The inhibitory Fc receptor, FcγRIIB, provides a signal that aborts B cell antigen receptor activation, blocking extracellular calcium influx. Because the protein-tyrosine phosphatase SHP-1 binds tyrosyl phosphorylated FcγRIIB and FcγRIIB-mediated inhibition is defective in motheaten (me/me) mice, which do not express SHP-1, it was proposed that SHP-1 mediates FcγRIIB signaling in B cells (D’Ambrosio, D., Hippen, K. L., Minskoff, S. A., Mellman, I., Pani, G., Siminovitch, K. A., and Cambier, J. C. (1995) Science 268, 293–297). However, SHP-1 is dispensable for FcγRIIB-mediated inhibition of FcεRI signaling in mast cells (Ono, M., Bolland, S., Tempst, P., and Ravetch, J. V. (1996) Nature 383, 263–266), prompting us to re-examine the role of SHP-1 in FcγRIIB signaling in B cells. We generated immortalized sIgM+ FcγRIIB+ cell lines from me/me mice and normal littermates. Co-ligation of FcγRIIB and the sIgM antigen receptor inhibits calcium influx in both cell lines. Inhibition is reversed by preincubation with anti-FcγRIIB antibodies, indicating that it is mediated by FcγRIIB. The inositol 5’ phosphatase SHIP is recruited to tyrosyl-phosphorylated FcγRIIB in both cell lines. FcγRIIB-mediated CD19 dephosphorylation also occurs in the presence or absence of SHP-1. Our results establish that SHP-1 is dispensable for FcγRIIB-mediated inhibition of sIgM antigen receptor signaling.

The sIgM antigen receptor (BCR) is a cell surface heteromeric structure consisting of a ligand binding component, surface immunoglobulin (sIg), and accessory signal transducing components, the Igα and Igβ chains. Activation of the BCR leads to B lymphocyte proliferation, differentiation, and antibody secretion. Upon BCR cross-linking, several protein-tyrosine kinases including Lyn, Fyn, Blk, Syk, and Btk become activated and cause the recruitment, tyrosine phosphorylation, and activation of secondary signaling molecules, including PI-3K, Shc, Gap, Vav, and phospholipase Cγ. BCR accessory components, such as CD19 and CD22, also become tyrosyl phosphorylated and recruit secondary signaling molecules, which either enhance or dampen the BCR signal. These initial events lead to Ras activation, PI3-K activation, phosphoinositide turnover, and an increase in intracellular free calcium (reviewed in Ref. 1).

By co-ligating the BCR and the inhibitory Fc receptor, FcγRIIB, immune complexes activate a negative feedback mechanism that inhibits antibody production (reviewed in Refs. 2 and 3). FcγRIIB-mediated inhibition in B lymphocytes can be studied experimentally by comparing responses evoked by cross-linking the BCR with Fab’γ anti-Mu, which engages the BCR alone, with those initiated with intact anti-Mu Ig, which co-ligates FcγRIIB and the BCR. Co-ligation results in inhibition of BCR-evoked inositol triphosphate production and extracellular calcium influx (4, 5). Sustained calcium influx is required for multiple cellular processes, including proliferation and differentiation (reviewed in Refs. 6 and 7). Low concentrations of calcium ionophores override FcγRIIB-mediated inhibition of A20 B cell activation, indicating that inhibition of calcium influx is a (the) key mechanism by which antigen-mediated signals are aborted by FcγRIIB signals (4). An analogous mechanism operates in mast cells, where co-engagement of FcγRIIB and FcεRI results in inhibition of FcεRI-generated responses (9–11).

A single tyrosyl residue, situated within the 13-amino acid ITIM (immunoreceptor tyrosine-based inhibitory motif) in the cytoplasmic domain of FcγRIIB, is required for FcγRIIB-mediated inhibition of BCR signaling (12). SHP-1, an SH2-domain containing protein-tyrosine phosphatase (PTP), binds to the tyrosyl-phosphorylated FcγRIIB ITIM motif both in vitro and in vivo (13). Because B cells from motheaten (me/me) and motheaten viable (me+/me+) mice, which have defective SHP-1 alleles, failed to demonstrate FcγRIIB-mediated inhibition, it was proposed that the FcγRIIB inhibitory signal is mediated by SHP-1 (13, 14). Consistent with this model, a recent report demonstrated that CD19 is dephosphorylated upon co-cross-linking of FcγRIIB and the BCR, leading to decreased binding of the p85 subunit of PI-3K to CD19 (15). This led to the proposal that SHP-1 regulates intracellular calcium levels by controlling CD19 tyrosyl phosphorylation, which in turn controls associated PI-3K activity (15). Interestingly, me/me mice exhibit a plethora of immune system defects, including hypergamaglobulinemia and a high level of circulating immune complexes (reviewed in Ref. 16). Conceivably, one or more of these disorders might be explained by defective FcγRIIB signaling.

However, the role of SHP-1 in FcγRIIB-mediated inhibitory signaling has been a subject of controversy. Mice lacking FcγRIIB do not exhibit uncontrolled antibody production when challenged with antigen (9). Moreover, as revealed by studies of mast cells from me/me mice, SHP-1 clearly is dispensable for
FcγRIIIB Inhibits B Cell Activation in the Absence of SHP-1

20039

FcyRIIIB-mediated inhibition of FceRI signaling (17). Ono et al. have proposed that the inositol phosphatase SHIP (SH2 domain containing 5'-inositol phosphatase), which also binds to the FcγRIIB ITIM, is the key mediator of FcγRIIB signaling (17). The situation is complicated further by a report that the other mammalian SH2-containing PTP, SHP-2, also binds to this ITIM, at least in vitro (14). Although it is possible that FcγRIIB employs unique signaling mechanisms in different cell types, these findings have raised questions as to whether SHP-1 actually is required for FcγRIIB-mediated inhibition in B cells.

To develop a model system for studying the role of SHP-1 in multiple B cell signaling pathways, we generated B cell lines from me/me (ME) and normal (N) littermate mice by immortalization with the retrovirus J2 (18, 19). N and ME cell lines express similar surface markers and respond to BCR stimulation and FcγRIIB/BCR co-ligation. We find that both cell lines exhibit comparably decreased calcium influx when FcγRIIB is co-cross-linked with the BCR. Consistent with previous reports, CD19 tyrosyl phosphorylation decreases upon co-cross-linking of FcγRIIB and the BCR in N cells. However, dephosphorylation also is observed in the ME line. Our results establish that SHP-1 is dispensable for both biochemical and biological responses to FcγRIIB engagement in B cells.

EXPERIMENTAL PROCEDURES

Generation of Immortalized me/me and Normal B Cells—J2 producer cells (20) were obtained from Dr. Christopher Myers of the University of Tennessee, Memphis, TN. Virus-containing supernatants were collected from J2 cells growing in log phase for 24 h in RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum, 135 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 10 mM HEPES, pH 7.4, 1.5 mM EGTA, followed by the addition of 5 mM CaCl2. The emission ratio (F/F0) of Fura-2 fluorescence was used to measure calcium influx, cells were activated with anti-Mu antibodies in the presence of Triton X-100. Immortalized B cell lines were obtained by co-cross-linking FcγRIIB engagement with the BCR. Consistent with previous reports, CD19 tyrosyl phosphorylation decreases upon co-cross-linking of FcγRIIB and the BCR in N cells. However, dephosphorylation also is observed in the ME line. Our results establish that SHP-1 is dispensable for both biochemical and biological responses to FcγRIIB engagement in B cells.

Calcium Assays—N and ME cells were loaded with 2 μM (final concentration) Fura-2 ( Molecular Probes, Eugene, OR) in a buffer containing 135 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 10 mM HEPES, pH 7.4, and 0.1% bovine serum albumin. Cells were washed once and resuspended at 2 × 106 cells/ml with medium containing J2 retrovirus. After 15–30 days, cells that had proliferated were expanded and analyzed for sIgM by flow cytometry. sIgM+ cells were subsequently clonied by limiting dilution, expanded, and maintained in RPMI 1640 supplemented as described above, except that the fetal calf serum concentration was decreased to 10%.

Flow Cytometry—Cells were stained with the appropriate antibodies in the presence of fully supplemented RPMI. Antibodies used for staining included anti-IgM and anti-FcγRIIB (CD32/CD16; 2.4G2), obtained from Pharmingen. Flow cytometric data were obtained on a FACScan using CellQuest acquisition and analytical software (Becton Dickinson, San Jose, CA).

Calcium Assays—N and ME cells were loaded with 2 μM (final concentration) Fura-2 ( Molecular Probes, Eugene, OR) in a buffer containing 135 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 10 mM HEPES, pH 7.4, and 0.1% bovine serum albumin. Cells were washed once and resuspended at 2 × 106 cells/ml. Assays were performed as described previously (21) using a Delta Scan spectrophotometer (Photon Technologies Inc., South Brunswick, NJ). Cells were activated with 5 μg/ml F(ab')2 goat anti-mouse Mu or with 10 μg/ml rabbit anti-mouse Mu (Jackson ImmunoResearch) either with or without preincubation with anti-FcR (2.4G2) antibody. To monitor calcium influx, cells were activated with anti-Mu antibodies in the presence of 1.5 mM EGTA, followed by the addition of 5 mM CaCl2. The emission values at 510 nm following excitation at 340 or 380 nm were collected and analyzed using FELIX software (Photon Technologies). Analyses of data and generation of graphs were performed using Quattropro.

Cell Activations and Immunoprecipitations—N and ME cells were activated by incubation with 5–10 μg/ml F(ab')2, anti-Mu or with 10 μg/ml rabbit anti-mouse Mu (Jackson ImmunoResearch) in the presence of 5 μg/ml F(ab')2, anti-Mu or with 10 μg/ml rabbit anti-mouse Mu (Jackson ImmunoResearch) at 37 °C. Activations were stopped by addition of lysis buffer containing 1% Nonidet P-40, 150 mM sodium chloride, 5 mM sodium fluoride, 1 mM EDTA, 2 mM sodium orthovanadate, 25 mM HEPES, pH 7.4, and the protease inhibitors leupeptin (10 μg/ml), aprotonin (10 μg/ml), pepstatin (1 μg/ml), antipain (1 μg/ml), and phenylmethylsulfonfluoride (10 μg/ml). Typically, 5 × 106 cells were activated per time point, and one-tenth of the resulting lysate was removed before immunoprecipitation and analyzed by anti-phosphotyrosine immunoblotting. In some cases, activations were stopped by the addition of ice-cold phosphate-buffered saline, and cell pellets were frozen in liquid N2 and stored at −80 °C prior to lysis. Lysates were immunoprecipitated for 1–3 h with the indicated antibodies, including anti-CD19 (Pharmingen), anti-FcγRIIB (CD32/CD16, Pharmingen), and anti-SHP (a gift from Dr. Mark Coggeshall, Ohio State University). Anti-FcγRIIB antibodies were covalently cross-linked to protein G-agarose beads prior to use. Immunoprecipitates were collected onto either protein A-Sepharose (Pharmacia Biotech Inc.) or protein G-agarose (Oncogene Sciences) beads, washed three times with lysis buffer, separated by SDS-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene difluoride membranes (Immobilon, Millipore).

Immunoblotting—For anti-phosphotyrosine immunoblotting, membranes were blocked with 5% bovine serum albumin (Boehringer Mannheim) in TBST buffer (10 mM Tris, pH 7.4, 150 mM sodium chloride, 0.1% Tween 20), followed by incubation with 1 μg/ml monoclonal anti-phosphotyrosine 4G10 antibodies (Upstate Biotechnology Inc.) and subsequent incubation with secondary antibodies (donkey anti-mouse) conjugated to horseradish peroxidase (Amersham Corp.). All other blots were blocked in 5% milk in TBST and were developed in a similar manner with the indicated antibodies. Antibodies used for immunoblotting included: anti-SHP-1 polyclonal antiserum (1:1000 dilution) (22, 23), polyclonal anti-PTP1C and anti-PTP1D (1 μg/ml; Signal Transduction Labs), polyclonal anti-CD19 (a gift from Dr. John Cambier, Denver Colorado), and anti-SHIP (1:1000). Immunoreactive proteins were detected by Enhanced Chemiluminescence (Amersham Corp.). For reprobing, polyvinylidene difluoride membranes were stripped of antibodies, according to the manufacturer's suggestions (Amersham Corp.).

RESULTS AND DISCUSSION

SHP-1 has been implicated as a negative regulator of antigen receptor (BCR) signaling in B cells and, in particular, in FcγRIIB signaling (13, 24, 25). Comparison of BCR signaling events in primary B cells from me/me mice is difficult because these cells are developmentally distinct and have reduced surface levels of sIgM and FcγRIIB compared with age-matched B cells from normal littermates (24). To circumvent such problems, we generated a set of immortalized B cell lines derived from me/me and normal littermate mice, using the murine retrovirus J2, which expresses the viral oncoproteins ras and myc (19, 20) (Fig. 1A). Previous work indicated that this virus can generate immortal, factor-independent macrophage and B cell lines in vivo and ex vivo (20, 26). Unlike B cell lines produced by Abelson MuLV, which are arrested at the pro- or early pre-B cell stage and thus cannot signal through the BCR, many J2-derived lines are sIgM+, sIgD+, and retain BCR signaling capacity (8, 20) (Figs. 2 and 3). We obtained several normal cell lines (N) and one me/me-derived line (ME) (Fig. 1). Southern analysis indicated that these lines are clonal (data not shown), and immunoblotting confirmed that the ME line lacks SHP-1 expression (Fig. 1B). Notably, however, the N and ME lines express equal levels of SHP-2 (Fig. 1B). The surface phenotypes of the N and ME cell lines are similar; of particular importance for the results discussed below, they express comparable levels of sIgM and FcγRIIB (Fig. 1, C–F). Both lines also display similar levels of sIgD, CD45 (B220), CD19, and CD22 but do not express CD5, CD11b, B7.1 or B7.2 (data not shown). Therefore, their surface phenotype more closely resembles primary B cells, rather than B-1a, germinal center or memory B cells. Other properties of these cell lines will be discussed in more detail elsewhere.

We assessed FcγRIIB-mediated inhibition in both cell lines by comparing their calcium response following BCR ligation alone or together with FcγRIIB. The change in the ratio (che-lated/free) of Fura-2 fluorescence was used to measure calcium responses to both stimuli. We first tested the N cell line to determine whether J2 immortalization interferes with BCR

2 M. J. S. Nadler, unpublished observations.

3 M. J. S. Nadler, B. G. Neel, and H. H. Worts, manuscript in preparation.
activation and/or FcγRIIB-mediated inhibition. N cells activated with F(ab')2 anti-Mu (engagement of BCR alone) exhibited a robust increase in intracellular free calcium. This response was markedly diminished in N cells stimulated with intact anti-Mu (Fig. 2A). Preincubation with anti-FcγRIIB antibodies (2.4G2) abrogated the ability of intact anti-Mu to inhibit the calcium response in N cells (Fig. 2B), indicating that inhibition occurred solely via engagement of FcγRIIB. N cells activated in the presence of EGTA showed little difference in peak calcium release from intracellular stores (Fig. 2C). Readdition of CaCl2 after the release phase to allow measurement of calcium influx revealed strong inhibition by intact anti-Mu (Fig. 2D). These results, which are consistent with those reported for primary B cells and several B cell lines (see Introduction), demonstrate that J2-immortalized B cells provide a valid model to study BCR signal transduction and its regulation by FcγRIIB.

To determine whether SHP-1 is required for negative regulation of calcium influx by FcγRIIB, we compared calcium responses in ME cells following ligation of the BCR with either F(ab')2 or intact anti-Mu. Surprisingly, ME cells activated with intact anti-Mu also showed complete inhibition of the calcium response compared with those stimulated with F(ab')2 anti-Mu (Fig. 2D); again, inhibition was reversed by preincubation with anti-FcγRIIB antibodies (Fig. 2E). As in N cells, there was little difference in peak calcium release from intracellular stores following ligation of ME cells with either antibody in the presence of EGTA, but extracellular calcium influx was markedly reduced with intact anti-Mu compared with F(ab')2 anti-Mu (Fig. 2F). As with N cells, we have recently confirmed these results using matched rabbit F(ab')2 and intact anti-Mu IgG (data not shown). Because ME cells exhibit FcγRIIB-mediated
inhibition of the BCR-induced calcium response, we conclude that, as in mast cells (17), SHP-1 is not required for FcR-mediated inhibition of B cell signaling.

Although SHP-1 is dispensable for FcγRIIB-mediated inhibition of BCR calcium influx, it remained possible that it influences FcγRIIB-evoked tyrosyl phosphorylation events. To address this issue, we first examined the tyrosyl phosphorylation of FcγRIIB itself. Previous studies established that tyrosyl phosphorylation of FcγRIIB occurs upon co-cross-linking to the BCR, but not when the BCR alone is engaged (12). Anti-phosphotyrosine immunoblots of FcγRIIB immunoprecipitates from N and ME cells activated with either F(ab')2 or by intact anti-Mu ligation revealed FcγRIIB tyrosyl phosphorylation only upon co-cross-linking (Fig. 3A, upper panel). FcγRIIB was not significantly tyrosyl phosphorylated either basally or upon F(ab')2 anti-Mu stimulation in ME cells, indicating that loss of SHP-1 does not lead to aberrant FcγRIIB tyrosyl phosphorylation (Fig. 3A, upper panel). To determine if absence of SHP-1 resulted in global differences in total cellular tyrosyl phosphorylation upon FcγRIIB/BCR co-cross-linking, we compared anti-phosphotyrosine immunoblots of total cell lysates from cells that were activated with either F(ab')2 or intact anti-Mu. In N cells, each stimulus yielded a comparable overall total cellular tyrosyl phosphorylation response (Fig. 3C), consistent with previous studies that reported no gross difference in total tyrosyl phosphorylation between intact versus F(ab')2 anti-BCR stimulation (12). Each stimulus also evoked comparable increases in total cellular phosphotyrosine in ME cells (Fig. 3C). A detailed
comparison of BCR signal transduction between N, ME, and ME cells reconstituted with wild type and mutant SHP-1 will be presented elsewhere.3

CD19 has been reported to display decreased tyrosyl phosphorylation upon FcγRIIB co-cross-linking with the BCR. It has been suggested that SHP-1 mediates the differential tyrosyl phosphorylation of this co-receptor (15). To address these issues, we examined CD19 tyrosyl phosphorylation in both cell lines. In N cells, anti-phosphotyrosine immunoblotting of CD19 immunoprecipitates revealed that tyrosyl phosphorylation of CD19 is slightly decreased when the BCR is co-cross-linked with FcγRIIB (Fig. 3D), consistent with the previous study (15). However, decreased CD19 tyrosyl phosphorylation also was observed when the BCR was co-cross-linked with FcγRIIB in ME cells (Fig. 3D). These data suggest that SHP-1 is not solely responsible for CD19 dephosphorylation in response to FcγRIIB engagement.

Our results clearly establish that SHP-1 is not required for FcγRIIB-mediated inhibition of BCR-induced calcium influx. These findings contrast with an earlier report, which suggested that SHP-1 is necessary for FcγRIIB-mediated inhibition in B cells (13). The earlier study compared signaling in primary lymphocytes from normal and me/me or me'/me’ mice. Primary B cells from me/me mice are developmentally distinct from those of their normal counterparts and are similar in phenotype to anergized B cells, exhibiting decreased levels of BCR and increased expression of activation markers (24). The failure of the earlier workers to observe effects of FcγRIIB engagement on the proliferative responses of me/me B cells may reflect developmental differences caused by the lack of SHP-1, rather than the direct effect of absence of SHP-1 on signaling via FcγRIIB. Alternatively, the high levels of circulating immune complexes in me/me mice may lead to desensitization of FcγRIIB signaling (although me/me B cells do retain surface FcγRIIB; data not shown).

Our results also establish that SHP-1 is not required for the FcγRIIB-evoked decrease in CD19 tyrosyl phosphorylation. Presumably, because there has been no demonstrated change in any protein-tyrosine kinase activity upon FcγRIIB engagement (12), a PTP other than SHP-1 promotes CD19 dephosphorylation. FcγRIIB has been shown to bind the FcγRIIB ITIM (14), raising the possibility that it may be responsible for CD19 dephosphorylation in vivo. In any case, the functional significance of CD19 dephosphorylation in response to FcγRIIB engagement remains unclear.

As in mast cells (17) and other B cell lines and primary B cells, SHIP, an SH2-domain containing inositol phosphatase, associates with tyrosyl phosphorylated FcγRIIB and becomes tyrosyl phosphorylated in both N and ME cells (Figs. 3A, lower panel, and B), raising the possibility that it mediates inhibitory signaling by this receptor. The mechanism by which an inositol phosphatase could promote inhibition of calcium influx is unclear. Hydrolysis of phosphatidylinositol 3,4,5-trisphosphate and/or phosphatidylinositol 4,5-bisphosphate could directly or indirectly modulate a calcium channel responsible for influx. Such a channel(s) remains to be identified in B cells. Dephosphorylation of CD19, with consequent diminished recruitment of PI-3K (15), might lead to similar effects on phosphatidylinositol-containing lipids, as recruitment of SHIP to FcγRIIB. Therefore, it remains possible that FcγRIIB may function by
recruiting both SHIP and at least SHP-1 or SHP-2. However, it is important to note that we have been unable to detect stable association of SHP-2 with tyrosyl phosphorylated FcγRIIB in B cells (data not shown). Clarifying the roles of SHIP and SHP-2 in FcγRIIB signaling in B cells will most likely require generation of cell lines or mice that lack expression of these proteins.

Acknowledgments—We thank Dr. Christopher Meyers for the J2 producer cell line and Dr. Paul McLean for assistance in the Southern analysis of the ME and N cell lines. We also thank Drs. Andrew Scharenberg and Jean-Pierre Kinet (Beth Israel Deaconess Medical Center, Boston) for helpful comments and for use of the Photon Technologies instrument.

Note Added in Proof—Dr. J. V. Ravetch’s group also has shown that SHP-1 is dispensable for FcγRIIB-mediated inhibition of B cell antigen receptor signaling, using DT40 B cells lacking SHP-1 as a consequence of homologous recombination (Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997) Cell, in press). In addition, Ono et al. show that SHP-1 is required for KIR-mediated inhibition.

REFERENCES
1. Cambier, J. C., Pleiman, C. M., and Clark, M. R. (1994) Annu. Rev. Immunol. 12, 457–486
2. Doody, G. M., Dempsey, P. W., and Fearon, D. T. (1996) Curr. Opin. Immunol. 8, 378–382
3. Ravetch, J. V. (1994) Cell 78, 553–560
4. Diegel, M. L., Rankin, B. M., Bolen, J. B., Dubois, P. M., and Kiener, P. A. (1994) J. Biol. Chem. 269, 11409–11416
5. Choquet, D., Partiseti, M., Amigorena, S., Bonnerot, C., Fridman, W. H., and Kern, H. (1995) J. Cell Biol. 121, 355–363
6. Clapham, D. (1995) Cell 80, 259–268
7. Lewis, R. (1995) Annu. Rev. Immunol. 13, 623–653
8. Chen, Y.-W., Lin, M.-S., and Vora, K. A. (1992) Int. Immunol. 4, 1293–1302
9. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. (1996) Nature 374, 346–349
10. Dearon, M., Malbec, O., Latour, S., Arock, M., and Fridman, W. H. (1995) J. Clin. Invest. 95, 577–585
11. Dearon, M., Latour, S., Malbec, O., Espinoza, E., Pina, P., Pasmans, S., and Fridman, W. H. (1995) Immunity 3, 635–646
12. Mata, T., Kurosaki, T., Misselwitz, K., Sanchez, M., Nussenzweig, M. C., and Ravetch, J. V. (1994) Nature 368, 70–73
13. D'Ambrosio, D., Hippen, R., Kullen, H., and Kinet, J. (1995) J. Immunol. 156, 4531–4534
14. Kieni, P. A., Lioubin, M., Ruhlschneider, L. R., Laidetter, J. A., Nadler, S. G., and Diegel, M. L. (1997) J. Biol. Chem. 272, 3334–3344
15. Tsui, F. W., and Tsui, H. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 81, 6868–6872
16. Cleveland, J. L., Rapp, U. R., Hartley, J. W., Fredrickson, T. N., Holmes, K. L., Morse, H. C. D., Jansen, H. W., Patschanzky, T., and Bister, K. (1985) J. Virol. 55, 23–33
17. Morse, H. C. I., Hartley, J. W., Fredrickson, T. N., Yetter, R. A., Majumdar, C., Cleveland, J. L., and Rapp, U. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 363–368
18. Diegel, M. L., Rankin, B. M., Bolen, J. B., Dubois, P. M., and Kiener, P. A. (1994) J. Biol. Chem. 269, 11409–11416
19. Choquet, D., Partiseti, M., Amigorena, S., Bonnerot, C., Fridman, W. H., and Kern, H. (1995) J. Cell Biol. 121, 355–363
20. Clapham, D. (1995) Cell 80, 259–268
21. Lewis, R. (1995) Annu. Rev. Immunol. 13, 623–653
22. Chen, Y.-W., Lin, M.-S., and Vora, K. A. (1992) Int. Immunol. 4, 1293–1302
23. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. (1996) Nature 374, 346–349
24. Dearon, M., Malbec, O., Latour, S., Arock, M., and Fridman, W. H. (1995) J. Clin. Invest. 95, 577–585
25. Dearon, M., Latour, S., Malbec, O., Espinoza, E., Pina, P., Pasmans, S., and Fridman, W. H. (1995) Immunity 3, 635–646
26. Mata, T., Kurosaki, T., Misselwitz, K., Sanchez, M., Nussenzweig, M. C., and Ravetch, J. V. (1994) Nature 368, 70–73
27. D'Ambrosio, D., Hippen, R., Kullen, H., and Kinet, J. (1995) J. Immunol. 156, 4531–4534
28. Kieni, P. A., Lioubin, M., Ruhlschneider, L. R., Laidetter, J. A., Nadler, S. G., and Diegel, M. L. (1997) J. Biol. Chem. 272, 3334–3344
29. Tsui, F. W., and Tsui, H. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 81, 6868–6872
30. Cleveland, J. L., Rapp, U. R., Hartley, J. W., Fredrickson, T. N., Holmes, K. L., Morse, H. C. D., Jansen, H. W., Patschanzky, T., and Bister, K. (1985) J. Virol. 55, 23–33
31. Morse, H. C. I., Hartley, J. W., Fredrickson, T. N., Yetter, R. A., Majumdar, C., Cleveland, J. L., and Rapp, U. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 363–368
32. Diegel, M. L., Rankin, B. M., Bolen, J. B., Dubois, P. M., and Kiener, P. A. (1994) J. Biol. Chem. 269, 11409–11416
33. Choquet, D., Partiseti, M., Amigorena, S., Bonnerot, C., Fridman, W. H., and Kern, H. (1995) J. Cell Biol. 121, 355–363
34. Clapham, D. (1995) Cell 80, 259–268
35. Lewis, R. (1995) Annu. Rev. Immunol. 13, 623–653
36. Chen, Y.-W., Lin, M.-S., and Vora, K. A. (1992) Int. Immunol. 4, 1293–1302
37. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. (1996) Nature 374, 346–349
38. Dearon, M., Malbec, O., Latour, S., Arock, M., and Fridman, W. H. (1995) J. Clin. Invest. 95, 577–585
39. Dearon, M., Latour, S., Malbec, O., Espinoza, E., Pina, P., Pasmans, S., and Fridman, W. H. (1995) Immunity 3, 635–646
40. Mata, T., Kurosaki, T., Misselwitz, K., Sanchez, M., Nussenzweig, M. C., and Ravetch, J. V. (1994) Nature 368, 70–73
41. D'Ambrosio, D., Hippen, R., Kullen, H., and Kinet, J. (1995) J. Biol. Chem. 269, 5918–5925
42. Lorenz, U., Ravichandran, K., Pei, D., Walsh, C. T., Burakoff, S. J., and Neel, B. G. (1984) Mol. Cell. Biol. 14, 1824–1834
43. Chen, H. E., Chang, S., Trub, T., and Neel, B. G. (1995) Mol. Cell. Biol. 16, 3685–3697
44. Cyster, J. G., and Goodman, C. C. (1995) Immunity 3, 1–20
45. Pani, G., Kozlowski, M., Cambier, J. C., Mills, G. B., and Siminovich, K. A. (1995) J. Exp. Med. 181, 2077–2084
46. Principato, M., Cleveland, J. L., Rapp, U. R., Holmes, K. L., Pieric, J. H., Morse, H. C. III, and Klinken, S. P. (1990) Mol. Cell. Biol. 10, 3562–3568