The Guanine-Nucleotide Exchange Factor Vav Is a Crucial Regulator of B Cell Receptor Activation and B Cell Responses to Nonrepetitive Antigens

Martin F. Bachmann, Lars Nitschke, Connie Krawczyk, Kerry Tedford, Pamela S. Ohashi, Klaus D. Fischer and Josef M. Penninger

J Immunol 1999; 163:137-142; 
http://www.jimmunol.org/content/163/1/137

References
This article cites 44 articles, 12 of which you can access for free at:
http://www.jimmunol.org/content/163/1/137.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
The Guanine-Nucleotide Exchange Factor Vav Is a Crucial Regulator of B Cell Receptor Activation and B Cell Responses to Nonrepetitive Antigens

Martin F. Bachmann,* Lars Nitschke, † Connie Krawczyk, § Kerry Tedford, ‡ Pamela S. Ohashi, ¶ Klaus D. Fischer, ‡ and Josef M. Penninger 2§

The proto-oncogene product Vav is required for receptor clustering, proliferation, and differentiation of T cells, and Vav was identified as a substrate in the TCR and B cell receptor signaling pathway. The role of Vav in B cell responses to Ag challenge in vivo is not known. In this study, we show that Vav regulates B cell proliferation following in vitro activation of Ag receptors, but Vav has no apparent role in CD40-, IL-4-, or LPS-induced B cell activation. Increased degrees of Ag receptor cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav−/− B cells. In vivo, vav−/− mice mounted protective antiviral IgM and IgG responses to infections with vesicular stomatitis virus and recombinant vaccinia virus expressing the vesicular stomatitis virus glycoprotein, which harbor repetitive surface epitopes that directly cross-link the Ag receptor and activate B cells in the absence of T cell help. vav−/− B cells also responded normally to the polyvalent, repetitive hapten Ag trinitrophenyl (TNP)-Ficoll that effectively cross-links B cell receptors. Moreover, increased degrees of Ag receptor cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav−/− B cells. However, vav−/− mice failed to mount immune responses to the nonrepetitive, T cell-dependent hapten Ag (4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP)-OVA. These results provide the first genetic evidence on the role of the guanine exchange factor Vav in immune responses to viral infections and antigenic challenge in vivo, and suggest that Vav adjusts the threshold for Ag receptor-mediated B cell activation depending on the nature of the Ag. *The Journal of Immunology, 1999, 163: 137–142.

The hematopoietic-specific proto-oncogene vav encodes a 95-kDa protein that contains a unique collection of protein interaction motifs, including a calponin homology domain, Dbh homology, and adjacent pleckstrin homology domains, and an SH2 domain flanked by two SH3 domains (1–4). Vav is rapidly phosphorylated following stimulation of various growth factor receptors and Ag receptors in T and B lymphocytes, and phosphorylated Vav associates with signaling molecules proximal to activated Ag receptors (2, 5, 6). Recent data suggest that Vav functions as a guanine-nucleotide (GTP/GDP) exchange factor for members of the Rho-like small GTPase family members RhoA, Rac1, and CDC42, which regulate cytoskeletal organization and activation of the p21-activated kinase and stress-activated protein kinase/e-Jun N-terminal kinase signaling pathways (7–11).

Studies in vav−/− mice have shown that Vav is essential for TCR capping and actin polymerization in response to Ag receptor activation (12, 13). Moreover, Vav is required for Ag receptor-induced proliferation of B and T cells in vitro and effective T cell selection (12–17). In T cells, coordinate activation of calcineurin and Vav pathways via the TCR and CD28 is a crucial requisite for IL-2 production, and overexpression of Vav enhances TCR-mediated NF-AT transcriptional activity and IL-2 expression (13, 18–20). Similar to T cells, Vav is rapidly phosphorylated following Ag receptor activation in B cells. Vav interacts with the B cell costimulatory molecule CD19 and the Bruton’s tyrosine kinase (Btk)3 (21, 22), and it was reported that Vav has an important role in CD19-mediated activation of lipid and protein kinases (23). Moreover, B cells from CD19 mutant mice display reduced Vav tyrosine phosphorylation following IgM ligation (24). Collectively, these observations point to a role of Vav at the interface of Ag-induced receptor signaling and GTPase-controlled actin rearrangements and Ag receptor clustering, and show that Vav is required for normal lymphocyte function (1). The in vivo role of Vav in B cell responses following Ag challenge is not known.

We report in vav−/− mice that Vav expression is important for BCR-induced proliferation, efficient T help-dependent IgG class switching, and Ab responses to T cell-dependent hapten Ags. However, vav−/− mice mount normal B cell responses to T cell-independent repetitive viral and polyvalent hapten Ags, implying that the Vav defect can be overcome by repetitive Ags that effectively cross-link BCR. Moreover, increased degrees of cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav−/− B cells. These results indicate that Vav has an important role in setting the threshold for Ag receptor-mediated stimulation of B lymphocytes.

1 J. M. P. is supported by the Medical Research Council (MRC) of Canada.
2 Address correspondence and reprint requests to Dr. Josef M. Penninger, Amgen Institute and Ontario Cancer Institute, Department of Medical Biophysics and Immunology, University of Toronto, 620 University Avenue, Suite 706, M5G 2C1 Toronto, Ontario, Canada. E-mail address: jpenning@amgen.com

Received for publication September 16, 1998. Accepted for publication April 12, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 Abbreviations used in this paper: Btk, Bruton’s tyrosine kinase; BCR, B cell receptor; LCMV, lymphocytic choriomeningitis virus; NIP, 1-(4-hydroxy-5-iodo-3-nitrophenyl)acetyl; sIg, soluble Ig; TNP, trinitrophenyl; VSV, vesicular stomatitis virus; Vacc-G, recombinant vaccinia virus expressing the VSV glycoprotein; VSV-G, VSV glycoprotein.
Materials and Methods

Mice

The generation of mice homozygous for the vav mutation has been described previously (12). Mice were analyzed for the vav mutation using PCR (vav<sup>+</sup> allele, sense primer, 5'-ATTAGGACCTGATGGTGCCAG CTT-3'; and antisense primer, 5'-GTCCCTCCTCTTCTGTCGGG-3'); vav<sup>+/−</sup> allele, sense primer, 5'-AAGGCCCTCCCTCACCCTG-3'; and antisense primer, 5'-GATGAAAAGCTGTGTCTGATA-3'). If not otherwise stated, all mice used for experiments were between 6 and 10 wk of age and backcrossed to a C57BL/6 background for four generations. Mice were kept under pathogen-free conditions in accordance with guidelines of the Canadian Medical Research Council.

Immunocytochemistry

Single cell suspensions from thymus, spleen, mesenteric lymph nodes, and bone marrow from vav<sup>+/−</sup> and vav<sup>−/−</sup> mice were prepared as described (25), resuspended in immunofluorescence staining buffer (PBS, 4% FCS, and 0.1% NaN₃), and incubated with appropriate Abs. The following mAbs were used: anti-B220 (FITC, PE, or biotinylated); anti-CD19 (biotin labeled); anti-CD43 (FITC labeled); anti-CD25/IL-2Rα (FITC labeled); anti-H-2K<sub>b</sub> (FITC labeled); anti-CD86 (biotinylated); anti-CD40 (FITC labeled); anti-sIgM (FITC labeled); anti-sIgD (biotinylated) (all biotinylated); anti-CD23 (biotinylated); anti-CD69 (FITC labeled); anti-CD5 (Jackson ImmunoResearch, West Grove, PA), soluble polyclonal anti-IgM (sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the LPS (Sigma, St. Louis, MO).

B cell stimulation assays

B cells were purified from vav<sup>+/−</sup> and vav<sup>−/−</sup> mice, as described (25). FACS analysis revealed that the remaining cells were >90% IgM<sup>−</sup>. Cells were placed into a round-bottom 96-well plate (Costar, Cambridge, MA) in IMDM and activated using soluble intact polyclonal goat anti-mouse IgM (Jackson ImmunoResearch, West Grove, PA), soluble polyclonal anti-IgM (Jackson ImmunoResearch), the soluble anti-IgM Ab (B7.6, murine rL-4 (Genzyme, Cambridge, MA), soluble anti-CD40 (Serotec), and LPS (Sigma, St. Louis, MO). For super-cross-linking, different concentrations of rabbit anti-goat Ig (Jackson ImmunoResearch) were bound to the plastic of a 96-well plate (4°C for 12 h), washed three times in PBS, and then incubated with a fixed concentration (5 μg/ml) of goat anti-mouse IgM (see above) for 12 h at 4°C. In addition, the same goat anti-mouse IgM Ab was bound to Sepharose beads. B cells were harvested at 1–4 days after a 12-h pulse with 1 μCi [3]Hthymidine/well.

Up-regulation of surface receptors in B cells

For CD40, anti-IgM, LPS-, and IL-4-mediated up-regulation of I-A<sub>β</sub>, CD86, ICAM-1, and CD23 (26), purified B cells (>90% IgM<sup>−</sup> B cells) were activated with anti-CD40 (1 μg/ml), anti-IgM (20 μg/ml), anti-CD40 (1 μg/ml) plus anti-IgM (2 μg/ml), mouse rL-4 (10 U/ml), or LPS (10 μg/ml) in IMDM (10% FCS, 37°C). After 24 h of activation, cells were harvested and double stained with Abs reactive against B220 (PE) and ICAM-1 (biotin), CD23 (biotin), I-A<sub>β</sub> (rat IgG, followed by goat anti-rat FITC), or CD86 (biotin). Biotinylated Abs were visualized using streptavidin-RED670, and staining of cells was analyzed using a FACScan (Becton Dickinson, Mountain View, CA).

Vesicular stomatitis virus (VSV) and Vacc-G infections and detection of neutralizing Abs

Mice were immunized with VSV-Indiana (2 × 10⁶ PFU, i.v.) or recombinant vaccinia virus expressing VSV glycoprotein (Vacc-G; 2 × 10⁶ PFU, i.v.). At the indicated time points, sera were collected, and neutralizing IgM and IgG Ab titers were determined, as described (27). In brief, 1/2 dilutions of 40-fold prediluted and heat-inactivated sera were incubated with VSV for 90 min. The presence of remaining infectious virus was determined by incubating the VSV serum samples with fibroblasts for another 24 h. Serum dilutions that reduced the number of viral plaques by 50% were taken as specific. IgG Ab titers were determined after preincubation of sera with 2-β-ME, a procedure that eliminates IgM (27).

Hapten immunization

Mice were immunized with 50 μg of the T-dependent haptenated protein NIP-OVA s.c. at the base of the tail and i.p. (100 μg total per mouse), or with the polyvalent T-independent hapten TNP-Ficoll (10 μg/mouse, i.p.) (28). NIP-specific serum IgG1 and IgG2a titers were determined 8 and 15 days later by ELISA on NIP-BSA-coated plates (Nunc-Immuno Plate; Nunc, Naperville, IL). NIP-OVA and NIP-BSA were kind gifts of A. Rolink (Basel Institute for Immunology, Basel, Switzerland). TNP-specific IgM and IgG3 Ab titers were determined by ELISA on day 0 and days 5 and 7 after immunization (28).

Results

Impaired BCR-mediated activation of vav<sup>−/−</sup> B cells

Previously, it has been shown in vav<sup>−/−</sup>rag<sup>−/−</sup> blastocyte complementation studies that Vav has a role in the development of conventional and peritoneal CD5<sup>+</sup> B1 B lymphocytes (16, 17). Fig. 1 shows that vav<sup>−/−</sup> mice exhibit normal numbers of B220<sup>+</sup> IgM<sup>+</sup> B cells and B220<sup>+</sup>CD43<sup>+</sup> B cell precursors in the bone marrow and peripheral lymphoid organs. B cell development also proceeded normally in the bone marrow, as measured by the expression of the early B cell differentiation markers CD25 and heat stable Ag (data not shown). Moreover, splenic B cells from vav<sup>−/−</sup> mice expressed normal levels of slgD, CD19, CD23, CD40, CD44, ICAM-1, CD95 (FAS), and H-2K<sup>β</sup> on the cell surface (data not shown), implying that Vav has no apparent role in the development of conventional B cells. However, the numbers of peritoneal CD5<sup>+</sup> B1 cells were 50–75% reduced in vav<sup>−/−</sup> mice as compared with vav<sup>+/−</sup> and vav<sup>+/+</sup> littermate controls (data not shown).

To determine the requirement of Vav for B cell activation, we measured proliferation of B cells in response to various stimuli. vav<sup>−/−</sup> B cells displayed impaired proliferative responses to cross-linking using a polyclonal goat anti-mouse IgM Ab, but responded normally to LPS, IL-4, and anti-CD40 (Fig. 2A). Whereas vav<sup>+/−</sup> and vav<sup>+/+</sup> B cells up-regulated MHC class II (I-A<sub>β</sub>) (Fig. 2B), CD86 (B7.2) (Fig. 2C), ICAM-1, and CD23 (data not shown) upon activation with anti-CD40, LPS, or IL-4, vav<sup>−/−</sup> B cells failed to up-regulate these molecules following anti-IgM cross-linking. Stimulation of vav<sup>−/−</sup> B cells with anti-CD40 plus anti-IgM partially rescued the BCR proliferation defect (Fig. 2A) and induced
up-regulation of MHC class II molecules (Fig. 2B). Although these results imply that Vav has no apparent role in LPS- and CD40-mediated B cell activation in vitro, our data do not preclude that Vav has an important function in CD40 and LPS signaling. Importantly, Vav expression is required for cell cycle progression and MHC class II expression following BCR engagement.

Because Vav regulates receptor clustering in T and B cells (12, 13), we analyzed whether the defect of BCR-mediated activation could be reversed by increased degrees of cross-linking. Indeed, increased doses of the polyclonal goat anti-mouse IgM F(ab')2 Ab could partially restore proliferation in vav−/− B cells (Fig. 2D). Increased doses of the anti-IgM mAb B7.6 could not restore proliferation (Fig. 2D), suggesting that enhanced cross-linking via polyclonal anti-IgM Abs, but not a monoclonal anti-IgM Ab, can overcome the proliferative defect in vav−/− B cells. Importantly, super-cross-linking of an anti-IgM Ab increased proliferation of vav−/− B cells in a dose-dependent fashion. In addition, increased degrees of cross-linking using different doses of anti-IgM coupled to beads enhanced proliferation of vav−/− B cells (Fig. 2E and F). Similar to negative regulation of B cell proliferation in wild-type and vav−/− B cells using the intact goat anti-mouse anti-IgM Ab, super-cross-linking of the goat anti-mouse IgM F(ab')2 Ab induced significantly higher proliferation in vav−/− B cells than super-cross-linking of the intact goat anti-mouse IgM Ab, indicating that this mechanism of negative regulation is still operational in vav−/− B cells. These data show that increased degrees of cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav−/− B cells.

**Vav is not required for IgM responses to repetitive viral and hapten Ags**

To examine the requirement for Vav in B cell responses in vivo, we challenged vav−/− and vav−/− mice with VSV, which has highly repetitive surface epitopes (29). VSV infections are controlled exclusively by neutralizing Abs (30). All neutralizing Abs are directed against the VSV glycoprotein that is present in a highly repetitive form in the viral envelope. Due to this repetitiveness, neutralizing IgM Abs are induced in complete absence of T cell help (31). However, the isotype switch from IgM to IgG is Th cell dependent (32, 33). Moreover, production of VSV-neutralizing IgG Abs and the formation of VSV-specific germinal centers are dependent on CD28 expression (25, 34). In vav−/− mice, VSV infections induced rapid, T cell-independent IgM production, followed by a Th cell and CD28 costimulation-dependent IgG response (Table I). The T cell-independent IgM response induced by VSV was not affected by the absence of Vav, indicating that efficient cross-linking mediated by highly repetitive Ags can overcome the defect in vav−/− B cells. However, the T cell-dependent

---

**FIGURE 2.** B cell stimulation in vav−/− mice. A–C, Activation of splenic B cells. Purified splenic B cells (10⁵/well) from vav−/− and vav+/+ littermate mice were seeded in medium containing no added stimulus (control), soluble polyclonal anti-IgM Ab (20 μg/ml), soluble anti-CD40 (1 μg/ml), IL-4 (10 U/ml), soluble anti-IgM Ab (2 μg/ml) plus soluble anti-CD40 (1 μg/ml), and LPS (10 μg/ml). After 24 h, the cells were pulsed for 12 h with 1 μCi [³H]thymidine/well (A), and stained for surface expression of I-AK and CD86 (B and C). [³H]Thymidine uptake of triplicate cultures is shown in cpm ± SD. I-AK and CD86 surface expression of triplicate samples is shown as mean fluorescence of anti-I-AK and anti-CD86 Ab staining ± SD. One result representative of three experiments is shown. D–F, Super-cross-linking of the B cell Ag receptor. Purified splenic B cells (10⁵/well) from vav−/− and vav−/− littermate mice were seeded in medium containing no added stimulus (Control), LPS (2 μg/ml) as positive control, different doses of polyclonal intact anti-IgM coupled to beads (anti-IgM-beads), and plate-bound polyclonal intact goat anti-IgM (5 μg/ml) super-cross-linked with different concentrations of rabbit anti-goat Ig (IgM-X-link) (E and F). [³H]Thymidine uptake of triplicate cultures is shown in cpm ± SD after 48-h (D and E) and 72-h (F) culture periods. One result representative of five experiments is shown.
To assess whether T cell-independent type 1 Ag due to its high degree of organization and normal distribution of T cells, B cells, macrophages, and follicular dendritic cells (data not shown).

VSV glycoprotein (VSV-G) in the viral envelope behaves as a T cell-independent type 1 Ag due to its high degree of organization (31). To assess whether vav−/− B cells could also be stimulated by a less repetitive form of VSV-G, vav−/− and vav−/− littermate mice were immunized with a recombinant vaccinia virus expressing VSV-G (Vac-G). This form of VSV glycoprotein has been shown to act as a type 2 T cell-independent Ag (29, 30). As observed after infection with VSV, vav−/− mice mounted normal VSV-G-specific IgM responses after immunization with Vac-G (Table II). The T cell-dependent VSV-G IgG response was reduced significantly in vav−/− mice, albeit clearly detectable (Table II). Moreover, immunization with the type 2 T cell-independent hapten TNP-Ficoll, a polyvalent Ag that can effectively cross-link the BCR (28), showed that the levels and kinetics of anti-TNP-specific IgM and IgG3 production are comparable among vav−/+ and vav−/− mice, albeit slightly lower in vav−/− mice (Fig. 3). Our in vivo results show that vav−/− B cells are able to respond to repetitive type 1 and type 2 T cell-independent viral and hapten Ags. However, Th cell-dependent neutralizing IgG responses to viral Ags are reduced in vav−/− mice.

Impaired B cell responses to a T cell-dependent hapten

To further analyze the role of Vav in B cell responses to nonrepetitive T cell-dependent Ags, vav−/− and vav−/− mice were immunized with the T cell-dependent hapten NIP conjugated to OVA (NIP-OVA). Whereas vav−/+ mice exhibit high titers of anti-NIP-specific IgG1 and IgG2a Abs, IgG1 and IgG2a Ab responses to NIP were absent in vav−/− mice (Fig. 4). In addition, germinal center formation was not observed in vav−/− mice following challenge with NIP-OVA (data not shown). These data show that vav−/− mice can mount biologically relevant responses against VSV and recombinant vaccinia VSV-G, and that Vav has no critical role in B cell responses to the polyvalent hapten Ag TNP-Ficoll. However, Vav expression is required to generate functional B cell responses to nonrepetitive hapten Ags in vivo.

Discussion

Previously, it has been shown in vav−/−rag−/− blastocyst complementation studies that Vav has a role in the development of conventional B cells and peritoneal CD5+ B1 B lymphocytes (16, 17). Our results in viable vav−/− mice demonstrate that Vav is dispensable for B cell differentiation and pre-BCR-driven expansion. Stimulation of vav−/− B cells by LPS, CD40, or IL-4 was normal.
However, peripheral B cells from vav<sup>−/−</sup> mice exhibited an impaired proliferative response to IgM ligation, indicating a critical role for Vav in Ag receptor signaling. In contrast to the normal development of conventional B cells, numbers of unconventional CD5<sup>+</sup> B1 cells in the peritoneal cavity were reduced significantly in vav<sup>−/−</sup> mice. This reduction could be a direct consequence of reduced Ag receptor-mediated signaling in the absence of Vav, because the size of the B1 cell population is dependent on their capacity for self-renewal (35). A significant reduction in the B1 B cell population has also been observed in CD19-deficient (36, 37) and Btk-deficient (38, 39) mice. Both CD19 and Btk interact with Vav (21, 22), and it was reported that Vav has an important role in CD19-mediated activation of lipid and protein kinases (23).

Importantly, vav<sup>−/−</sup> cells display impaired proliferation and up-regulation of surface MHC class II molecules following IgM stimulation in vitro and vav null mice do not respond to the non-repetitive hapten NIP-OVA in vivo. However, vav<sup>−/−</sup> mice mount a protective immune response to viral infections, and vav<sup>−/−</sup> B cells respond to viral and haptenated Ags that have repetitive, polyvalent structures. We have reported previously that Vav has no role for Vav in Ag receptor signaling. In contrast to the normal responses of conventional B cells, numbers of unconventional B1 cells in the peritoneal cavity were reduced significantly in vav<sup>−/−</sup> mice. This reduction could be a direct consequence of reduced Ag receptor-mediated signaling in the absence of Vav, because the size of the B1 cell population is dependent on their capacity for self-renewal (35). A significant reduction in the B1 B cell population has also been observed in CD19-deficient (36, 37) and Btk-deficient (38, 39) mice. Both CD19 and Btk interact with Vav (21, 22), and it was reported that Vav has an important role in CD19-mediated activation of lipid and protein kinases (23).

Importantly, vav<sup>−/−</sup> B cells display impaired proliferation and up-regulation of surface MHC class II molecules following IgM stimulation in vitro and vav null mice do not respond to the non-repetitive hapten NIP-OVA in vivo. However, vav<sup>−/−</sup> mice mount a protective immune response to viral infections, and vav<sup>−/−</sup> B cells respond to viral and haptenated Ags that have repetitive, polyvalent structures. We have reported previously that Vav has no role for Vav in Ag receptor signaling. In contrast to the normal responses of conventional B cells, numbers of unconventional B1 cells in the peritoneal cavity were reduced significantly in vav<sup>−/−</sup> mice. This reduction could be a direct consequence of reduced Ag receptor-mediated signaling in the absence of Vav, because the size of the B1 cell population is dependent on their capacity for self-renewal (35). A significant reduction in the B1 B cell population has also been observed in CD19-deficient (36, 37) and Btk-deficient (38, 39) mice. Both CD19 and Btk interact with Vav (21, 22), and it was reported that Vav has an important role in CD19-mediated activation of lipid and protein kinases (23).

FIGURE 4. Impaired responses to the T cell-dependent hapten NIP-OVA. A–D, vav<sup>−/−</sup> and vav<sup>+/+</sup> littermate mice were immunized with NIP-OVA, and serum IgG1 and IgG2a titers were determined 8 and 15 days later by ELISA on NIP-BSA-coated plates. Arbitrary units of OD of NIP-OVA, and serum IgG1 and IgG2a titers were determined 8 and 15 days later by ELISA on NIP-BSA-coated plates. Arbitrary units of OD of NIP-specific IgG1 and IgG2a titers were shown for individual mice. Titers represent 2-fold dilutions of sera starting from 1/80. One result representative of two experiments is shown.

However, peripheral B cells from vav<sup>−/−</sup> mice exhibited an impaired proliferative response to IgM ligation, indicating a critical role for Vav in Ag receptor signaling. In contrast to the normal development of conventional B cells, numbers of unconventional CD5<sup>+</sup> B1 cells in the peritoneal cavity were reduced significantly in vav<sup>−/−</sup> mice. This reduction could be a direct consequence of reduced Ag receptor-mediated signaling in the absence of Vav, because the size of the B1 cell population is dependent on their capacity for self-renewal (35). A significant reduction in the B1 B cell population has also been observed in CD19-deficient (36, 37) and Btk-deficient (38, 39) mice. Both CD19 and Btk interact with Vav (21, 22), and it was reported that Vav has an important role in CD19-mediated activation of lipid and protein kinases (23).

Importantly, vav<sup>−/−</sup> B cells display impaired proliferation and up-regulation of surface MHC class II molecules following IgM stimulation in vitro and vav null mice do not respond to the non-repetitive hapten NIP-OVA in vivo. However, vav<sup>−/−</sup> mice mount a protective immune response to viral infections, and vav<sup>−/−</sup> B cells respond to viral and haptenated Ags that have repetitive, polyvalent structures. We have reported previously that Vav has no apparent role in TCR-mediated signaling pathways such as overall tyrosine phosphorylation, mitogen-activated protein kinase, and stress-activated protein kinase/c-Jun N-terminal kinase activation (12, 13). However, Vav was found to associate with the cytoskeletal membrane anchors Talin and Vinculin and to coordinate recruitment of the actin cytoskeleton to the Ag receptor complex. Consistent with a role for Vav in transducing Ag receptor signals to the actin cytoskeleton, vav<sup>−/−</sup> mice T cells displayed impaired actin polymerization in response to Ag receptor activation and exhibit defective clustering (patching and capping) of the TCR (12, 13). Moreover, gene-targeted mice with a mutation in the Wiskott-Aldrich syndrome protein (WASP), a cytoskeletal protein that associates with the Vav target CDC42 and regulates cytoskeletal reorganization, display a T cell phenotype similar to vav<sup>−/−</sup> mice, i.e., wasp<sup>−/−</sup> T cells exhibit impaired TCR capping, proliferation, and IL-2 production following TCR stimulation (40). Based on these results, it has been suggested that TCR-mediated cytoskeletal reorganization and receptor clustering are crucial prerequisites for T cell maturation, IL-2 production, and cell cycle progression. Cytoskeletal rearrangements and formation of caps probably relocate the signaling machinery to the site of receptor engagement and thus organize compartmentalized, actin-scaffolded signaling highways (12).

Similar to T cells, Ab-mediated cross-linking of the BCR on B lymphocytes induces the formation of cap structures localizing at one pole of the cell, and formation of the caps is partially dependent on Vav expression (13). Although the functional relevance of cap formation is equivocal in B cells, IgM-associated Igα together with Lyn and Syk translocate to the membrane skeleton following BCR cross-linking (41, 42) and p21<sup>ras</sup> has been shown to co-cap with surface Ig molecules in mouse splenic B lymphocytes (43). A potential role of BCR caps in the generation of B cell responses is in line with our findings that increased degrees of Ag receptor cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav<sup>−/−</sup> B cells in vitro, and that vav<sup>−/−</sup> B cells can be activated in vivo with repetitive Ags that effectively cross-link BCR. However, vav<sup>−/−</sup> B cells do not respond to non-repetitive hapten Ags and low doses of anti-IgM Ab stimulation. Strong antigenicity of repetitive Ags has been described previously, and B cell responses against these molecules normally do not require T cell help (31). By contrast, B cell responses to non-repetitive weak Ags and Ig class switching are Th cell dependent.

The impaired responses of vav<sup>−/−</sup> mice to the hapten NIP-OVA could be due to a defect in T cell help and/or an intrinsic defect in BCR-mediated stimulation. We have shown previously that peripheral T cells from vav<sup>−/−</sup> mice have a defect in IL-2 production and cell cycle progression following TCR activation (12). Thus, the defective response of vav<sup>−/−</sup> B cells to the T cell-dependent hapten Ag NIP-OVA and reduced T cell-dependent Ig class switching following VSV and Vacci-G infections in vav<sup>−/−</sup> mice can be attributed to compromised T cell help. However, vav<sup>−/−</sup> mice can mount protective Th cell-dependent IgG responses to VSV and Vacci-G infections, indicating that T cell help must be, at least in part, functional in the absence of Vav. Moreover, vav<sup>−/−</sup> B cells have an impaired response to IgM cross-linking in vitro, indicating that Vav has a direct role in BCR-mediated activation.

The relative importance of the Vav deficiency in B and T cells in vivo needs to be further examined using adoptive transfer experiments. Recently, it has been shown that Vav regulates CD19-mediated PIP5 kinase activation in B cells (23). Interestingly, in vivo immune responses of CD19<sup>−/−</sup> mice resemble immune responses in vav<sup>−/−</sup> mice, i.e., CD19<sup>−/−</sup> mice exhibit nearly normal Ig responses following infections with repetitive VSV, but impaired B cell responses to challenge with nonrepetitive LCMV (44). These results suggest that the positive regulatory B cell co-receptor CD19 and Vav mediate similar signaling pathways required for B cell activation in vivo.

We report that the splenic B cells lacking the guanine-nucleotide exchange factor Vav do not respond to IgM cross-linking and do not respond to challenge with the T cell-dependent hapten NIP-OVA. By contrast, vav<sup>−/−</sup> mice mounted protective antiviral IgM and IgG responses to infections with VSV and Vacci-G, which harbor repetitive surface epitopes that directly cross-link the Ag receptor and activate B cells in the absence of T cell help. vav<sup>−/−</sup>
B cells also responded normally to the polyvalent, T cell-independent hapten Ag TNP-Ficol in vivo. Increased degrees of Ag receptor cross-linking can partially reverse the proliferative defect in the anti-IgM response of vav−/− B cells. These results suggest that Vav has an important role in setting the threshold for Ag receptor-mediated stimulation of T and B lymphocytes depending on the nature of the Ag.

Acknowledgments

We thank C. Paige and A. Rolink for reagents and M. Nghiem, K. Bachmaier, A. Hakem, and L. Zhang for critical comments. We also thank Christiane Ruedel and Manfred Kopf for critically reading the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-LaRoche (Basel, Switzerland).

References