Vav Proteins Regulate the Plasma Cell Program and Secretory Ig Production

Linda M. Stephenson, Ana V. Miletic, Tracie Kloeppel, Shana Kusin and Wojcieh Swat

*J Immunol* 2006; 177:8620-8625; doi: 10.4049/jimmunol.177.12.8620

http://www.jimmunol.org/content/177/12/8620

References

This article cites 28 articles, 8 of which you can access for free at:
http://www.jimmunol.org/content/177/12/8620.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Vav Proteins Regulate the Plasma Cell Program and Secretory Ig Production

Linda M. Stephenson, Ana V. Miletic, Tracie Kloeppel, Shana Kusin, and Wojciech Swat

Plasma cell (PC) development is initiated following B cell activation and controlled by a B lymphocyte-induced maturation protein (Blimp)-1-dependent program involving the concerted action of several proplasma transcriptional regulators. However, the factors that control Blimp-1 expression remain largely unknown. In this context, mice deficient for all three of the Vav family of proteins (Vavnull) develop substantial B cell populations, including marginal zone B cells, yet have a virtual absence of serum Igs, indicating that control Blimp-1 expression remain largely unknown. In this context, mice deficient for all three of the Vav family of proteins (Vavnull) develop substantial B cell populations, including marginal zone B cells, yet have a virtual absence of serum Igs, indicating that Vav may be specifically required in PC development and Ig production. We show in this study that mature marginal zone B cells from Vavnull mice proliferate following stimulation with TLR ligands but exhibit severe defects in PC differentiation and Ig secretion. Under conditions inducing PC differentiation, Vavnull B cells fail to efficiently induce Blimp-1, X box-binding protein-1, J chain, or secretory Ig μ transcripts but express IFN-regulatory factor-4 at levels similar to wild-type cells. These data indicate a previously unknown role for Vav as an upstream regulator of Blimp-1. The Journal of Immunology, 2006, 177: 8620–8625.

Plasma cells (PC) are terminally differentiated B cells dedicated to the synthesis and secretion of large quantities of soluble Igs. Differentiation of PC is initiated following activation of B cells through Ag or TLRs and requires both proliferation and the induction of the PC transcriptional program. Although the exact role of proliferation is unclear, the induction of the PC program is preceded by a proliferative burst with the probability of commitment to the PC fate increasing with each round of division, suggesting a mechanistic link between these processes (1).

The mechanism of PC development is incompletely understood. Gene ablation studies indicate that at least three transcription factors are critical for PC differentiation, including IFN-regulatory factor (IRF)-4, B lymphocyte-induced maturation protein (Blimp)-1, and X box-binding protein (XBP)-1 (2–4). Although mice deficient for IRF-4 have severe defects in PC development, IRF-4+/− B cells do not proliferate to LPS in vitro, indicating a critical role for IRF-4 in proliferation induction (1, 2). Commitment to the PC lineage is initiated by Blimp-1, a transcriptional repressor both necessary and sufficient to promote PC development. Accordingly, inactivation of Blimp-1 disrupts PC development, leading to a paucity of both short- and long-lived PC and of serum Ig (4). Blimp-1 controls multiple transcriptional programs in PC, including the induction of XBP-1 (5), required for the induction of the secretory phenotype (3, 6).

Marginal zone (MZ) B cells can recognize bacterial pathogens through TLRs early in the immune response and develop into PC secreting low-affinity Abs bridging innate and acquired immunity (7). MZ B cells are uniquely suited for this niche as they are extremely sensitive to bacterial toxins and rapidly proliferate and differentiate into PC following TLR stimulation (8). Accordingly, MZ B cells induce Blimp-1 within 24 h of in vitro LPS stimulation and produce the bulk of IgM and IgG3 detected within 3–4 days of in vivo challenge (9).

The Vav family proteins, thought to be critical mediators of signaling downstream of several surface receptors, contain multiple domains characteristic of proteins involved in signal transduction, including calponin homology, dbl-homology, Src homology 2, and Src homology 3, and acidic regions implicated in nuclear and cytoplasmic scaffolding activities (10–13). Nevertheless, a precise mechanistic understanding of Vav function in different cell types remains to be elucidated. Recent studies suggested that Vav may also be involved in epigenetic control of gene expression via interactions with chromatin remodeling and/or transcriptional repressor proteins (11, 14, 15). We have previously demonstrated that mice deficient in all three Vav proteins (Vavnull) exhibit perturbations in B cells that predominantly affect the mature follicular B cell compartment; however, development of newly formed and immature B cells is not perturbed and Vavnull mice generate substantial numbers of MZ B cells (16). Given these partial defects in B lineage cells in Vavnull mice, which contrast sharply with our finding of the total lack of serum Igs, we hypothesized that Vav proteins may be critical for the differentiation of Ig-producing cells, beyond their role in B cell development.

Materials and Methods

Mice

The generation of Vavnull mice has been previously described (16). Vavnull control mice, and all breedings were maintained in the specific pathogen-free facility of Washington University School of Medicine in accordance with institutional policies for animal care and usage.

Flow cytometry

Single-cell suspensions were prepared from the spleens of 6- to 8-wk-old mice and stained with Abs according to standard protocols. Ab conjugates against the following markers were used: rat IgG2a (isotype control), CD21/CD35, CD23, B220, IgM, IgD, CD86, CD43, CD19, AA4.1, syndecan-1, CD180 (BD Biosciences), and TLR4 (eBioscience). Biotinylated
FIGURE 1. Analyses of all serum Igs, including IgM, in Vavnull mice. An absence of serum Igs in Vavnull mice is shown. Serum Igs were analyzed by ELISA as described in Materials and Methods. Each data point represents an individual animal. The horizontal bars represent mean concentrations. \( p < 0.05 \) for total Ig, IgG1, and IgG2A; \( p < 0.0001 \) for IgM, IgG2B, and IgG3.

Abs were detected with streptavidin-CyChrome (BD Biosciences). MZ B cells were isolated by cell sorting using anti-CD21/CD35 and anti-CD23. For intracellular staining of IgM, cells were surface stained with Abs against IgM and syndecan-1, fixed with 2% paraformaldehyde (Polysciences), and permeabilized in 0.1% saponin. Samples analyzed for intracellular Ig were again stained with anti-IgM. The change in mean fluorescence intensity (ΔMFI) is defined as MFI of intracellularly stained samples minus MFI from surface stained samples. Labeling with 1 μM CFSE (Molecular Probes) was performed on sorted MZ B cells. Labeled cells were washed in complete medium and cultured with the indicated stimuli for 72 h.

Cell culture, proliferation assays, and retroviral transduction

Unless otherwise indicated, MZ B cells were plated at 2–3 × 10⁶/ml in complete DMEM-10% FBS, stimulated with either LPS (0.5 μg/ml; Sigma-Aldrich) or 0.6 μg/ml nuclease-resistant phosphorothioate backbone oligodeoxynucleotide (CpG S-ODN) 1826: 5’TCCATGACGTTCCTGACGTT-3’ (Integrated DNA Technologies) and cultured at 37°C in a 5% CO₂-humidified incubator. Proliferation assays using [3H]thymidine were performed on sorted MZ B cells. Although Vavnull mice exhibit severe defects in the generation of B1 B cells, which also show defects in B1 B cell generation, have normal levels of serum Igs (21). This finding suggests that the defective generation of B1 B cells may contribute to, but is not sufficient to explain, the striking absence of serum Igs in Vavnull mice (16, 18, 19, 21). Thus, the severe paucity of serum Ig in Vavnull mice (Fig. 1) implicates Vav in the regulation of Ig production beyond a requirement in B cell development.

MZ B cells from Vavnull mice are phenotypically mature and proliferate in response to TLR ligands

To examine whether Vav is specifically required in PC differentiation and Ig production, we focused on MZ B cells as the development of these cells appears to be largely independent of Vav (16). Indeed, analyses of WT and Vavnull splenocytes showed that populations of MZ B cells (CD21/CD35high CD23low/weak) were present in both types of mice, with only modest decreases in numbers and percentages in Vavnull mice (16) (Fig. 2. A and B). Moreover, analyses of the expression of a panel of surface markers showed that MZ B cells from Vavnull mice were of similar size as MZ B cells from WT mice, expressed equivalent levels of IgM, IgD, B220, CD19, and CD86, but did not express AA4.1 and CD43, markers of immature B cells (Fig. 2A). These data indicate that the MZ B cells in Vavnull mice are mature and phenotypically indistinguishable from WT.

To determine whether proliferative responses of Vavnull MZ B cells to TLR ligands were altered, FACS-sorted MZ B cells from either WT or Vavnull mice were stimulated with either the TLR4 ligand LPS or the TLR9 ligand CpG, and their proliferation was measured by multiple assays. Given that proliferation is thought to be a mechanistic requirement for secretory Ig production (1), we reasoned that potential defects in TLR signaling and defective proliferation of Vavnull MZ B cells could underscore any defects in Ig production of these cells.

MZ B cells from Vavnull mice were stimulated with the TLR4 ligand LPS, and the TLR9 ligand CpG, and their proliferation was measured by multiple assays.
production. Surprisingly, LPS or CpG-stimulated Vavnull MZ B cells proliferated similarly to WT, as determined by three different assays. First, the percentages of blasting cells, as defined by forward and side scatter, were similar between WT and Vavnull cells (Fig. 2C). Second, [3H]thymidine incorporation by WT and Vavnull MZ B cells, in response to various doses of LPS or CpG, gave indistinguishable results (Fig. 2D). Third, analyses of proliferation by CFSE dye-dilution indicated that both WT and Vavnull MZ B cells underwent similar numbers of cell divisions upon TLR4 or TLR9 stimulation (Fig. 2E). Thus, we conclude that LPS- or CpG-induced proliferative responses of Vavnull MZ B cells are similar to responses in WT MZ B cells as assessed by several independent criteria.

In this context, a recent report indicated a requirement for Vav in B cell proliferative responses to LPS (22). In agreement with this report, we found that proliferation of total splenic (B220+/H11001) Vavnull B cells to LPS was decreased as compared with WT (Fig. 3). Because the majority of B220+ cells in Vavnull mice are immature (16), these results are consistent with the notion that immature B cells proliferate less vigorously in response to TLR4 stimulation than mature B cells (23). However, we cannot rule out the possibility that the requirement, or lack thereof, for Vav in LPS-induced proliferative responses distinguishes MZ B cells from other B cell subsets, a precedence of which has been reported (24). Nevertheless, our results clearly indicate that in murine MZ B cells, Vav proteins are superfluous in mitogenic responses triggered by TLR4 or TLR9.

MZ B cells from Vavnull mice are severely impaired in PC differentiation

Having determined that MZ B cells from Vav null mice are competent to proliferate in response to LPS or CpG (Fig. 2, C–E), we next analyzed the ability of these cells to differentiate into PC and produce secretory Ig. To this end, FACS-sorted MZ B cells from WT and Vavnull mice were stimulated with LPS or CpG and then analyzed for the expression of syndecan-1 (CD138), a PC marker induced during PC differentiation but absent on the surface of naive B cells (25). As expected, WT MZ B cells cultured in the presence of LPS or CpG induced expression of syndecan-1 (CD138), a PC marker induced during PC differentiation but absent on the surface of naive B cells (25). As expected, WT MZ B cells cultured in the presence of LPS or CpG up-regulated the expression of syndecan-1 (Fig. 4A). Strikingly, MZ B cells from Vavnull mice showed reduced percentages of syndecan-1-expressing cells under the same culture conditions (Fig. 4A). To directly assess the ability of MZ B cells to produce secretory Ig, we analyzed WT and Vavnull culture supernatants for soluble IgM and IgG3. In contrast to the abundant IgM and IgG3 detected in the supernatant from WT MZ B cells, ~10-fold less IgM and 4-fold less IgG3 were detected in the supernatant from LPS-stimulated Vavnull MZ B cells (Fig. 4B). Similarly, CpG-stimulated Vavnull MZ B cells showed a significant reduction of supernatant IgM as compared with WT cells. Minimal IgM was detected in wells of MZ B cells cultured in the absence of LPS or CpG, nor did these cells express syndecan-1 (data not shown). These data indicate that Vav proteins are critical for LPS or CpG-induced syndecan-1 expression and secretory Ig production by MZ B cells.
PC differentiation results in the concomitant decrease of surface IgM and the increase in secreted IgM, presumably due to differential splicing of μH chain transcripts encoding membrane-bound or soluble IgM, respectively (1). Although our data (Fig. 4, A and B) suggested a requirement for Vav in soluble Ig production, we reasoned that it is possible that Vav may instead be selectively required for the secretion of soluble Igs. To rule out this possibility, we analyzed surface vs intracellular expression of IgM in WT and Vavnull MZ B cells, comparing the change in MFI. In this assay, WT MZ B cells cultured in the presence of LPS showed reduced expression of surface IgM, whereas the cytoplasmic Ig staining revealed a dramatic up-regulation of total Ig levels (Fig. 4C, top left). Strikingly, although Vavnull MZ B cells expressed higher levels of surface IgM than WT, these cells contained little cytoplasmic Ig protein, suggesting that the defect in soluble Ig production is due to an impairment in PC differentiation rather than a selective defect in Ig secretion (Fig. 4C). Of note, we consistently detected small populations of syndecan-1-positive cells in Vavnull MZ B cell cultures (Fig. 4A), suggesting that the in vitro PC differentiation defect in Vavnull MZ B cells is not absolute, even though Vavnull mice produce virtually no detectable Ig in their serum. In this regard, Vavnull syndecan-1-positive cells contained intracellular IgM and expressed lower levels of surface IgM (Fig. 4C). Taken together, these data indicate that under conditions of in vitro stimulation with TLR ligands, Vavnull MZ B cells exhibit a profound, albeit leaky, defect in PC differentiation and implicate Vav as a critical factor for B cell conversion into Ig-secreting cells.

To ascertain that the PC differentiation defect of Vavnull MZ B cells was due to a lack of Vav protein expression and not due to developmental defects preceding PC differentiation, we tested whether the reintroduction of recombinant Vav could rescue PC differentiation in these cells. To this end, we infected LPS-stimulated WT and Vavnull MZ B cells with retrovirons encoding a GFP-appended Vav1 or control (GFP only) and analyzed for the induction of syndecan-1. Although a majority of WT cells expressing either GFP alone or GFP-Vav1 expressed syndecan-1, only Vavnull cells transduced with Vav1 expressed syndecan-1 at levels similar to WT (Fig. 4D). Vavnull cells infected with the control vector showed no increase in syndecan-1 expression (Fig. 4D). Thus, although we were unable to obtain sufficient numbers of transduced cells to measure soluble Ig production in these experiments, these data indicate that Vavnull MZ B cells are competent to differentiate into PC upon reintroduction of Vav1 protein, albeit it is likely that all three Vav proteins contribute to achieve optimal B cell function (16, 18, 19).

Vav is an upstream regulator of the Blimp-1-mediated PC developmental program

Having established that Vav proteins are critical for PC differentiation, but not for proliferative expansion of PC progenitors, we hypothesized that Vav may control a specific mechanistic step during the induction of the “plasmacytic program.” In this regard, PC development is dependent on the up-regulation of the transcriptional repressor Blimp-1. Although factors that control its own expression remain unknown at present, Blimp-1 induces the expression of several critical PC-specific genes, including XBP-1, J chain, and secretory Ig μ (4). To determine whether Vav is required for the induction of Blimp-1 or Blimp-1-dependent genes, FACS-sorted WT and Vavnull MZ B cells were cultured in the presence of LPS or CpG and then analyzed by RT-PCR and quantitative RT-PCR for their ability to induce PC-specific genes. Strikingly, these analyses revealed that, when compared with WT, Blimp-1 induction was severely decreased in Vavnull MZ B cells cultured in the presence of LPS or CpG (Fig. 5A), with a greater than 4-fold decrease as assayed by quantitative RT-PCR (Fig. 5B). Furthermore, consistent with Blimp-1 defects in these cells, expression of XBP-1 and J chain were also impaired in Vavnull MZ B cells (Fig. 5A). Because Blimp-1 is required for the differential splicing of H chain transcripts from membrane-bound IgM (μm) to secretory IgM (μs) (4), we tested for the presence of these transcripts. We found the levels of membrane-bound transcripts were similar, or even slightly higher, in Vavnull cells, whereas transcripts of secretory IgM were lower (Fig. 5A), in agreement with the levels of membrane vs secretory Ig H chain protein (Fig. 4C). These data indicate that the Vav family is essential for the inducible expression of Blimp-1 and Blimp-1-regulated effectors. Although BCL-6 has been previously implicated in the negative regulation of Blimp-1 expression (1), we found that the levels of BCL-6 transcripts were similar between WT and Vavnull MZ B cells under both stimulated and unstimulated conditions, suggesting that Vav does not regulate the expression of BCL-6 (Fig. 5A).
FIGURE 5. Impaired induction of Blimp-1 and the Blimp-1-mediated transcriptional program in Vavnull MZ B cells. A, Semiquantitative RT-PCR analyses of MZ B cell RNA with 4-fold serial dilutions of cDNA, as indicated. One representative experiment for three performed is shown. B, Quantitative RT-PCR analysis of LPS induction of genes. Data shown are mean values (n = 3 experiments) normalized to GAPDH.

However, we observed a modest but reproducible increase in expression of Pax-5 in LPS-stimulated Vavnull B cells (Fig. 5, A and B), consistent with a requirement for Blimp-1 in Pax-5 down-regulation (26). We also note that as syndecan-1 expression is thought to be controlled by Blimp-1 (27), the decrease in syndecan-1 observed on Vavnull MZ B cells (Fig. 4A) may be due to a decrease in Blimp-1.

In contrast to Blimp-1 and Blimp-1-regulated genes, the induction of IRF-4 transcripts was indistinguishable between WT and Vavnull MZ B cells. Because IRF-4 transcripts are increased in B cells following LPS stimulation and B cells from IRF-4-deficient mice exhibit profound defects in proliferation, IRF-4 is thought to act early in PC development (1, 2). Thus, induction of IRF-4 in Vavnull MZ B cells is consistent with the ability of these cells to proliferate and suggests that either Vav acts downstream of IRF-4 or that the regulation of IRF-4 is independent of Blimp-1. Together, these data indicate that Vav is an essential upstream mediator of Blimp-1 expression and PC differentiation. Moreover, defective induction of Blimp-1 and Blimp-1-dependent effectors (Fig. 5, A and B), but normal proliferation of Vavnull MZ B cells (Fig. 2, C–E), indicate a dichotomy in pathways emanating from TLRs distinguished by the dependence on Vav.

Discussion

In this report we show that the Vav family of Rho guanine nucleotide exchange factors is critical for differentiation of Ig-producing cells and Ig production in vitro and in vivo. We also demonstrate that Vav acts as an upstream regulator of Blimp-1. However the mechanism by which Vav promotes Blimp-1 induction remains to be elucidated. TLR induction of Blimp-1 may be dependent on the activation of NF-κB and the engagement of NF-κB sites located upstream of the transcriptional start site of prdm1, the gene encoding Blimp-1 (1). However, although Vav has been implicated in NF-κB activation via IkB kinase, the degradation of IkB following LPS stimulation of total splenic Vavnull B cells was similar to WT (data not shown). In this context, several recent studies suggested an intriguing possibility that Vav may regulate gene transcription via interactions with nuclear factors such as NF-κB and Ezh2, and raises the possibility that Vav may regulate Blimp-1 gene expression through an epigenetic mechanism involving direct effects on chromatin. Vav1 protein, which contains nuclear localization signals and has been found in the cell nucleus where it associates with transcriptionally active complexes including NF-κB, as well as Ezh2, may be responsible for epigenetic modifications of chromatin via effects on nuclear actin polymerization (11, 14, 15, 28). Although any potential mechanism still requires experimental validation, this report clearly establishes that Vav is critical in PC differentiation and Ab production, even though it may be superfluous in mediating mitogenic effects of TLR ligands, at least in MZ B cells. Thus, these data underscore an essential role for Vav proteins in the generation of humoral immune responses.

Acknowledgments

We thank Amy Boyet for flow cytometric assistance and Drs. P. Allen, K. Murphy, A. Shaw, B. Sleckman, D. Graham, J. Sedy, and C.-Y. Huang for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References


