Platelet endothelial cell adhesion molecule-1 (PECAM-1) is 130-kDa member of the immunoglobulin gene superfamily that localizes to cell-cell borders of confluent endothelial cells and has been shown to play a role in the control of endothelial sheet migration and leukocyte transmigration through the endothelium. The cytoplasmic tail plays an important role in the modulation of PECAM-1 function. Mutation of tyrosine 663 or 686 in the cytoplasmic tail reduces phosphorylation and mutation of 686 is associated with a reduction in PECAM-1-mediated inhibition of cell migration (1). We have previously noted that these two tyrosine residues are surrounded by consensus sequences for Src homology 2 (SH2) domain binding (1, 2), and the experiments presented explore the potential for PECAM-1-Src and PECAM-1-SH2 domain interactions. PECAM-1 is more highly phosphorylated in endothelial cells overexpressing c-Src, and in *in vitro* kinase assays, c-Src can phosphorylate a glutathione S-transferase (GST)-PECAM cytoplasmic tail fusion protein. The phosphorylated fusion protein associates with the bead-bound c-Src. This association appears to be mediated by Src-SH2 domain, because PECAM-1 can be precipitated by a GST-Src-SH2 affinity matrix. The binding to the GST-Src-SH2 affinity matrix correlates directly with the level of PECAM-1 phosphorylation, because more PECAM-1 is precipitated from c-Src overexpressors and from wild-type rather than Tyr-663→Phe and Tyr-686→Phe mutant PECAM-1 expressors. Yet unidentified phosphoproteins can also be coimmunoprecipitated with wild-type but not mutant PECAM-1. Finally, we note the similarity of the PECAM-1 cytoplasmic domain sequence to the immunoreceptor tyrosine-based activation motif. Our data begin to delineate how tyrosines 663 and 686 may play a role in mediating PECAM-1 signal transduction.

Platelet endothelial cell adhesion molecule-1 (PECAM-1), a variant member of the ITAM family, reveals similarities that support the inclusion of PECAM-1 as a potential mediator of signal transduction (9). The experiments presented in this paper explore the potential for Src phosphorylation of and association with PECAM-1. We also present experiments in murine embryonic vascular development (8) both modulate PECAM-1 phosphoryrosine content. Tyrosines 663 and 686 appear to be critical to PECAM-1 phosphorylation, because mutation of either results in a drastic reduction in phosphorylation. Mutation of 686 is also associated with a reversal of the PECAM-1-mediated inhibition of cell migration (1). Deletion of exon 14, which includes tyrosine 686, is also associated with changes in PECAM-1 binding characteristics (6).

Tyrosine phosphorylation is a potential mode of signal transduction for PECAM-1, because integrin engagement (1) and murine embryonic vascular development (8) both modulate PECAM-1 phosphoryrosine content. Tyrosines 663 and 686 appear to be critical to PECAM-1 phosphorylation, because mutation of either results in a drastic reduction in phosphorylation. Mutation of 686 is also associated with a reversal of the PECAM-1-mediated inhibition of cell migration (1). Deletion of exon 14, which includes tyrosine 686, is also associated with changes in PECAM-1 binding characteristics (6).

Tyrosines 663 and 686 are followed by TEV and XEV residues, which confer selectivity for binding by the SH2 domain of the cytoplasmic kinase Src (1, 2). Binding of SH2 domains to phosphotyrosine residues and consequent protein-protein interactions has been shown to be a powerful mediator of signal transduction (9). The experiments presented in this paper explore the potential for Src phosphorylation of and association with PECAM-1. We also demonstrate that phosphorylated PECAM-1 can be coprecipitated with a variety of phosphorylated proteins and that this association is dependent on tyrosines 663 and 686. Finally, a comparison of the PECAM-1 cytoplasmic tail with that of proteins containing the ITAM reveals similarities that support the inclusion of PECAM-1 as a variant member of the ITAM family.

**MATERIALS AND METHODS**

**Antibodies**—Polyclonal anti-human PECAM-1 (Houston) and anti-bovine PECAM-1 (Elsie) were generous gifts of Steve Albeda and Horace DeLisser (University of Pennsylvania), and monoclonal anti-human PECAM-1 (JC70A) was obtained from DAKO. Polyclonal antibody to the PECAM-1 cytoplasmic tail (BooBoo) was generated in our laboratory (1). This antibody recognizes a 130-kDa protein on Western blots of lysates from human endothelial cells and 3T3 cells transfected with PECAM-1 cDNA (1). Monoclonal anti-phosphotyrosine (4G10) and anti-c-Src (PY20) were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Rabbit polyclonal anti-ZAP-70, anti-Syk, and anti-SH-tyrosine S-transferase; HUVEC, human umbilical vein endothelial cell; ITAM, immunoreceptor tyrosine-based activation motif; SH2, Src homology 2; PECAM-1cyt, recombinant cytoplasmic domain of PECAM-1; PAGE, polyacrylamide gel electrophoresis; DFP, difosfophoinositide.
PTP2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**Fusion Protein**—The GST-PECAM cytoplasmic tail fusion protein (GST-PECAM-1cyto) was made by using polymerase chain reaction to generate the coding sequence for PECAM-1 cytoplasmic tail starting at the glutathione-S-transferase leader followed by a thrombin cleavage site immediately 5' to the multicloning site. HB101 was transformed with the construct or the vector alone and induced to express the proteins with 0.5 mM isopropyl-1-thio-galactopyranoside. The bacteria were lysed by a freeze-thaw cycle, and the fusion protein or the GST is isolated by incubating the lysate with glutathione-agarose beads (Sigma) and five washes with 15 volumes of phosphate-buffered saline. The PECAM cytoplasmic tail could be removed from the GST leader by cleavage with thrombin (25 units/ml beads, from Sigma) and collection of the releasate in four washes of the beads. The relative purity of the purified fusion protein and PECAM-1 cytoplasmic tail was visualized by resolving on 10% SDS-PAGE and staining with Coomassie Blue.

**Cells**—Human umbilical vein endothelial cells (HUVECs) (10) were obtained from Jordan Pober (Yale Medical School) and were cultured in gelatin-coated flasks in M199 supplemented with 20% fetal bovine serum, endothelial cell growth factor, and heparin. Bovine aortic endothelial cells (BAECs) were isolated and cultured as described (11). BAECs transduced with a Moloney retroviral construct containing c-Src (c-Src), the kinase negative mutant met295 (Kin−), or the neomycin resistance gene (HIPPO) alone were previously generated and characterized (12). 3T3 cells expressing wild-type PECAM-1 and the tyrosine resistance gene (HIPPO) alone were previously generated and characterized (13). They were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Jurkat cells were purchased from ATCC (Clone E6–1, ATCC TIB 152; ATCC, Rockville, MD) and cultured according to the vendor's instructions.

**Immunoprecipitation and Western Blots**—Cell trypsinization, platting (onto tissue culture plastic Petri dishes), immunoprecipitation, and Western blotting were all performed as described previously (1). In summary, HUVEC, BAEC, and 3T3 cells were pretreated with 25 μg/ml cycloheximide and then trypsinized and plated on tissue culture Petri dishes for varying amounts of time before scraping into cold lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 1% sodium deoxycholate, and either 2 mM phenylmethylsulfonyl fluoride or 1 mM DFP). Immunoprecipitates were resolved on 6–7.5% SDS-PAGE under reducing conditions and then transferred to nitrocellulose. Western blots were visualized with use of the ECL system (Amersham Corp.).

Co-immunoprecipitation of phosphoproteins with PECAM-1 from transfected 3T3 cells was performed by preincubation with 0.5 mM sodium orthovanadate for 2 h and plating onto tissue culture plastic for 2 h more in serum-free medium containing 0.5 mM sodium orthovanadate at subconfluent densities to induce adhesion-mediated phospho-rylation of PECAM-1. Cells were lysed with 0.1% Triton X-100, 50 mM NaCl, 50 mM Tris, pH 7.2, 1 mM DFP, 0.25 μg/mL apotinin units/ml aprotinin, and PECAM-1 was immunoprecipitated with anti-PECAM-1 antibody (Houston). Immune complexes were washed three times with the lysis buffer, solubilized with 2 × Laemmli solubilization buffer without reducing agents, and resolved on 10% SDS-PAGE.

Jurkat cells were harvested by centrifugation and washed twice in phosphate-buffered saline prior to lysing in 3–4 volumes of lysis buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 0.5 mM NaVO<sub>4</sub>, 1 mM EDTA, and Complete™ protease inhibitor mixture (Boehringer Mannheim). Western blotting was performed using 10 μg of cellular protein/lane resolved on 7.5% SDS-PAGE and incubated 2 h to overnight in primary antibody (anti-ZAP-70, anti-Syk, and anti-SH-PTP2; 0.5 μg/ml in Blotto (50% nonfat dry milk in TBS-T) (50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20)). polyclonal anti-human PECAM-1 (BooBoo) at 1:1000 in Blotto, anti-c-Src (PY20) at 1:1000 in 2.5% bovine serum albumin in TBS-T). The results were visualized using ECL detection reagents (Amersham Corp.). Immunoprecipitation of Jurkat ZAP-70, Syk, and SH-PTP2 were performed using 250 μg of lysate and 10 μg of antibodies against human PECAM-1 (BooBoo) 1:133, ZAP-70, Syk, and SH-PTP2 1:50. Immunoprecipitates were resolved using 7.5% SDS-PAGE and visualized by western blotting or used in Far Western assays (13).

**Fusion Protein Binding Assays**—For binding to GST-PECAM-SH2, 3T3 cells expressing the different forms of PECAM-1 were processed and used for commounprecipitations described above. GST-PECAM-SH2 bound to glutathione-agarose beads were prepared as described for GST-PECAM-1cyto and were incubated with the lysates for 60 min at 4°C. Protein complexes were then washed three times with lysis buffer and resolved on 6% SDS-PAGE under reducing conditions. Binding of PECAM-1 was assessed by Western blotting with anti-PECAM-1 antibody (JCT0A).

**Kinase Assay**—In vitro kinase assays were performed essentially as described previously (12). Briefly, BAECs overexpressing c-Src were lysed with RIPA buffer (50 mM Tris, pH 7.4–7.6, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.2 mM sodium orthovanadate), and 500 μg of the lysate was used to immunoprecipitate c-Src with anti-Src antibody and protein A-Sepharose. The immunoprecipitates, including the 20 μl of protein A-Sepharose was mixed with 20 μl of reaction mix to a final concentration of 50 mM HEPES, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 25 μg unlabeled ATP, 10 μl of [γ-32P]ATP, 0.5 mg/ml substrate. The substrate was the GST-PECAM fusion protein, GST alone, or the PECAM cytoplasmic tail cleaved from the GST leader. Mixtures were incubated for 20 min at 30°C, and the reaction was stopped by the addition of Laemmli solubilization buffer. For experiments involving separation of liquid and solid phase, the supernatant was removed from the reaction mixture after the incubation, and beads were washed three times with 50 mM HEPES or communcipitation buffer (see below). Solubilization buffer was added to both the supernatant and the washed beads. All samples were resolved on 10–12% SDS-PAGE, and radioactivity was visualized by autoradiography.

**RESULTS AND DISCUSSION**

**PECAM-1 Is an in Vitro Substrate for c-Src and Is More Highly Phosphorylated in c-Src Overexpressors**—Previous studies have shown that in the absence of phosphatase inhibitors, PECAM-1 tyrosine phosphorylation in endothelial cells can be maximized by plating for a short period of time on tissue culture plastic (1). PECAM-1 phosphorylation in BAECs overexpressing c-Src, the kinase inactive mutant met295, or a vector control (HIPPO) was assessed in this manner, and PECAM-1 was found to be more highly phosphorylated in c-Src overexpressors (Fig. 1). met295 cells showed no consistent differences in the level of PECAM-1 phosphorylation compared with control, and PECAM-1 protein levels were constant in all three cell types. These data suggested that c-Src may phosphorylate PECAM-1 in vivo.

In addition to the short term experiments described above, PECAM-1 phosphorylation in these cells was assessed after longer time periods in culture (at least 24 h) at which time the cells had become confluent and had deposited an underlying extracellular matrix. In these studies PECAM-1 was also found to be more highly phosphorylated in c-Src overexpressors (Fig. 1). met295 cells showed no consistent differences in the level of PECAM-1 phosphorylation compared with control, and PECAM-1 protein levels were constant in all three cell types. These data suggested that c-Src may phosphorylate PECAM-1 in vivo.

In **in vitro** kinase assays with c-Src, the kinase negative mutant met295, or a vector control (HIPPO) was assessed in this manner, and PECAM-1 was found to be more highly phosphorylated in c-Src overexpressors (Fig. 1). met295 cells showed no consistent differences in the level of PECAM-1 phosphorylation compared with control, and PECAM-1 protein levels were constant in all three cell types. These data suggested that c-Src may phosphorylate PECAM-1 in vivo.

**PECAM-1 Binds SH2 Domain**

**FIG. 1. Western blot with anti-phospho-tyrosine (upper panel) of PECAM-1 immunoprecipitated from BAECs plated on tissue culture plastic Petri dishes.** The blot was stripped of antibodies and reprobed with anti-PECAM-1 (lower panel). Hippo, BAEC transfectant clone containing a vector only construct. c-Src, BAEC transfectant clone overexpressing pp60 c-Src. Kin−, BAEC transfectant clone overexpressing a kinase negative mutant of pp60 c-Src.
PECAM-1 binds SH2 domain

**PECAM-1 Binds SH2 Domain**

**PECAM-1 Cytoplasmic Tail Contains an ITAM-like Sequence**

Not Mutant PECAM-1—Binding of SH2 domain containing proteins to phosphoproteins leads to a cascade of phosphorylation and association with other phosphoproteins (9). The ability of phosphorylated PECAM-1 to associate with an SH2-containing protein in vitro suggested that PECAM-1 could be coprecipitated with other phosphoproteins. PECAM-1 immunoprecipitation under reduced stringency conditions coprecipitated at least two phosphoproteins in 3T3 cells expressing wild-type PECAM-1 but not in cells expressing either F663 or F686 (Fig. 6). The specificity for the wild-type PECAM-1 suggested that the association is dependent on PECAM-1 phosphorylation. Western blotting with anti-Src did not consistently identify the approximately 60-kDa band as being Src; the identity of these bands will require further investigation.

**Phosphoproteins Can Be Co-precipitated with Wild-type but substrate enolase but not the GST leader by itself, suggesting that PECAM-1 is an in vitro substrate for c-Src (Fig. 2). There was no consistent pattern in the level of phosphorylation of the fusion protein versus the cytoplasmic tail alone upon multiple repititions of this experiment.

**PECAM-1 Binding to c-Src and c-Src SH2 Is Dependent on PECAM Phosphorylation and Tyrosines 663 and 686**—To assess any physical association between the PECAM-1 cytoplasmic tail and c-Src, the supernatant was separated from the phosphomimetic c-Src after the kinase assay. Coomassie Blue staining of the gel revealed that the vast majority of the fusion protein and all of the cytoplasmic tail and GST leader remained in the supernatant, but a substantial portion of the phosphorylated fusion protein remained bound to the immunoprecipitated c-Src (Fig. 3), suggesting that the phosphorylated fusion protein could bind c-Src, presumably via the c-Src SH2 domain. This portion also appeared to be more slowly migrating, perhaps reflecting a more highly phosphorylated state in the bound portion. Despite levels of phosphorylation equal to or surpassing that of the fusion protein, the cytoplasmic tail alone did not precipitate with c-Src. These data may indicate that flanking sequence(s) are necessary to stabilize the interaction between the PECAM-1 cytoplasmic tail and c-Src.

A GST-c-Src-SH2 domain fusion protein was used as an affinity matrix for PECAM-1 in lysates of BAECs overexpressing c-Src, met295, and HIPPO. PECAM-1 from all three lysates precipitated c-Src (Fig. 3), suggesting that the phosphorylated cytoplasmic tail and GST protein is not phosphorylated. The numbered marks represent the positions of the molecular mass standards.

**Western blot with anti-PECAM-1 of proteins bound to a GST-Src-SH2 domain affinity matrix.** Lysates from BAEC transfectants plated on tissue culture plastic dishes for 2 h were incubated with GST-Src-SH2 domain affinity matrix. Bound proteins were resolved on SDS-PAGE and blotted with anti-PECAM-1 antibodies to assess quantity of PECAM-1 bound. Hippo, BAEC transfectant clone containing a vector only construct. c-Src, BAEC transfectant clone overexpressing pp60 c-Src. Kin', BAEC transfectant clone overexpressing a kinase negative mutant of pp60 c-Src. The level of PECAM binding was correlated to the level of tyrosine phosphorylation of PECAM as determined in Fig. 1.

**Phosphorylation of c-Src and phosphorylation of enolase, the fusion protein, and the cytoplasmic tail of PECAM-1 but not of GST alone.** The kinase and substrates are the same as in Fig. 2. The phosphorylated fusion protein appears preferentially associated with the bead-bound c-Src, whereas the phosphorylated cytoplasmic domain of PECAM-1 (PECAMcyto) appears predominately in the supernatant fraction, and GST protein is not phosphorylated. The numbered marks represent the positions of the molecular mass standards.
FIG. 6. Immunoprecipitation of tyrosine phosphorylated PECAM-1 co-precipitates other phosphoproteins. Left panel, 3T3 cells expressing wild type (wt), PECAM<sup>−/−</sup>, and PECAM<sup>+/−</sup> were pretreated with sodium orthovanadate for 2 h, trypsinized, plated on tissue culture plastic dishes for 2 h, and then lysed. Lysates were then incubated with anti-PECAM-1 antibodies, and the precipitates were resolved on a 10% SDS-polyacrylamide gel under nonreducing conditions. After blotting, the membranes were probed with anti-phosphotyrosine antibodies. The top band (150 kDa) represents the immunoglobulin used for immunoprecipitation. The 130-kDa band represents PECAM-1. The lower bands (denoted by asterisks) represent phosphoproteins co-precipitating with PECAM-1. The 55-kDa band is nonspecifically co-precipitated with normal rabbit serum as well (data not shown). Right panel, the blot is stripped and reprobed with anti-PECAM-1 to show that similar amounts of PECAM-1 are immunoprecipitated.

and Exhibits ITAM-like Properties—The ITAM is a sequence of amino acids, (D/E)XXX...XX(D/E)XXX...XX(L/I), identified in subunits of the T-cell receptor, B-cell receptor, and Fc receptors that function in signal transduction (13). Upon antigen recognition of the T-cell receptor at the cell surface, the ζ subunit becomes tyrosine phosphorylated by Src-related lck. This phosphorylation leads to association with ZAP-70, a tyrosine kinase that has two SH2 domains that work cooperatively to bind the phosphorylated tyrosines in the ITAM domain. ITAMs function in a similar manner in the B-cell receptor and Fc receptors, becoming phosphorylated by a member of the Src kinase family and subsequently bound by a member of the ZAP-70/Syk family.

Part of the PECAM-1 cytoplasmic tail resembles the ITAM (Fig. 7). It has two consensus sites for binding SH2 domains, as noted earlier (1, 2), although valines instead of leucine/isoleucine occupy the 3+ position, and the two tyrosines have 23 rather than 10 intervening amino acids. Divergence from the 10-residue spacing is also found in the 15-, 23-, and 29-residue spacings of the FcgRII (14), brain immunoglobulin tyrosine-based activation motif-like molecule (15) and natural killer-associated transcripts (16), respectively. The presence of the aspartates are somewhat variable in the ITAM-containing proteins, but PECAM-1 has two aspartate residues at −3 and −11 from the first tyrosine. The sequence is fairly well conserved in mice, substituting an isoleucine for the second valine and Gln for the −11 aspartate (17).

Genetically, the tyrosine residues in the ITAMs are encoded by two different exons, and the first aspartate/glutamate inconsistently is the product of yet another exon (18). Similarly, exons 12, 13, and 14 of PECAM-1 encode the first aspartate, the second aspartate and tyrosine 663, and tyrosine 686, respectively (19).

As with PECAM-1, mutation of either tyrosine of the ITAM ablates the majority of phosphorylation (1, 20). Although some studies suggest that the two ITAM tyrosines are equivalent in mediating function, differential phosphorylation of the two tyrosines (21) and differential binding of proteins to singly versus doubly phosphorylated ITAMs (22) have been observed, suggesting potential for individual roles for the two ITAM tyrosines. Similarly, effects on cell migration and PECAM-1 binding characteristics are observed with point mutation of and splicing out of PECAM-1 tyrosine 686 but not of tyrosine 663 (1, 6), suggesting differential functions for the two tyrosines.

REFERENCES

1. Lu, T., Yan, L., and Madri, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11808–11813

2. M. Barreuther, T. Lu, S. Davis, and J. A. Madri, unpublished observations.
PECAM-1 Binds SH2 Domain

2. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., Neel, B. G., Birge, R. R., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B., and Cantley, L. C. (1993) Cell 72, 767–778
3. Newman, P. J., Berndt, M. C., Gorski, J., White, G. C., Lyman, S., Paddock, C., and Muller, W. A. (1990) Science 247, 1219–1222
4. Tanaka, Y., Albeida, S. M., Horgan, K. J., Seventer, G. A. v., Shimizu, Y., Newman, W., Hallam, J., Newman, P. J., Buck, C. A., and Shaw, S. (1992) J. Exp. Med. 176, 245–253
5. Schimmenti, L. A., Yan, H., Madri, J. A., and Albeida, S. M. (1992) J. Cell. Physiol. 153, 417–428
6. Yan, H. C., Baldwin, H. S., Sun, J., Buck, C. A., Albeida, S. M., and DeLisser, H. M. (1995) J. Biol. Chem. 270, 23672–23680
7. DeLisser, H. M., Chilkotowsky, J., Yan, H. C., Daise, M. L., Buck, C. A., and Albeida, S. M. (1994) J. Cell Biol. 124, 195–203
8. Pinter, E., Barreuther, M., Lu, T., and Madri, J. A. (1997) Am. J. Pathol., in press
9. Pawson, T. (1995) Nature 373, 573–580
10. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745–2756
11. Madri, J. A., Pratt, B. M., and Yannariello-Brown, J. (1988) Am. J. Pathol. 132, 18–27
12. Bell, L., Luthringer, D. J., Madri, J. A., and Warren, S. L. (1992) J. Clin. Invest. 89, 315–320
13. Cambier, J. (1996) J. Immunol. 155, 3281–3285
14. Van den Herik-Oudijk, I. E., Capel, J. A., van der Bruggen, T., and Van de Winkel, J. G. J. (1995) Blood 85, 2202–2211
15. Ohnishi, H., Kubota, M., Ohtake, A., Sato, K., and Sano, S. (1996) J. Biol. Chem. 271, 25569–25574
16. Colonna, M., and Samaridis, J. (1995) Science 268, 405–408
17. Xie, Y., and Muller, W. A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5569–5573
18. Wegener, A., Letourneur, F., Howeveler, Breast, T., Luton, F., and Malissen, B. (1992) Cell 68, 83–95
19. Kirschbaum, N. E., Gumina, R. J., and Newman, P. J. (1994) Blood 84, 4028–4037
20. Irving, B., Chan, A., and Weiss, A. (1993) J. Exp. Med. 177, 1092–1103
21. Flaswinkel, H., and Reth, M. (1994) EMBO J. 13, 83–89
22. Osman, N., Lucas, S., and Cantrell, D. (1995) Eur. J. Immunol. 25, 2863–2869
23. Chan, A. C., Desai, D. M., and Weiss, A. (1994) Annu. Rev. Immunol. 12, 555–592
24. Weiss, A. (1993) Cell 73, 209–212
25. Berman, M. E., Xie, Y., and Muller, W. (1996) J. Immunol 156, 1515–1524
26. Levesley, D. I., Oliver, J. M., Swart, B. W., Berndt, M. C., Haylock, D. N., and Simmons, P. J. (1994) J. Immunol. 152, 4073–4083
27. Newman, P. J. (1977) J. Clin. Invest. 59, 3–8