated by the addition of anti-IL-6, although MΦs secrete significant amounts of IL-6 (36.1 ± 6.8 ng/mL) (Figure 1A).8 To investigate whether other factors secreted by MΦs repressed autoantibody secretion, we prepared CM from LPS-stimulated MΦs from IL-6 deficient (IL-6−/−) mice. MΦ CM prepared from IL-6−/− mice repressed 47% of Ig secretion (Figure 1A), indicating that in addition to IL-6, MΦs secrete other factors that repress Ig secretion by autoreactive B cells.

To identify the other factor(s), we neutralized CM from IL-6−/− MΦs with a panel of antibodies. The addition of neutralizing anti-CD40L completely restored Ig secretion (Figure 1B). To confirm these findings, we added rsCD40L to cultures containing Sm-specific B cells. As shown in Figure 1C, rsCD40L (100 ng/mL) inhibited 74% of Ig secretion. This was a direct effect of sCD40L on B cells because sorted B cells (99% pure) were similarly repressed (data not shown). We were concerned that 100 ng/mL rsCD40L was not physiologically relevant and might be due to low bioactivity. To assess this, we treated purified Sm-specific B cells with supernatant from a CHO cell line expressing CD40L. As little as 9 pg/mL (1:128 dilution) repressed 63% of secretion. Repression was due to sCD40L because supernatant from nontransfected parental CHO-K1 cells failed to repress Ig secretion. The trimeric form of sCD40L contains the most biological activity, although monomers do have low levels of activity.22 Because the rsCD40L preparation is likely to contain a mixture of both oligomerized and nonoligomerized forms, we treated Sm-specific B cells with monomeric and oligomeric sCD40L to determine which form repressed Ig secretion. LPS-stimulated Ig secretion was similar in the presence or absence of monomeric sCD40L (rsCD40L); however, when a crosslinking antibody was added to oligomerize the sCD40L, Ig secretion was repressed 63% (data not shown). This indicates that the oligomerized form of sCD40L represses autoantibody secretion. The amount of trimer in the rsCD40L preparation was below the limit of detection by silver staining, confirming the low bioactivity of the recombinant protein. To determine whether MΦs secrete repressive factors other than IL-6 and sCD40L, we neutralized LPS-activated MΦ CM with anti-IL-6...
and anti-CD40L. Neutralization with either anti–IL-6 or anti-CD40L failed to restore Ig secretion (Figure 1D). However, neutralization with both antibodies restored 95% of secretion. Collectively, the data indicate that MΦs repress autoantibody secretion through the production of IL-6 and sCD40L. Although both factors are equally competent to regulate secretion, only one factor is required.

We previously reported that only chronically antigen-experienced B cells are regulated by IL-6, suggesting that persistent antigen exposure reprograms the IL-6 receptor to repress LPS-induced Ig secretion.8 To determine whether sCD40L exhibited the same specificity, we compared the effects of rsCD40L on naive and chronically antigen-experienced B cells. rsCD40L repressed 53% of Ig secretion by HEL-specific B cells that had been continuously exposed to shHEL (HEL-Ig × shHEL) (Figure 2A). In contrast, rsCD40L did not affect LPS-induced Ig secretion by naive HEL-specific B cells (HEL-Ig). To assess whether sCD40L regulated other chronically antigen-experienced B cells, we measured Ig secretion by Ars-specific B cells. Ars/A1 IgTg mice express a heavy and light chain pair that binds Ars; however, B cells from these mice cross-react with single-stranded DNA, conferring an anergic phenotype.11,23 Similar to the effects on HEL-specific B cells, rsCD40L repressed 43% of LPS-induced Ig secretion by purified Ars-specific B cells (Figure 2B). To determine whether autoreactive B cells in a population of nonautoreactive cells could be regulated by sCD40L, we measured the amount of nucleosome-specific Ig produced by C57BL/6 B cells. As shown in Figure 2C, LPS-induced anti-nucleosome secretion was repressed 40% by rsCD40L, whereas total IgM secretion in the same cultures was unaffected. These data indicate that during innate immune responses, B cells continuously exposed to self-antigen are repressed by sCD40L. Further, the repressive effects of MΦs are not restricted to Sm-specific B cells.

Our data show that multiple soluble factors secreted by DCs and MΦs regulate autoreactive B cells, suggesting a possible redundant function. Alternatively, DCs and MΦs might exhibit specialized functions with different soluble factors regulating unique B-cell subsets. To examine this, we isolated FO and MZ B cells from 2-12H mice and assessed the effects of DCs, MΦs, rsCD40L, and rIL-6 on their differentiation into ASCs. The 2-12H mice express the same heavy chain as the 2-12H/Vκx8 mice, but it pairs with endogenous light chains, resulting in antigen receptors of various affinities for Sm.10 We used these mice because the number of MZ B cells is higher compared with 2-12H/Vκx8 mice.8,17 As shown in Figure 2D, differentiation of FO B cells into ASC was significantly reduced by the addition of DCs, MΦs, rIL-6, and rsCD40L to the cultures. In contrast, MZ B cells were unaffected by DCs and rIL-6; however, addition of MΦs or rsCD40L reduced the number of ASCs by 41% and 42%, respectively. This indicates that MZ B cells are partially regulated by MΦ secretion of sCD40L, but not IL-6. Collectively, the data indicate that the use of multiple factors is redundant in the repression of FO B cells but sCD40L plays a unique role in repressing MZ B cells.

Because termination of cell division is required for plasma cell differentiation, we reasoned that impaired cell-cycle arrest may be the mechanism through which sCD40L repressed Ig secretion. To assess this, we labeled naive (C57BL/6) and autoreactive (2-12H/Vκx8) B cells with CFSE and calculated the proliferative index (PI); an indication of the average number of divisions per cell (Figure 3A). LPS-stimulated C57BL/6 B cells treated with rsCD40L (PI = 6.6 ± 0.26) showed enhanced proliferation compared with untreated cells (PI = 4.7 ± 0.18). Likewise, LPS-stimulated 2-12H/Vκx8 B cells treated with rsCD40L (PI = 5.1 ± 0.25) proliferated more than untreated cells (PI = 3.7 ± 0.39), revealing that rsCD40L has similar affects on the proliferation of naive and autoreactive B cells. This was consistent with comparable increases in the numbers of viable B cells in rsCD40L-treated C57BL/6 and 2-12H/Vκx8 cultures after 3 days (data not shown). Thus, the ability of sCD40L to repress Ig secretion was not due to prolonged proliferation.

Germinal center B cells stimulated through CD40 are blocked from terminally differentiating into ASCs.6,24,25 To determine whether sCD40L regulated autoreactive B cells by a mechanism similar to cell fate decisions in the germinal center, we quantitated intracellular IgM and enumerated plasma-cell formation. As shown in Figure 3C, 7% of Sm-specific B cells became intracellular IgM+ after 3 days of LPS-stimulation. Addition of rsCD40L reduced the number of intracellular IgM+ cells by 50%. Paralleling this decrease, the presence of rsCD40L inhibited the number of ASC by 49% (Figure 3D). In contrast, the number of intracellular IgM+ cells and the number of ASCs in LPS-stimulated C57BL/6 cultures was minimally affected by treatment with rsCD40L (Figure 3C,D). It is noteworthy that the number of intracellular IgM+ cells in nonautoreactive mice (C57BL/6) is significantly higher than auto-reactive mice (2-12H/Vκx8). This might indicate that despite removal of the regulatory mechanisms conferred by DCs/MΦs, some cells maintain intrinsic regulatory mechanisms that repress Ig secretion. These data indicate that sCD40L prevents Ig secretion by inhibiting the differentiation of autoreactive B cells into plasma cells.

**Figure 2.** Soluble mediators selectively repress chronically Ag-experienced B cells and differentially regulate autoreactive FO and MZ B cells. B cells (1 × 10⁵) from HEL-Ig × shHEL and HEL-Ig (A), Ars/A1 (B), or C57BL/6 mice (C) were stimulated with LPS (30 μg/mL) in the presence or absence of rsCD40L (100 ng/mL) for 4 days. Anti-HEL IgM (HEL-Ig and HEL-Ig × shHEL), IgM+ (Ars/A1), IgM, and anti-nucleosome Ig (C57BL/6) were quantitated by ELISA. LPS-stimulated B cells (100%) secreted 9-15 ng/mL (HEL-Ig), 16-47 ng/mL (HEL-Ig × shHEL), 2-9 ng/mL (Ars/A1), 56-156 ng/mL anti-nucleosome Ig (C57BL/6), and 19-43 ng/mL IgM+ (C57BL/6) (100%) secreted. (D) 1 × 10⁵ Sm-specific (2-12H) FO and MZ B cells were sorted and stimulated with LPS (30 μg/mL) in the absence or presence of BMDCs (1 × 10⁵), rIL-6 (10 ng/mL), or rsCD40L (100 ng/mL). The number of ASCs was determined on day 3 using an Sm-specific enzyme-linked immunosorbent spot (ELISPOT). LPS-stimulated FO B cells (100%) yielded 2.8 ± 1.0 to 1 × 10⁶ spots/10⁵ cells, whereas MZ B cells (100%) yielded 2.3 ± 1.3 × 10⁶ spots/10⁵ cells. Statistical analysis was performed using 1-sample t test by comparing treated and untreated cultures. Data represent at least 3 experiments. Error bars represent plus or minus SEM. (*P < .05.)
Terminal differentiation of B cells requires expression of the transcriptional activators, Blimp-1, and XBP-1. We reasoned that sCD40L might prevent autoantibody production by directly or indirectly regulating Blimp-1 and XBP-1. Real-time PCR analysis showed that Blimp-1 (Figure 3E) and XBP-1 (Figure 3F) mRNA was up-regulated by LPS stimulation of autoreactive B cells. However, treatment with rsCD40L reduced Blimp-1 and XBP-1 mRNA levels by 47% and 58%, respectively. Collectively, the data show that despite maintaining comparable proliferation, sCD40L blocks the formation of ASCs through the regulation of transcription factors required for terminal differentiation. This allows MΦs to regulate autoreactive MZ and FO B cells during innate immune responses.

The defects underlying autoimmune disease are poorly defined. Our data identify a unique mechanism of tolerance wherein MΦs selectively repress Ig secretion through the secretion of IL-6 and sCD40L. To distinguish defects in the production of soluble mediators from the effects of cell-cell contact, we assessed the ability of CM from MRL/lpr MΦs to regulate Ig secretion. Similar to the results obtained from intact cells, CM from C57BL/6 MΦs repressed 77% of Ig secretion, whereas CM from MRL/1pr MΦs repressed 48% (Figure 4B). Because the defect in MΦ CM was comparable with the defect found when intact MΦs were cocultured with Sm-specific B cells, we reasoned that defects in the production of soluble mediators might be responsible. To address this, we quantitated IL-6 and CD40L levels. Consistent with previous reports, MΦs derived from MRL/lpr mice secreted significantly less IL-6 than MΦs from C57BL/6 mice (Figure 4C). We were unable to detect sCD40L in CM from C57BL/6 or MRL/lpr MΦs by ELISA; however, it was detectable by immunostaining. Unstimulated C57BL/6 and MRL/lpr MΦs showed negligible CD40L staining (data not shown). In contrast, LPS stimulation induced 55% of the C57BL/6 MΦs to express CD40L compared with 18% of MRL/lpr MΦs (data not shown). Most importantly, the amount of CD40L staining by LPS-stimulated MRL/lpr MΦs was 3-fold lower than LPS-stimulated C57BL/6 MΦs (Figure 4D). We further examined MΦ defects by comparing the repressive ability of CM from ex vivo MΦ isolated from C57BL/6, C57BL/6;lpr, and predisease and postdisease MRL/lpr mice. As shown in Figure 4E, C57BL/6;lpr MΦ CM repressed Ig secretion comparable with C57BL/6 MΦ CM (1.1- vs 1-fold). In contrast, MRL MΦ CM was less repressive (0.64-fold). MRL/lpr MΦ CM derived from predisease and postdisease mice were equally defective at repressing Sm-specific Ig secretion (0.71- vs 0.72-fold). Thus, the data indicate that the MΦ defects are associated with the MRL background and that regardless of disease status, defects in regulating autoreactive B cells occur coincident with failure to secrete soluble mediators that repress terminal differentiation.

**Discussion**

Balancing tolerance and immunity requires strict regulation of Ig production by B lymphocytes. During T-dependent humoral immune responses, the binding of foreign antigen promotes immunity through Ig secretion. In contrast, persistent ligation of self-reactive BCRs ensures tolerance by desensitizing autoreactive receptors to subsequent stimulation. Likewise, innate immune responses require that autoreactive B cells remain unresponsive during polyclonal B-cell activation. Our data show that DCs and MΦs are key regulators of autoreactive B cells during innate immune responses. DCs and MΦs maintain low-affinity Sm-specific B cells in an unresponsive state, in part through the secretion of IL-6. Here we show that in addition to IL-6, MΦs secrete sCD40L to regulate autoreactive B cells. Similar to IL-6, sCD40L selectively represses Ig secretion by B cells continuously exposed to self-antigen but had no affect on naive B cells. The ability of IL-6 and sCD40L to selectively repress chronically antigen experienced B cells was
Figure 4. Repression of Ig secretion by MRL/pr Mφs is defective coincident with a failure to secrete soluble mediators. Sm-specific B cells (1 × 10⁶) were stimulated with LPS (30 μg/mL) and cocultured with the indicated number of C57BL/6 (n = 5) or MRL/pr (n = 9) BMMφs A or LPS-activated Mφ CM from C57BL/6 (n = 5) or MRL/pr (n = 9) mice (B) for 4 days. IgM%A was determined by ELISA. LPS-stimulated B cells (100%) secreted 1.2-20 μg/mL. (C) IL-6 levels in LPS-activated Mφ CM from C27BL/6 (C) or MRL/pr (D) Mφs were determined by ELISA. (D) Mφs derived from C57BL/6 and MRL/pr mice were LPS-stimulated (15 μg/mL) for 3 hours then stained with anti-CD40L. The quantitative data from 5 experiments (100 cells/experiment) is shown. The absolute number of LPS-stimulated C57BL/6 Mφs (n = 5), predisease MRL/lpr (n = 5), or postdisease MRL/lpr (n = 6) mice for 4 days. IgM%A was determined by ELISA. LPS-stimulated B cells (100%) secreted 1-4 μg/mL. Each circle represents an individual mouse. The horizontal bars mark the mean secretion. Statistical analysis was performed using the exact Wilcoxon rank-sum test to compare all experimental groups to LPS-stimulated C57BL/6 Mφs (panels A-C) or the one-sample t-test to compare unstimulated cultures to stimulated cultures (panel D) or experimental groups to C57BL/6 Mφ CM (panel E) (*p ≤ .05).

demonstrated with IgG and non-Tg B cells, indicating that repression of Ig secretion occurs in mixed cell populations. Repression by sCD40L did not reflect changes in proliferation, but rather a reduction in the number of B cells that differentiated into ASCs. The importance of Mφs in maintaining B-cell tolerance was apparent in lupus-prone, MRL/pr mice. We found that compared with C57BL/6 mice, Mφs from MRL/pr mice were significantly less efficient at repressing Ig secretion coincident with diminished production of IL-6 and CD40L. The defect was apparent in all MRL/pr mice, regardless of their disease status. Likewise, defects in MRL/pr DCs are associated with the MRL background. The data indicate that Mφs regulate autoreactive B cells by secreting repressive factors that inhibit the formation of ASCs. This mechanism of tolerance is diminished in lupus-prone mice, suggesting its role in the autoimmune associated with SLE.

The ability of sCD40L to repress Ig secretion by autoreactive B cells is reminiscent of cell fate decisions in the germinal center. Ligation of CD40 enhances immunity by inducing proliferation, germinal center formation, and class switch recombination; however, CD40 signal transduction also inhibits differentiation of B cells and reduces Ig secretion by B-cell hybridomas. Furthermore, sustained CD40 signaling in germinal center B cells selects for memory cell formation by inhibiting differentiation of ASCs. The findings that sCD40L represses autoantibody production during innate immune responses expands our understanding of the pleiotropic nature of CD40L by identifying that CD40/CD40L interactions are important in more than T-dependent immune responses. Thus, molecules that have historically been thought to promote immunity also protect from autoimmunity by differentially regulating Ig secretion.

The diverse roles of CD40L in adaptive and innate immune responses suggest that other receptors influence the outcome of CD40 signal transduction. Our data showed that repression of Ig secretion by IL-6 and sCD40L selectively occurred in B cells continuously exposed to self-antigen. This suggests that the BCR regulates the outcome of signal transduction through other receptors (Figure 2). Although the BCR-derived signals remain to be elucidated, others have reported that persistent BCR stimulation induces Fas-mediated apoptosis, whereas cells undergoing transient stimulation remain refractory to Fas stimulation. In addition, persistent BCR stimulation regulates TLR9-mediated Ig secretion in an Erk-dependent manner. Cross-talk between the BCR and CD40 also lowers the signaling threshold for B cell activation and regulates BCR-mediated apoptosis. Thus, some aspects of B-cell fate are directed by the ability of persistent BCR ligation to alter the expression of Fas receptor or the outcome of CD40, TLR9, and IL-6 receptor ligation.

Activation of the innate immune response induces Mφs to secrete multiple soluble mediators that repress autoantibody secretion. Although IL-6 and sCD40L contribute to repression, either is sufficient, indicating that IL-6 and sCD40L possess redundant function in repressing autoantibody production (Figure 1D). Our data also showed that B cell subsets are differentially susceptible to repression by each of the soluble mediators (Figure 2D). This indicates that IL-6 and sCD40L possess specialized function wherein Mφs partially repress MZ B cells, whereas DCs and Mφs repress FO B cells. MZ B cells play an important role in T-independent immunity and express Ig receptors that recognize multiple antigens, including self-antigens. Although the identity of the Mφ subtype that represses Ig secretion remains undefined, it is interesting to speculate that the cells required for retention of MZ B cells in the spleen may regulate MZ B cell activation. Studies are under way to characterize repression of other B-cell subsets, including the pre-plasma cells that become dysregulated in lupus-prone mice. It is also noteworthy that the magnitude of Mφ-mediated repression of 2-12H MZ B cells (40%) was less than FO cells (87%), indicating that MZ B cells may be regulated by additional mechanisms (Figure 2D). Because 2-12/Vκ light B cells do not have a significant population of MZ B cells, they are efficiently repressed by Mφs (96%) (Figure 2A). However, another possibility is that B cells expressing different affinity receptors may be more or less susceptible to DC/Mφ-mediated tolerance. This is evident when repression of HEL-specific B cells is compared with Sm-specific B cells. Both of these models contain comparable percentages of MZ B cells, yet IL-6 and sCD40L repress approximately 55% of Ig secretion by high-affinity HEL-specific B cells and 70% of secretion by low-affinity 2-12/H/VκB cells (Figures 1C, 2A). Thus, our data identify DC/Mφ-mediated tolerance as a mechanism that regulates autoreactive B cells during innate immune responses and reveals that multiple factors differentially regulate unique B-cell subsets, raising the possibility that the location determines the factors required to regulate Ig secretion.

The identification of Mφs as regulatory cells that maintain B-cell tolerance raises the possibility that defects in Mφ function predisposes to autoimmunity and possibly SLE. Here, we show that Mφs from autoimmune-prone mice are deficient in the production of IL-6 and sCD40L. Previous studies identified that Mφs exhibit defects in IL-6 secretion that is triggered by apoptotic cells.
Coincidently, Mφs from autoimmune mice and SLE patients are defective in phagocytosis of apoptotic cells. Thus, the diminished clearance of apoptotic cells may chronically suppress the secretion of tolerogenic factors, such as IL-6 and sCD40L. Reduced secretion of tolerogenic factors during an innate immune response would allow activation and terminal differentiation of autoreactive B cells. Alternatively, failure of lupus-prone B cells to reprogrammed, such that CD40 and IL-6 receptor ligation does not repress Ig secretion, may lead to autoimmunity. Whether these mechanisms are dysregulated in vivo remains to be determined.

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References

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Authorship
Contribution: M.A.K. directed and performed experiments, analyzed data, and wrote the manuscript; N.J.W. performed experiments and analyzed data; A.L.G. performed experiments; L.L. performed statistical analysis; K.A. and L.J.W. provided mice; and B.J.V. directed experiments and wrote the manuscript.

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Macrophages prevent the differentiation of autoreactive B cells by secreting CD40 ligand and interleukin-6

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