doi:10.1006/jaut.2001.0570, available online at http://www.idealibrary.com on IDEAL® Journal of Autoimmunity (2002) **18,** 105–117

Absence of CD5 Dramatically Reduces Progression of Pulmonary Inflammatory Lesions in SHP-1 Protein-Tyrosine Phosphatase-Deficient 'Viable Motheaten' Mice

Melissa J. Joliat[1], Pamela A. Lang[1], Bonnie L. Lyons[1], Lisa Burzenski[1], Michael A. Lynes[2], Taolin Yi[3], John P. Sundberg[1] and Leonard D. Shultz[1]

[1]*The Jackson Laboratory, Bar Harbor, ME 04609*
[2]*Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269*
[3]*Department of Cancer Biology, The Cleveland Clinic Foundation, Cleveland, OH 44194*

Received 10 September 2001
Accepted 23 October 2001

Key words: cell surface molecules, inflammation, kinases/phosphatases, monocytes/macrophages, mouse

Mice homozygous for the viable motheaten (*Hcph*$^{me\text{-}v}$) mutation are deficient in SHP-1 protein-tyrosine phosphatase, resulting in severe systemic autoimmunity and immune dysfunction. A high percentage of B-cells in viable motheaten mice express the cell surface glycoprotein CD5, in contrast to wild type mice that express CD5 on only a small percentage of B-cells. CD5$^+$ B-cells have been associated with autoantibody production. To determine the role of CD5 in the development of the inflammatory disease in *me*^{v}/*me*^{v} mice, we created a stock of *CD5*^{null} *me*^{v}/*me*^{v} mice. The longevity of *CD5*^{null} *me*^{v}/*me*^{v} mice was increased 69% in comparison to *me*^{v}/*me*^{v} mice on a similar (B6;129) background. The increased lifespan was associated with a marked reduction in pulmonary inflammation. Flow cytometry analysis of spleen cells from *CD5*^{null} *me*^{v}/*me*^{v} mice at 9–12 weeks of age revealed significant decreases in percentages of IgM/B220 double positive B-cells, Mac-1/Gr-1 double positive cells and CD4$^+$ T-cells compared with *me*^{v}/*me*^{v} mice. *CD5*^{null} *me*^{v}/*me*^{v} mice also had significantly lower serum IgM levels in comparison to *me*^{v}/*me*^{v} mice. Study of *CD5*^{null} *me*^{v}/*me*^{v} mice may provide further insight into the role of CD5 in cell signaling and may help explain the observed association of CD5$^+$ B-cells with autoimmune disease.

Introduction

Mice homozygous for either of the two recessive allelic mutations in the hematopoietic cell phosphatase (*Hcph*) gene on Chromosome 6 are severely immunodeficient, express high levels of serum autoantibodies [1–4] and die at an early age from inflammatory lesions in the lungs and elsewhere. The *Hcph* gene encodes the Src homology region 2 (SH2) domain-containing protein tyrosine phosphatase-1 (SHP-1) [5, 6], which is a negative regulator of signaling through a number of hematopoietic growth factor receptors [7–9]. The two mutant alleles of the *Hcph* gene that arose spontaneously in C57BL/6J mice are 'motheaten', (*Hcph*^{me}, abbreviated *me*) and 'viable motheaten' (*Hcph*$^{me\text{-}v}$, abbreviated *me*^{v}). The *me* mutation is due to a cytosine deletion that results in the total absence of the SHP-1 protein due to creation of a termination codon. Death of *me/me* mice occurs at approximately 3 weeks of age. The *me*^{v} mutation is a thymine to adenine transversion that results in creation of alternative splicing sites and an 80% to 90% reduction in SHP-1 activity compared with wild type mice. Homozygous *me*^{v}/*me*^{v} mice live to approximately 9 weeks of age and are thus the more commonly used model to study the consequences of deficiency in SHP-1 protein tyrosine phosphatase [10].

In addition to autoimmunity and severe pulmonary disease, *me*^{v}/*me*^{v} mice have many additional cellular abnormalities, including an increase in myelopoiesis [1, 11] and multiple defects in lymphocyte development and function [12–15]. A large percentage of B-cells present in *me*^{v}/*me*^{v} mice express CD5 [16], which is normally found only on T-cells and a small percentage of B-cells in wild type mice.

CD5 (Ly-1) is a monomeric 67-kD membrane glycoprotein expressed on all mature T-cells and on a subset of B-cells, termed B-1a cells. Classified structurally as a member of the scavenger receptor family, CD5 consists of a cysteine rich extracellular region and a cytoplasmic domain containing motifs compatible with phosphorylation by tyrosine and serine/threonine kinases. CD5 is part of the TCR complex. It is expressed at low levels on immature, CD4$^+$CD8$^+$

Correspondence to: Leonard D. Shultz, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609. Fax: (207) 288-6079. E-mail: lds@jax.org

0896–8411/02/020105+13 $35.00/0

thymocytes and is upregulated in differentiated T-cells [17]. Tyrosine residues within the CD5 cytoplasmic region are phosphorylated upon TCR stimulation [18]. Recently, it has been reported that CD5 functions as a negative regulator of signal transduction through the TCR, affecting thymocyte differentiation as well as mediating TCR signaling in mature cells [19–21]. CD5 has been found to be constitutively associated with SHP-1 in Jurkat cells and normal phytohemagglutinin-expanded T-lymphoblasts [22], indicating that the negative regulatory role of CD5 in T-cells may be mediated through SHP-1.

CD5 is associated with the B-cell receptor (BCR) on B-1a cells [23]. In wild type adult mice, cells expressing CD5 constitute a significant percentage of the B-cell population in the peritoneal and pleural cavities, while they are scarcely found in the splenic B-cell pool [24]. Although the function of CD5 on B-cells is poorly understood, B-1a cells, that express CD5, are associated with production of natural autoantibodies [25], specifically of the IgM isotype [26]. An increase in the number of B-cells expressing CD5 is often associated with increased production of autoantibodies (reviewed in Pers *et al*. [27]). In *me/me* and me^v/me^v mice, virtually all of the B-lymphocytes, including the splenic populations, express CD5 [28].

CD5 deletion in otherwise wild type mice does not interfere with the ability of these mice to mount an efficient immune response [24]. T-cells from $CD5^{null}$ mice are hyperreactive in response to TCR mediated signals *in vitro* [19], but this increased reactivity has no apparent detrimental effect on T-cell function *in vivo*. Thus, mice lacking CD5 are healthy and have normal distribution of lymphoid and myeloid cells. To explore the effect that CD5 expression has on the inflammatory disease of viable motheaten mice, we crossed 129-$Cd5^{tm1Cgn}$ ($CD5^{null}$) mice with C57BL/6J-me^v mice to create a stock of mice homozygous for the CD5 deletion and segregating for the viable motheaten mutation. Comparison of the phenotypes of me^v/me^v mice with or without CD5 revealed a marked role of this molecule in the development of immunopathologic changes in me^v/me^v mice.

Materials and Methods

Mice

All mice were raised at the Jackson Laboratory (Bar Harbor, ME). 129-$Cd5^{tm1Cgn}$ ($CD5^{null}$) mice [24] were first crossed with heterozygous C57BL/6J-+/me^v mice. Offspring were heterozygous for $CD5^{null}$ and were typed for the presence of the me^v mutation. Mice that were heterozygous for both the me^v mutation and $CD5^{null}$ were intercrossed. Homozygous $CD5^{null}$ mice from these matings that were heterozygous for the me^v mutation were then intercrossed to create a (B6;129)-$CD5^{null}$ me^v strain in which the CD5 null allele was fixed to homozygosity and the mice were segregating for the me^v mutation. Concurrently, 129P3/J wild type mice were bred with C57BL/6-+/me^v mice in order to produce me^v/me^v and +/? control mice on a matched segregating background that expressed normal CD5 levels. Mice were typed for the CD5 null allele by PCR, using primers for the inserted neomycin resistance gene (*neo*13: 5′-CTTGGGTGGAGAGGCTATTC-3′, *neo*14: 5′-AGGTGAGATGACAGGAGATC-3′). Determination of the genotype at the *Hcph* locus (me^v/me^v, +/me^v or +/+) was also conducted by PCR, using primers flanking the viable motheaten mutation (me^v-F: 5′-CGTGTCATCGTCATGACT-3′, me^v-R: 5′-AGGAAGTTGGGGCTTTGCCGT-3′). Before electrophoresis, the amplification products were digested with *Rsa*I to distinguish among +/+, +/me^v and me^v/me^v mice [29]. Mice were housed in conventional pathogen-free animal facilities.

Histopathology

Groups of mice from 4–18 weeks of age were euthanized by CO_2 asphyxiation. Tissues were fixed in Fekete's acid alcohol formalin, embedded in paraffin and sectioned at 6 μm. Slides were stained with Mayer's hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) for histological examination. Immunohistochemistry was carried out on lung and kidney sections using biotinylated rabbit anti-mouse Ig (DAKO, Carpinteria, CA) for identification of immune complexes. Non-specific staining was blocked on deparaffinized sections with peroxidase blocking reagent (DAKO). Tissues were then incubated in primary antibody for 1 h at room temperature in a humidified chamber. After three 5 min washes in PBS, tissues were incubated with avidin/horseradish-peroxidase conjugate for 45 min, followed by an additional trio of 5 min washes in PBS. Tissues were then incubated in diaminobenzidine (DAB) for 5 min at room temperature and washed in tap water for 5–15 min. Tissues were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

Flow cytometric analysis

Monoclonal antibodies, conjugated with phycoerythrin, FITC or biotin, were used for characterization of spleen cells from 9–12-week-old and 15–18-week-old mice. Streptavidin-Red 6-70 was used as a secondary fluorophore for biotinylated antibodies. The following antibodies were obtained from PharMingen, Inc. (San Diego, CA): anti-CD4, clone RM4-5; anti-pan granulocyte (Gr-1), clone RB6-8C5 [30]; anti-Mac-1, clone M1/70 [31]; anti-CD23, clone B3B4 [32, 33]; anti-CD43, clone S7 [34–36] and anti-CD19, clone 1D3 [37–39]. Additional antibodies, as follows, were purified from hybridoma cell lines as ascites; anti-CD3, clone 145-2C11 [40]; anti-CD8, clone 53-6.72 [41, 42]; anti-IgM, clone R6-60.2; anti-B220, clone RA3-6B2 [43]; anti-pan macrophage, clone F4/80; anti-MHC class I, clone M1/42 [44]; anti-MHC class II, clone M5/114 [45]; anti-pan erythrocyte, clone Ter119 [46]; and anti-CD5,

clone 53-7.3 [41, 42]. Single cell suspensions were prepared from individual spleens by extrusion of cells through Nytex 110 mesh bags (TETKO Inc., Elmsford, NY) into cold HBSS containing 5% FBS and 0.1% sodium azide. Erythrocytes were lysed in buffered ammonium chloride, and leukocyte populations were phenotyped as previously described [28] using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). B-1 B-cells were identified by labeling cells with antibodies against IgM, CD19 and CD43. Cells that were IgM^{high}, $CD19^{+}$, $CD43^{+}$ were considered B-1 B-cells [47, 48].

Ig levels

Levels of IgM, IgG1 and IgG3 in serum from 9–12-week-old individual mice were assayed by ELISA as previously described [28]. Plates were coated with goat anti-mouse isotype specific antibody (Southern Biotechnology Associates, Birmingham, AL). Alkaline phosphatase labeled goat anti-mouse κ-chain (Southern Biotech) was used as the detection antibody. Isotype specific standards (PharMingen) were run with each assay. Plates were developed with p-nitrophenyl phosphate (Sigma Chemical Co., St Louis, MO) and read with the EL 312e Bio-Kinetics Reader (Bio-Tek Instruments, Winooski, VT). Ig levels were determined from the standard curves.

Autoantibody determinations by ELISA

Circulating autoantibodies against histone proteins were determined using a modification of a protocol described by Amoura *et al*. [49]. In brief, individual wells of 96 well microtiter plates were first coated with 100 µl of 2 µg/µl calf thymus histone protein (Type IIS, Sigma) in PBS for 1 h at 37°C. The wells were then washed in an automated microplate washer with PBS containing 0.25% Tween 20 (PBS/T20), and then blocked with 200 µl PBS containing 1% BSA for 1 h at 37°C. The wells were washed, and 100 µl of each serum dilution was added to the appropriate wells. After incubation for one additional hour at 37°C, the wells were washed three times, and then 100 µl of a previously titered goat anti-mouse IgG (H+L)-alkaline phosphatase conjugate was added to each well. Following incubation at 37°C for 1 h, the plates were washed, and then 100 µl substrate (p-nitrophenyl phosphate, Sigma) dissolved at 1 mg/ml in diethanolamine substrate buffer was added to each well. The rate of substrate conversion to colored product at 37°C was monitored at 405 nm and recorded as mOD/min. Autoantibodies against double stranded DNA were detected according to a modification of a protocol described by Zouali *et al*. [50]. In brief, microtiter wells were coated with calf thymus DNA at 10 µg/ml in TBS (10 mM Tris base, 150 mM NaCl, pH 7.4) for 2 h at room temperature. Following this incubation, the plates were washed with PBS/T20 in an automated microplate washer, and then processed as described above.

Hematology

Blood was collected from the retro-orbital sinus using heparinized capillary tubes. Leukocytes and erythrocytes were counted using a model ZBI Coulter counter (Hialeah, FL). Mean corpuscular volumes (MCVs) were calculated from the packed red cell volumes and total RBC counts as previously described [1]. Blood smears were stained with Wright-Giemsa (Sigma) for examination of cells for morphological abnormalities. Reticulocytes were counted using the ADVIA 120 Hematology system (Bayer Corp, Tarrytown, NY). Confirmation of reticulocyte percentages was conducted manually following staining with New Methylene Blue stain (Mallinckrodt Baker Inc., Phillipsburg, NJ). Equal amounts of blood and stain were incubated together in a capillary tube for 15 min and smears were prepared. The smears were then air dried and coverslipped with Permount (Fisher).

Spleen cell lysates and immunoblotting

Spleens were dissected from mutant and control mice at 5–8 weeks of age and at 12–15 weeks of age. Single cell suspensions were prepared after lysis of red blood cells following established procedures [51]. For immunoblotting, the spleen cells were lysed at 4°C for 30 min in cold lysis buffer (20 mM Tris, pH 7.4; 1 mM EDTA; 10% glycerol; 1% Triton X-100; 100 mM NaCl; 1 µg of leupeptin per ml; 1 µg of aprotinin per ml; 1 mM bezamidine and 5 mM of iodoacetic acid). Cell lysates were clarified by centrifuging for 10 min at 10,000 *g* at 4°C. Protein levels were measures using the DC Protein Assay (Bio-Rad, Hercules, CA). 20 µg of each sample was resolved in a 12.5% SDS-PAGE gel and transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membranes were probed, as described previously [52], with monoclonal antibody against phosphotyrosine (4G10, Upstate Biotechnology Inc., Lake Placid, NY). Specific antibody signals were detected using an enhanced chemiluminescence kit (ECL, Amersham Pharmacia Biotech, Uppsala, Sweden).

Bone marrow macrophage cultures

Bone marrow plugs from femurs and tibias were harvested by extrusion with cold HBSS. Marrow plugs were disrupted by passage through a 25 g needle. The resulting single cell suspensions were washed 2× in sterile HBSS (Sigma), counted with a model ZBI Coulter counter and resuspended in complete media (RPMI-1640 (Sigma) containing 2 mM l-glutamine, 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin). A total volume of 5 ml medium containing 10^6 cells/ml was added to 25 cm^2 tissue culture flasks with either 500 or 1,000 units/ml of recombinant human (rHu) CSF-1 (Cetus) or recombinant murine (rMu) GM-CSF (R&D Systems, Minneapolis, MN). Cultures were incubated at 37°C in 5% 'CO_2 for 24 h to allow adherent cells to attach to the flask. The

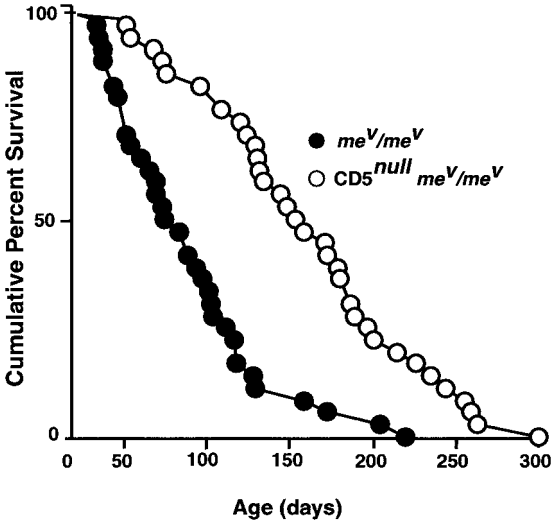


Figure 1. Cumulative percent survival of (B6;129)-me^v/me^v and (B6;129)-$CD5^{null}$ me^v/me^v mice as a function of age. Thirty-five male or female mice from each genotype were monitored from 3 weeks of age.

non-adherent cells were transferred to duplicate flasks and cultured for 7 days with periodic changes of media, maintaining the appropriate growth factor concentrations. To recover adherent cells from flasks, medium containing any non-adherent cells was removed and transferred to 50 ml tubes. The remaining adherent cells were washed 2× with 10 ml warm HBSS, then incubated for 10 min with 10 ml 2 mM EDTA/PBS at 37°C. Cultures were vigorously shaken to detach all adherent cells. The adherent cells were then combined with the non-adherent cells in 50 ml tubes, washed 2× in HBSS and used for FACS analysis as previously described. Preliminary FACS analysis of adherent and non-adherent cells separately showed that they had similar staining profiles. In subsequent FACS analyses, adherent and non-adherent were combined to increase the numbers of cells available for analysis.

Statistics

All measures of variance are presented as SEM. Student's *t* tests were performed to determine significance of difference of means. Significance was assumed for P values <0.05.

Results

Longevity

Thirty-five (B6;129) $CD5^{null}$ me^v/me^v mice and an equal number of (B6;129) me^v/me^v mice were monitored from 3 weeks of age (Figure 1). There was no significant effect of gender on survival of these mice. The mean lifespan of (B6;129)-$CD5^{null}$ me^v/me^v mice (162±10 days) was significantly increased in comparison to (B6;129)-me^v/me^v mice, which had a mean lifespan of 89±8 days (P<0.0001). Our previous studies have shown that C57BL6-me^v/me^v mice had a mean lifespan of 61±2.4 days [1], indicating a significant effect of strain background on lifespan of these mice. All subsequent data compares me^v/me^v mice, $CD5^{null}$ me^v/me^v mice and wild type +/+ controls on the (B6;129) segregating background.

Immunopathology

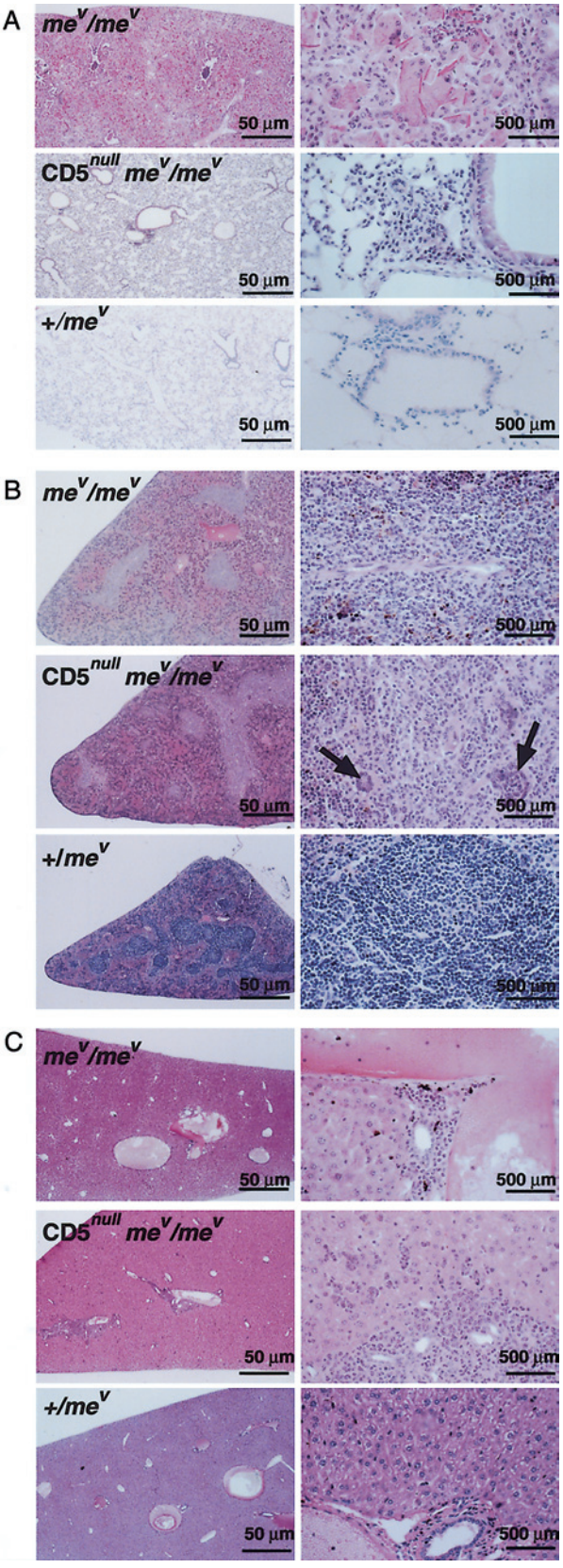
In order to determine the effect of the CD5 null allele on the characteristic pathologic lesions of me^v/me^v mice, total necropsies were performed on mutant ($CD5^{null}$ me^v/me^v and me^v/me^v) and control ($CD5^{null}$ +/? and +/?) mice and all tissues were examined histologically. Several striking differences were noted in (B6;129) $CD5^{null}$ me^v/me^v mice when compared to (B6;129) me^v/me^v mice.

Development of pulmonary lesions was significantly delayed in $CD5^{null}$ me^v/me^v mice in comparison to me^v/me^v mice. At 9 weeks of age, this difference was evident grossly at the time of necropsy. Lungs of me^v/me^v mice were mottled tan in color and firm, while lungs of $CD5^{null}$ me^v/me^v mice resembled the lungs of wild type mice and were compliant and evenly light pink in color. Histologically, the me^v/me^v mice showed rapid increase in severity of acidophilic macrophage pneumonia [53, 54]. Moderate disease was evident by 4 weeks of age in me^v/me^v mice and pneumonia was severe by 9 weeks. Pulmonary lesions were minimal or absent in $CD5^{null}$ me^v/me^v mice at 4 weeks of age, and by 9 weeks of age, only mild peribronchiolitis or pneumonitis was observed (Figure 2A). By 21 weeks of age, most $CD5^{null}$ me^v/me^v mice developed mild to moderate acidophilic macrophage pneumonia, while one $CD5^{null}$ me^v/me^v mouse necropsied at 43 weeks of age had only mild pulmonary disease (data not shown).

Spleens in $CD5^{null}$ me^v/me^v mice were markedly enlarged. Spleen weight expressed as a ratio of spleen to body weight ($S:B^{wt}$) was increased 42% in $CD5^{null}$ me^v/me^v mice in comparison to me^v/me^v mice (P<0.05) at 9 weeks of age. The $S:B^{wt}$ of me^v/me^v mice at 9 weeks of age was increased almost seven fold over a control group of +/+ and +/? mice combined (data not shown). The $S:B^{wt}$ of both $CD5^{null}$ me^v/me^v mice and me^v/me^v mice increased with age from 5–18 weeks, while in +/? and $CD5^{null}$ +/? mice, this ratio did not change significantly with age. The spleens in $CD5^{null}$ me^v/me^v mice at 9 weeks of age had poorly defined follicles and contained numerous multinucleated giant cells with abundant cytoplasm. While the splenic follicles of me^v/me^v mice were poorly delineated, the spleens lacked the extensive granulomatous lesions, consisting of multinucleated giant cells, found in $CD5^{null}$ me^v/me^v mice (Figure 2B). With increasing age, multinucleated giant cells in $CD5^{null}$ me^v/me^v mouse spleens became even more abundant, while the relative number of these cells in spleens of me^v/me^v mice increased only slightly.

The livers in $CD5^{null}$ me^v/me^v mice showed marked extramedullary myelo- and erythropoiesis at 9 weeks

of age. In contrast, the livers in me^v/me^v mice exhibited only mild extramedullary hematopoiesis (EMH), mostly myeloid in nature (Figure 2C). By 16 weeks of age, EMH in livers of $CD5^{null}$ me^v/me^v mice had increased considerably, while EMH in livers of me^v/me^v mice remained mild to moderate (data not shown).



$CD5^{null}$ me^v/me^v mice did not develop hyperplasia of the non-glandular stomach, a feature commonly seen in me^v/me^v mice at 9 weeks of age. Inflammation of the glandular stomach was observed in both $CD5^{null}$ me^v/me^v mice and me^v/me^v mice (data not shown). Renal glomeruli of $CD5^{null}$ me^v/me^v mice were enlarged and increased in cellularity at 9 weeks of age, indicating a mild membranoproliferative glomerulonephritis. The me^v/me^v mice showed glomerulonephritis, but the condition was less severe (data not shown). Immune complexes were detected in the glomeruli of both $CD5^{null}$ me^v/me^v and me^v/me^v kidneys by PAS staining and by immunohistochemistry using anti-mouse Ig (data not shown). Blood urea nitrogen (BUN) levels were not significantly elevated in either $CD5^{null}$ me^v/me^v or me^v/me^v mice ranging from 6 to 12 weeks of age (data not shown).

Flow cytometry analyses of cell populations

The observed splenomegaly in $CD5^{null}$ me^v/me^v and me^v/me^v mice was accompanied by a significant increase in total numbers of nucleated cells in the spleen in comparison to $CD5^{null}$ +/? and +/? mice, respectively ($P<0.05$), as expected. Average numbers of nucleated splenocytes in $CD5^{null}$ me^v/me^v were increased in comparison to me^v/me^v mice, but the data lacked statistical significance. An elevation in the number of splenic myeloid cells contributes to the splenomegaly observed in me^v/me^v mice. While an increase in ratio of spleen weight to body weight ($S:B^{wt}$) was observed in $CD5^{null}$ me^v/me^v mice in comparison to me^v/me^v mice, the reduction in pulmonary inflammation observed in $CD5^{null}$ me^v/me^v mice suggested a general decrease in myeloid cells numbers. To determine the proportions of cell populations present in the spleens of $CD5^{null}$ me^v/me^v mice, flow cytometric analysis was performed.

Figure 2. Photomicrographs of tissue sections from 62–64-day-old (B6;129) mice, genotypes as labeled. A. Lung shows severe eosinophilic macrophage pneumonia in me^v/me^v mice, in contrast to minor peribronchiolar aggregation of lymphocytes in $CD5^{null}$ me^v/me^v mice and normal appearance in $+/me^v$ control mice. B. Spleens of me^v/me^v and $CD5^{null}$ me^v/me^v mice have poorly defined lymphoid follicles that are markedly depleted of lymphoid cells. Spleens of $CD5^{null}$ me^v/me^v mice contain many multinucleated giant cells (arrows) that are not present in me^v/me^v spleens. In $+/me^v$ mice, spleens reveal well developed follicles. C. Livers of me^v/me^v mice exhibit mild extramedullary hematopoiesis, mostly myeloid in nature. In contrast, high levels of extramedullary myelo- and erythropoiesis are evident in livers of $CD5^{null}$ me^v/me^v mice. The $+/me^v$ livers appear normal. All tissues were fixed in Fekete's acid alcohol formalin and stained with hematoxylin and eosin.

Table 1. *Flow cytometry analyses of spleen cells from 9–12-week-old (B6;129) CD5*^{null} me^{v}/me^{v} *mice*

Cell population	(B6;129) me^{v}/me^{v}	(B6;129) CD5^{null} me^{v}/me^{v}	(B6;129) +/?	(B6;129) CD5^{null} +/?
CD3(+) CD4(+)	8.9±1.0	3.2±1.5*	16.1±1.8	17.5±3.5
CD3(+) CD8(+)	2.6±0.8	1.1±0.5	8.2±1.0	9.9±1.1
Mac-1(+) Gr-1(+)	40.2±3.0	14.2±2.5*	6.1±1.2	5.1±1.7
Mac-1(+) Gr-1(−)	21.2±3.4	29.9±3.9	12.5±7.9	15.7±5.4
Mac-1(−) Gr-1(+)	4.7±1.5	1.1±0.4*	4.3±0.6	6.3±2.0
IgM(+) B220(+)	19.5±2.2	7.1±2.7*	56.1±5.2	52.4±5.2
†B-1 cells† (as % of total B-cells)	79.6±5.0	83.6±5.7	5.4±0.6	6.6±0.6
Ter119(+)	12.6±2.9	56.2±7.8*	2.2±1.1	2.2±0.8
Mean # of nucleated spleen cells×10^{8}	3.4±0.6	4.4±0.7	1.7±0.3	1.1±0.1

Data are expressed as a mean percent±SEM.
n=6 for (B6;129) CD5^{null} me^{v}/me^{v}; n=5 for (B6;129) me^{v}/me^{v}; n=4 for (B6;129) CD5$^{+/+}$ +/? and (B6;129) +/?.
*Indicates significant (P<0.05) difference in spleen cell populations between CD5^{null} me^{v}/me^{v} and me^{v}/me^{v} mice.
†B-1 cells were identified as IgM^{high}, CD19^{+}, CD43^{+}.

Table 2. *Peripheral blood analysis of 9–12-week-old CD5*^{null} me^{v}/me^{v} *mice*

Cell population	(B6;129) me^{v}/me^{v}	(B6;129) CD5^{null} me^{v}/me^{v}	(B6;129) +/?	(B6;129) CD5^{null} +/?
Erythrocytes (×10^{9} cells/ml)	7.0±0.6	6.4±0.4	9.3±0.8	9.7±0.7
Leukocytes (×10^{6} cells/ml)	16.6±3.0	14.2±2.0	7.5±0.8	9.1±1.4
Hematocrit (%)	44.0±2.8	39.4±1.4	50.3±1.5	51.5±0.7
Reticulocytes (as % of total red blood cells)	17.3±4.5	30.3±5.2*	2.9±0.1	2.8±0.2

Data are expressed as stated±SEM.
n>3 for reticulocyte percentages; n>6 for all other assays.
*Indicates significant (P<0.05) difference in peripheral blood cell populations between CD5^{null} me^{v}/me^{v} and me^{v}/me^{v} mice.

Percentages of splenic Mac-1^{+} Gr-1^{+} monomyeloid cells were significantly reduced in CD5^{null} me^{v}/me^{v} mice compared to me^{v}/me^{v} mice, as were percentages of IgM^{+} B220^{+} B-cells and CD3^{+} CD4^{+} T-cells (Table 1). However, CD5^{null} me^{v}/me^{v} mice had a significant increase in percentages of splenic Ter119^{+} erythrocyte precursor cells compared to me^{v}/me^{v} mice, suggesting that the augmented splenomegaly in CD5^{null} me^{v}/me^{v} mice is a result of an increase in erythropoiesis.

To determine whether CD5 is necessary for development of B-1 cells, percentages of these cells were determined using antibodies against IgM, CD43 and CD19. Percentages of IgM^{+} B220^{+} B-cells that had a B-1 phenotype did not vary significantly between CD5^{null} me^{v}/me^{v} mice and me^{v}/me^{v} mice, indicating that CD5 expression is not required for development of the B-1 cell population.

Total nucleated cell numbers in the spleen did not change significantly with age in CD5^{null} me^{v}/me^{v}, me^{v}/me^{v}, CD5^{null} +/? or +/? mice. The only significant change in percentages of spleen cell populations in CD5^{null} me^{v}/me^{v} with age was a decrease in the percentage of Mac-1^{+} Gr-1^{-} cells (P<0.05) in 15–18-week-old mice in comparison to 9–12-week-old mice (data not shown). In contrast, several significant differences were seen when comparing spleens from 9–12-week-old me^{v}/me^{v} mice to spleens of 15–18-week-old me^{v}/me^{v} mice. Percentages of CD3^{+} CD4^{+} T-cells, Mac-1^{+} Gr-1^{+} cells and IgM^{+} B220^{+} B-cells were lower in 15–18-wk-old me^{v}/me^{v} mice when compared to 9–12-wk-old me^{v}/me^{v} mice (P<0.05), while the percentage of Ter119^{+} cells was increased (P<0.01) (data not shown).

Peripheral blood analysis

Analysis of reticulocyte levels in peripheral blood showed a significant increase in reticulocyte percentages in CD5^{null} me^{v}/me^{v} mice when compared to me^{v}/me^{v} mice (P<0.05) (Table 2). Reticulocyte percentages of both me^{v}/me^{v} and CD5^{null} me^{v}/me^{v} mice were significantly higher than in wild type controls (P<0.05). In contrast, peripheral blood red cell numbers were significantly reduced in both CD5^{null} me^{v}/me^{v} and me^{v}/me^{v} mice in comparison to wild type (+/+ and +/?) controls at 9–12 weeks of age (P<0.05). Although there was a significant increase in numbers of Ter-119^{+} splenic nucleated erythroid cells in CD5^{null} me^{v}/me^{v} mice in comparison to me^{v}/me^{v} mice (Table 1), there was not a corresponding increase in peripheral erythrocyte counts in CD5^{null} me^{v}/me^{v} mice (Table

2). Moreover, there were no significant differences in peripheral erythrocyte or leukocyte counts between CD5^{null} *me^{v}/me^{v}* and *me^{v}/me^{v}* mice, although the average erythrocyte and leukocyte numbers as well as hematocrit percentages were slightly lower in CD5^{null} *me^{v}/me^{v}* mice in comparison to *me^{v}/me^{v}* mice. Hematocrit percentages of *me^{v}/me^{v}* mice were lower on average than those of wild type (+/+ and +/?) mice, but the difference lacked statistical significance by a small margin (P=0.055). Although the decrease in hematocrit percentages of CD5^{null} *me^{v}/me^{v}* versus *me^{v}/me^{v}* mice was also not statistically significant, CD5^{null} *me^{v}/me^{v}* mice did have significantly lower hematocrits than +/+ or +/? mice (P<0.0001). Erythrocyte mean cell volumes (MCV) did not differ significantly in any of the genotypes studied (data not shown). Peripheral blood leukocyte numbers were comparably increased in both CD5^{null} *me^{v}/me^{v}* mice and *me^{v}/me^{v}* mice compared to wild type controls. The majority of these cells were monocytes and granulocytes (data not shown). There was no significant difference in either leukocyte or erythrocyte numbers in CD5^{null} +/? mice versus wild type +/? mice.

Serum Ig levels

B-cells in *me^{v}/me^{v}* mice are hyperresponsive to stimulation through the B-cell receptor (BCR) [55]. This hyperreactivity to BCR mediated signals in the absence of SHP-1 helps explain the otherwise counterintuitive observation that serum levels of IgM and IgG in *me^{v}/me^{v}* mice are significantly higher than in wild type mice, while their mature B-cell numbers are reduced. Since *CD5^{null} me^{v}/me^{v}* mice have significantly fewer B-cells than *me^{v}/me^{v}* mice, we assayed serum Ig levels in *CD5^{null} me^{v}/me^{v}* and control mice (Figure 3).

IgM levels were increased over 30 fold in *me^{v}/me^{v}* mice when compared with wild type (+/+ and +/*me^{v}*) mice. In *CD5^{null} me^{v}/me^{v}* mice, IgM levels were 70% lower than in *me^{v}/me^{v}* mice but still almost 10 fold higher than in wild type (+/+ and +/*me^{v}*) mice. Although previous reports have indicated that serum IgG1 levels in C57BL/6J-*me^{v}/me^{v}* mice are not significantly elevated over normal while serum IgG3 levels are significantly elevated in comparison with littermate controls [28], we found the opposite in our studies. IgG1 levels were 2.5 times higher in the serum of *me^{v}/me^{v}* mice than in +/? littermates. These levels were lower in *CD5^{null} me^{v}/me^{v}* mice than in *me^{v}/me^{v}* mice, but the change was not statistically significant. No significant variation was observed in IgG3 levels between mutant and control mice.

It is interesting to note that levels of IgG1 were significantly reduced in *CD5^{null}* +/? mice in comparison to +/? mice with a functional CD5 gene. Production of IgG1 is T-cell dependent and CD5 expression by T-cells may be required to induce class switching or secretion of IgG1. Levels of IgM and IgG3 were lower in *CD5^{null}* +/? compared with +/? mice, but the differences were not statistically significant.

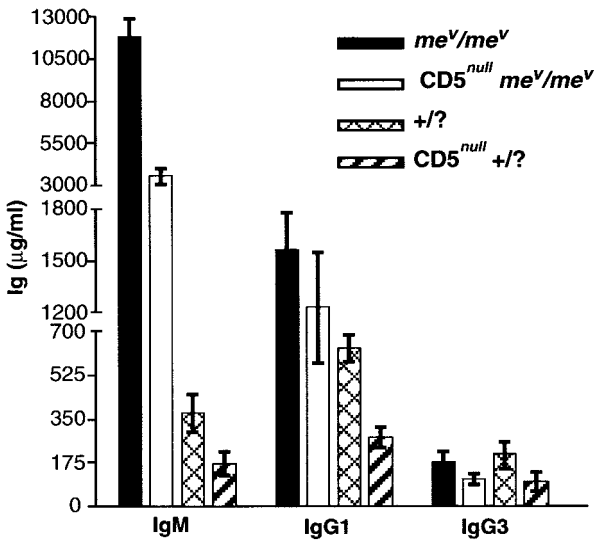


Figure 3. Serum Ig levels. Levels of serum IgM are significantly reduced in CD5^{null} *me^{v}/me^{v}* mice in comparison to *me^{v}/me^{v}* mice (p<0.05). Absence of CD5 did not result in a significant reduction of IgG1 levels or IgG3 levels in CD5^{null} *me^{v}/me^{v}* mice in comparison to *me^{v}/me^{v}* mice. CD5^{null} +/? mice showed a consistent reduction in Ig levels in comparison to wild type (+/?) mice. However, the difference was only statistically significant when comparing IgG1 levels of CD5^{null} +/? mice versus +/? mice with intact CD5.

Autoantibody assays

To assess the presence of autoantibodies in *CD5^{null} me^{v}/me^{v}* and *me^{v}/me^{v}* mice, serum levels of anti-double stranded DNA (anti-dsDNA) and anti-histone antibodies were assayed by ELISA. Levels of these autoantibodies were elevated in the serum of both *CD5^{null} me^{v}/me^{v}* and *me^{v}/me^{v}* mice, when compared to wild type (+/+ and +/*me^{v}*) controls (data not shown). In contrast to IgM levels, that were significantly reduced in *CD5^{null} me^{v}/me^{v}* mice when compared to *me^{v}/me^{v}* mice, levels of both anti-dsDNA and anti-histone antibodies did not vary significantly between *CD5^{null} me^{v}/me^{v}* and *me^{v}/me^{v}* mice. Levels of anti-histone and anti-dsDNA antibodies in *CD5^{null}* +/? mice showed no variation from +/+ and +/*me^{v}* controls (data not shown).

Anti-phosphotyrosine Western blotting

Variation in levels of tyrosine phosphorylation between two experimental samples can indicate a possible alteration in cell signaling. In order to explore whether the pathological changes observed in *CD5^{null} me^{v}/me^{v}* mice compared with *me^{v}/me^{v}* mice were accompanied by a difference in tyrosine phosphorylation, we examined total splenic protein by anti-phosphotyrosine western blotting (Figure 4). Spleen cell lysates were analyzed from mice at 12–15 weeks of age as well as from mice at 5–8 weeks (data from younger mice not shown). In *me^{v}/me^{v}* mice at 12–15 weeks of age, there was an increase in tyrosine

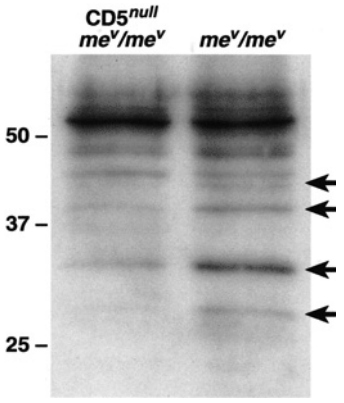


Figure 4. Characterization of tyrosine phosphorylation of proteins in spleen cell lysates. Spleen cell lysates from male C57BL/6J-CD5^{null} me^{v}/me^{v} and C57BL/6J-me^{v}/me^{v} mice at 4 months of age were analyzed by SDS-PAGE/immunoblotting with anti-phosphotyrosine antibody. The positions of protein size markers (kDa) are indicated on the left. Arrows indicate bands that exhibit enhanced tyrosine phosphorylation in C57BL/6J-me^{v}/me^{v} mice in comparison to C57BL/6J-CD5^{null} me^{v}/me^{v} mice.

phosphorylation of proteins in the range of 28–45 kd. Highly phosphorylated proteins in this range were not seen in *CD5*^{null} me^{v}/me^{v} mice of any age, nor were they seen in younger me^{v}/me^{v} mice.

Bone marrow macrophage cultures

Further support of the hypothesis that CD5 on macrophages plays a role in the development of the macrophage pneumonia seen in me^{v}/me^{v} mice required confirmation of the presence of CD5 directly on macrophages. Takahashi *et al*. [56] reported development of CD5 expression on macrophages in cell cultures grown with CSF-1 or GM-CSF. To verify this, we established cultures of bone marrow cells from C57BL/6J-me^{v}/me^{v} mice as well as from C57BL/6J-+/? controls and from C57BL/6J-*CD5*^{null} mice. Cultures were grown in the presence of either Rhu-CSF-1 or Rmu-GM-CSF. Adherent and non-adherent cells from cultures had similar cell surface marker expression profiles and were pooled for analysis. Flow cytometric analysis revealed that both C57BL/6J-+/? and C57BL/6J-me^{v}/me^{v} bone marrow contained cells that expressed CD5 after 7 days of culture with either 500 or 1000 units per ml of CSF-1 or GM-CSF (Figure 5). There was no difference in percentages of CD5^{+} cells with various amounts of cytokine, so results were pooled. Table 3 shows the mean results of three separate experiments. Following stimulation with GM-CSF, >90% of bone marrow cells that expressed CD5 were found to co-express Mac-1. Bone marrow from *CD5*^{null} mice grown under similar conditions, as expected, did not express CD5. Under all conditions, Gr-1 was expressed on <1.3% of CD5^{+} cells, and <0.9% of CD5^{+} cells expressed CD19 or CD3.

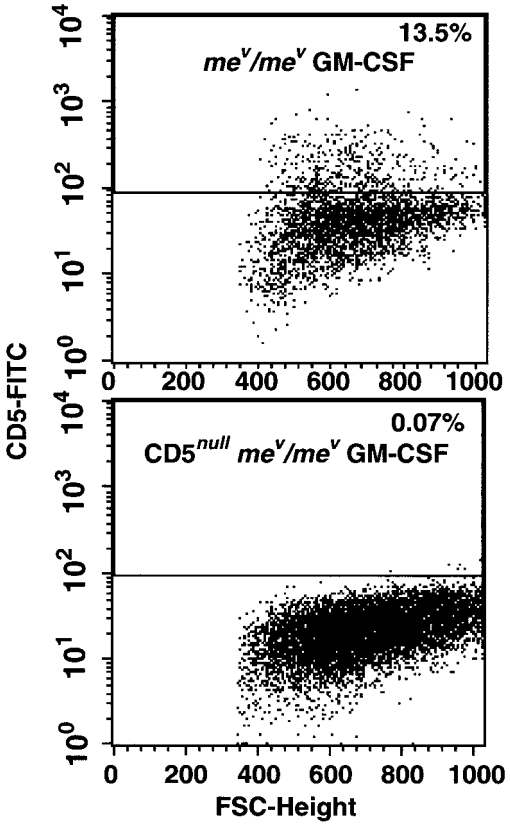


Figure 5. Expression of CD5 on bone marrow cells grown in culture for 7 days with 1,000 units per ml GM-CSF. Top panel shows expression of CD5 on me^{v}/me^{v} cells, bottom panels shows lack of CD5 expression on CD5^{null} cells. Dead cells were gated out using propidium iodide exclusion. Live cells were analyzed for CD5 expression. Numbers shown are percentages of CD5^{+} cells.

Discussion

This study focused on the role of CD5 in immunopathologic changes in me^{v}/me^{v} mice. Absence of CD5 expression in me^{v}/me^{v} mice resulted in significantly increased lifespan and numerous phenotypic changes in comparison to me^{v}/me^{v} mice with an intact CD5 gene. This increased longevity of *CD5*^{null} me^{v}/me^{v} mice was associated with a marked delay in development of pulmonary disease in these mice when compared to me^{v}/me^{v} mice. The reduced levels of macrophage infiltration in the lungs of *CD5*^{null} me^{v}/me^{v} mice were associated with a significant decrease in myeloid cell numbers in the spleen. Although the reduced acidophilic macrophage pneumonia may be a consequence of altered interactions of B- or T-cells with myeloid cells due to the absence of CD5 on the surface of the lymphocytes, mature B- and T-cells are not necessary for the development of pulmonary inflammation in viable motheaten mice [57]. Thus, the absence of CD5 function alone in either B- or T-cells would be unlikely to lead to such dramatic reduction of lung lesions as well as reduced myeloid cell populations in *CD5*^{null} me^{v}/me^{v} mice.

Recently, CD5 has been found to be constitutively expressed on a population of macrophages in me^{v}/me^{v}

Table 3. *Flow cytometry analysis of bone marrow cultures*

Cytokine	CSF-1		GM-CSF	
Genotype	+/?	me^v/me^v	+/?	me^v/me^v
Percent of total cells that are $CD5^+$	6.1±1.1	5.6±0.9	4.0±2.7	9.7±2.7
Percent of $CD5^+$ cells that are Mac-1^+	49.1±8.1	51.2±6.3	61.8±10.0	92.7±3.0*

Data are expressed as a mean percent±SEM.
$n>5$.
*Indicates significant ($P<0.01$) difference in percent of $CD5^+$ cells expressing Mac-1 between me^v/me^v cultures grown with GM-CSF and +/? cultures grown with GM-CSF as well as +/? and me^v/me^v cultures grown with CSF-1.

mice [11]. $CD5^+$ macrophages can be induced in the peritoneal cavity of wild type mice by treatment with high levels of GM-CSF. This cytokine is elevated in peritoneal fluid of me^v/me^v mice [56], and myeloid cells from me^v/me^v mice show enhanced signaling through the GM-CSF receptor [7]. Our observation that $CD5^{null}$ me^v/me^v mice have reduced numbers of splenic myeloid cells and less severe myeloid cell mediated pulmonary lesions compared with me^v/me^v mice may indicate a role for CD5 in response of cells to myeloid growth factors such as GM-CSF. We confirmed the development of $CD5^+$ macrophages in cultures of bone marrow from both me^v/me^v and wild type mice supplemented with either GM-CSF or CSF-1, providing support for the hypothesis that CD5 expression is involved with development or activation of macrophages through myeloid growth factor receptors. Although bone marrow cells from $CD5^{null}$ mice grown in culture with GM-CSF or CSF-1 lacked CD5 expression, myeloid cell antigen (Mac-1 and Gr-1) expression profiles on these bone marrow cells were similar to cells from mice with an intact CD5 gene (data not shown). Additionally, no quantifiable or qualifiable differences were apparent in colony assays of bone marrow cells from $CD5^{null}$ me^v/me^v mice or me^v/me^v mice grown in methylcellulose media with either CSF-1 or GM-CSF (data not shown). This data suggests that CD5 may play a role in later stages of myelopoiesis.

For many years, the presence of a common progenitor for B-cells and macrophages has been suggested [58–61]. Reports of biphenotypic B/macrophage cells have appeared in current literature [62–64] and $CD5^+$ B-cells have been shown to develop macrophage characteristics [65, 66], often in association with B-cell malignancy [67, 68] (reviewed in Borrello and Phipps [61]). It has been shown that treatment of wild type mice with GM-CSF resulted not only in an increase in numbers of $CD5^+$ macrophages, but also in an elevation in numbers of $CD5^+$ hematopoietic progenitor cells and $CD5^+$ B-cells [56], supporting the presence of a common precursor for $CD5^+$ (B-1a) B-cells and $CD5^+$ macrophages. Lack of CD5 on biphenotypic B/macrophage cells or on macrophages believed to have evolved from B-1 B-cells may contribute to the reduced severity of lung lesions seen in $CD5^{null}$ me^v/me^v mice in comparison to me^v/me^v mice. CD5 expression may play a role in lineage commitment, possibly through altering responses to growth factors as suggested above.

There are three reported ligands for CD5 that are expressed on B-cells. These ligands include CD72, CD5 ligand, and cell surface Ig. Interactions between CD5 and these ligands are thought to play a role in communication between T and B-cells. CD72 is expressed constitutively on B-cells [69, 70], while CD5 ligand (CD5L, gp40-80) is expressed on activated B lymphocytes in the spleen [71] and is constitutively expressed on peritoneal B-cells and B lymphoma cell lines [72]. Poposil *et al*. [73] suggested that CD5 can also interact with certain sequences in the VH framework of cell surface Igs. Ligation of T-cell surface CD5 to gp40-80 on B-cells has been implicated in the stimulation of B-cell proliferation through the gp40-80 receptor [72].

Until recently, CD5 ligands were reported to be present only on B-cells. However, several studies have now provided evidence that CD5 ligands are present on a variety of cell types in addition to B-cells, indicating that the interaction of CD5 with its various ligands may play a more diverse role in cellular communication than is currently thought. Agostini *et al*. reported the presence of CD72 on alveolar macrophages isolated from human sarcoidosis patients [74]. A recombinant soluble protein based on the human CD5 extracellular region has been reported to bind to a variety of cells of myeloid as well as lymphoid origins [75], indicating the presence of a novel, widespread CD5 ligand. Just as $CD5^+$ B-cells also express CD72, it is possible that a CD5 ligand is expressed on certain monomyeloid cells and that it may play a role in the activation of these cells through binding with CD5. Alternatively, interaction of CD5 with an unknown ligand could be involved in adhesion of monomyeloid cells to other cell types. The extracellular region of CD5 is closely homologous to that of CD6 [76], a scavenger receptor family member involved in cell-cell adhesion through its ligand, CD166 (ALCAM) [77, 78]. Interactions of CD5 with as yet unidentified ligands may be responsible for accumulation of macrophages in the lungs of motheaten mice due to dysregulated adhesion rather than solely dysregulated proliferation of pulmonary macrophages.

Red cells and mature myeloid cells arise from a common multipotential precursor, termed CFU-GEMM based on the ability of these progenitor cells to form granulocyte, erythroid, monocyte and megakaryocyte colonies in culture in response to the appropriate cytokines. CFU-GEMMs can differentiate into either erythroid burst forming units (BFU-Es) in the presence of erythropoietin (Epo), or into granulocyte/macrophage colony forming units (CFU-GMs) in the absence of Epo but in the presence of myeloid growth factors such as GM-CSF. Spleens of me^v/me^v mice have previously been reported to contain high numbers of CFU-GMs in comparison to wild type mice, while the frequency of CFU-GMs in the bone marrow is similar in me^v/me^v and wild type mice [12]. SHP-1 is involved in the negative regulation of Epo mediated signals [79]. Both bone marrow and spleens of me^v/me^v mice contain increased numbers of erythroid precursors (CFU-Es) in comparison to wild type controls. CFU-Es from me^v/me^v mice are hyperresponsive to Epo and a subpopulation of me^v/me^v CFU-Es has lost their dependence on exogenous Epo [12]. The increase in red cell precursors with a corresponding decrease in relative numbers of granulocytes in spleens of $CD5^{null}$ me^v/me^v mice versus me^v/me^v mice suggests the possibility that CD5 plays a significant role in the myeloid differentiation pathway in the absence of SHP-1. Dysregulated Epo signaling resulting from the absence of SHP-1 accompanied by insufficient myeloid growth factor mediated signals in the absence of CD5 could potentially explain the apparent shift from myelo- to erythropoiesis observed in $CD5^{null}$ me^v/me^v mice in comparison to me^v/me^v mice. The lack of CD5 may cause changes in lineage commitment in the myeloid/erythroid branch of cellular development as a result of a reduced response to myeloid growth factors.

Although CD5 has been reported to be a negative regulator of signaling through both the T-cell receptor [19] and the B-cell receptor [80], earlier studies indicated a costimulatory role for CD5 in TCR signal transduction [81–83]. Recently, additional studies have corroborated the reports of CD5 providing positive signals in both B- and T-cells, through a distinct cascade of second messengers including acidic sphingomyelinase and protein kinase C-ζ [84]. Our finding that proteins in the range of 28–45 kd showed increased phosphorylation on tyrosine residues in spleens of older me^v/me^v mice but not in $CD5^{null}$ me^v/me^v mice of the same age may provide additional evidence of a significant role in signaling for CD5. In the absence of the negative regulatory effects of SHP-1, the signals conveyed through CD5 may result in the phosphorylation of one or more novel signaling molecules in the 28–45 kd range. Identification of these molecules could help further elucidate the relationship between CD5 and SHP-1 and facilitate the resolution of the pathways in which these molecules interact.

Clear examination of the role of CD5 in the development of viable motheaten pathological lesions is problematic on the (B6;129) segregating background. A polymorphic variation between 129 and C57BL/6J linked to the *CD20* (*Ms4a2*) gene, which maps near the *CD5* locus, has been shown to affect peritoneal B-1 cell populations [85]. Although the phenotypic differences observed in (B6;129)-$CD5^{null}$ me^v/me^v mice in comparison to (B6;129)-me^v/me^v mice do not appear to be related to reduced B-1 cell numbers alone, the effect of strain specific polymorphisms is an important consideration in analyzing any targeted mutation on a segregating background. We have recently completed backcrossing the CD5 mutation on to the C57BL/6J background and have developed a colony of inbred C57BL/6J-$CD5^{null}$ me^v/me^v mice. Spleen cell lysates from C57BL/6J-$CD5^{null}$ me^v/me^v and C57BL/6J-me^v/me^v mice were utilized in the α or anti-phosphotyrosine western blots shown in Figure 4. This experiment mirrored what was seen in extensive western blotting studies using (B6;129)-$CD5^{null}$ me^v/me^v mice and (B6;129)-me^v/me^v mice. We are currently in the process of expanding our colony of C57BL/6J-$CD5^{null}$ me^v/me^v mice. With these mice, we will re-examine the immunopathologic changes observed in our previous studies using (B6;129) mice, as well as continue our investigation into the signaling alterations suggested by our reported western blotting results. Confimation of the role of CD5 in immunopathologic changes in me^v/me^v mice using inbred C57BL/6J-$CD5^{null}$ me^v/me^v mice will mitigate the complications of variability associated with background modifying genes and polymorphic diversity.

Acknowledgements

This work is supported in part by National Institutes of Health Grants CA20408 (L.D.S.), CA79891-01 and GM58893 (T.Y.), T32 CA09217 (M.J.R.), AR43801 (J.P.S.) and CA34196 to The Jackson Laboratory.

References

1. Shultz L.D., Coman D.R., Bailey C.L., Beamer W.G., Sidman C.L. 1984. Viable motheaten, a new allele at the motheaten locus. I. Pathology. *Am. J. Path.* **116:** 179–192
2. Shultz L.D., Green M.C. 1976. Motheaten, an immunodeficient mutant of the mouse. II. Depressed immune competence and elevated serum immunoglobulins. *J. Immunol.* **116:** 936–943
3. Sidman C.L., Shultz L.D., Unanue E.R. 1978. The mouse mutant motheaten. II. Functional studies of the immune system. *J. Immunol.* **121:** 2399–2404
4. Davidson W.F., Morse H.C.D., Sharrow S.O., Chused T.M. 1979. Phenotypic and functional effects of the motheaten gene on murine B and T lymphocytes. *J. Immunol.* **122:** 884–891
5. Yi T., Gilbert D.J., Jenkins N.A., Copeland N.G., Ihle J.N. 1992. Assignment of a novel protein tyrosine phosphatase gene (Hcph) to mouse chromosome 6. *Genomics* **14:** 793–795
6. Yi T.L., Cleveland J.L., Ihle J.N. 1992. Protein tyrosine phosphatase containing SH2 domains: characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-p13. *Mol. Cell Biol.* **12:** 836–846

7. Jiao H., Yang W., Berrada K., Tabrizi M., Shultz L., Yi T. 1997. Macrophages from motheaten and viable motheaten mutant mice show increased proliferative responses to GM-CSF: detection of potential HCP substrates in GM-CSF signal transduction. *Exp. Hematol.* **25:** 592–600
8. Tapley P., Shevde N.K., Schweitzer P.A., Gallina M., Christianson S.W., Lin I.L., Stein R.B., Shultz L.D., Rosen J., Lamb P. 1997. Increased G-CSF responsiveness of bone marrow cells from hematopoietic cell phosphatase deficient viable motheaten mice. *Exp. Hematol.* **25:** 122–131
9. Zhang J., Somani A.K., Siminovitch K.A. 2000. Roles of the SHP-1 tyrosine phosphatase in the negative regulation of cell signalling. *Semin. Immunol.* **12:** 361–378
10. Shultz L.D., Schweitzer P.A., Rajan T.V., Yi T., Ihle J.N., Matthews R.J., Thomas M.L., Beier D.R. 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* **73:** 1445–1454
11. Nakayama K., Takahashi K., Shultz L.D., Miyakawa K., Tomita K. 1997. Abnormal development and differentiation of macrophages and dendritic cells in viable motheaten mutant mice deficient in haematopoietic cell phosphatase [published erratum appears in Int J Exp Pathol 1997 Oct;78(5):364]. *Int. J. Exp. Path.* **78:** 245–257
12. Van Zant G., Shultz L. 1989. Hematologic abnormalities of the immunodeficient mouse mutant, viable motheaten (mev). *Exp. Hematol.* **17:** 81–87
13. Sidman C.L., Marshall J.D., Allen R.D. 1989. Murine viable motheaten mutation reveals a gene critical to the development of both B and T lymphocytes. *Proc. Natl Acad. Sci. USA* **86:** 6279–6282
14. Komschlies K.L., Greiner D.L., Shultz L., Goldschneider I. 1987. Defective lymphopoiesis in the bone marrow of motheaten (me/me) and viable motheaten (mev/mev) mutant mice. III. Normal mouse bone marrow cells enable mev/mev prothymocytes to generate thymocytes after intravenous transfer. *J. Exp. Med.* **166:** 1162–1167
15. Greiner D.L., Goldschneider I., Komschlies K.L., Medlock E.S., Bollum F.J., Schultz L. 1986. Defective lymphopoiesis in bone marrow of motheaten (me/me) and viable motheaten (mev/mev) mutant mice. I. Analysis of development of prothymocytes, early B lineage cells, and terminal deoxynucleotidyl transferase-positive cells. *J. Exp. Med.* **164:** 1129–1144
16. Sherr D.H., Dorf M.E., Gibson M., Sidman C.L. 1987. Ly-1 B helper cells in autoimmune viable motheaten mice. *J. Immunol.* **139:** 1811–1817
17. Azzam H.S., Grinberg A., Lui K., Shen H., Shores E.W., Love P.E. 1998. CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity. *J. Exp. Med.* **188:** 2301–2311
18. Davies A.A., Ley S.C., Crumpton M.J. 1992. CD5 is phosphorylated on tyrosine after stimulation of the T-cell antigen receptor complex. *Proc. Natl Acad. Sci. USA* **89:** 6368–6372
19. Tarakhovsky A., Kanner S.B., Hombach J., Ledbetter J.A., Müller W., Killeen N., Rajewsky K. 1995. A role for CD5 in TCR-mediated signal transduction and thymocyte selection. *Science* **269:** 535–537
20. Pena-Rossi C., Zuckerman L.A., Strong J., Kwan J., Ferris W., Chan S., Tarakhovsky A., Beyers A.D., Killeen N. 1999. Negative regulation of CD4 lineage development and responses by CD5. *J. Immunol.* **163:** 6494–6501
21. Chan S., Waltzinger C., Tarakhovsky A., Benoist C., Mathis D. 1999. An influence of CD5 on the selection of CD4-lineage T cells. *Eur. J. Immunol.* **29:** 2916–2922
22. Perez-Villar J.J., Whitney G.S., Bowen M.A., Hewgill D.H., Aruffo A.A., Kanner S.B. 1999. CD5 negatively regulates the T-cell antigen receptor signal transduction pathway: involvement of SH2-containing phosphotyrosine phosphatase SHP-1. *Mol. Cell. Biol.* **19:** 2903–2912
23. Lankester A.C., van Schijndel G.M., Cordell J.L., van Noesel C.J., van Lier R.A. 1994. CD5 is associated with the human B cell antigen receptor complex. *Eur. J. Immunol.* **24:** 812–816
24. Tarakhovsky A., Müller W., Rajewsky K. 1994. Lymphocyte populations and immune responses in CD5-deficient mice. *Eur. J. Immunol.* **24:** 1678–1684
25. Hayakawa K., Hardy R.R., Parks D.R., Herzenberg L.A. 1983. The 'Ly-1 B' cell subpopulation in normal immunodefective, and autoimmune mice. *J. Exp. Med.* **157:** 202–218
26. Hayakawa K., Hardy R.R., Honda M., Herzenberg L.A., Steinberg A.D. 1984. Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl Acad. Sci. USA* **81:** 2494–2498
27. Pers J., Jamin C., Predine-Hug F., Lydyard P., Youinou P. 1999. The role of CD5-expressing B cells in health and disease (Review). *Int. J. Mol. Med.* **3:** 239–245
28. Sidman C.L., Shultz L.D., Hardy R.R., Hayakawa K., Herzenberg L.A. 1986. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. *Science* **232:** 1423–1425
29. Pani G., Siminovitch K.A., Paige C.J. 1997. The motheaten mutation rescues B cell signaling and development in CD45-deficient mice. *J. Exp. Med.* **186:** 581–588
30. Fleming T.J., Fleming M.L., Malek T.R. 1993. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J. Immunol.* **151:** 2399–2408
31. Springer T., Galfre G., Secher D.S., Milstein C. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol.* **9:** 301–306
32. Waldschmidt T.J., Conrad D.H., Lynch R.G. 1988. The expression of B cell surface receptors. I. The ontogeny and distribution of the murine B cell IgE Fc receptor. *J. Immunol.* **140:** 2148–2154
33. Rao M., Lee W.T., Conrad D.H. 1987. Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J. Immunol.* **138:** 1845–1851
34. Hardy R., Hayakawa K. 1992. Generation of Ly-1 B cells from developmentally distinct precursors. Enrichment by stromal-cell culture or cell sorting. *Ann. NY Acad. Sci.* **651:** 99–111
35. Hardy R.R., Carmack C.E., Shinton S.A., Kemp J.D., Hayakawa K. 1991. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J. Exp. Med.* **173:** 1213–1225
36. Gulley M.L., Ogata L.C., Thorson J.A., Dailey M.O., Kemp J.D. 1988. Identification of a murine pan-T cell antigen which is also expressed during the terminal

phases of B cell differentiation. *J. Immunol.* **140:** 3751–3757

37. Sato S., Ono N., Steeber D.A., Pisetsky D.S., Tedder T.F. 1996. CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. *J. Immunol.* **157:** 4371–4378
38. Tedder T.F., Zhou L.J., Engel P. 1994. The CD19/CD21 signal transduction complex of B lymphocytes. *Immunol. Today* **15:** 437–442
39. Krop I., de Fougerolles A.R., Hardy R.R., Allison M., Schlissel M.S., Fearon D.T. 1996. Self-renewal of B-1 lymphocytes is dependent on CD19. *Eur. J. Immunol.* **26:** 238–242
40. Leo O., Foo M., Sachs D.H., Samelson L.E., Bluestone J.A. 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl Acad. Sci. USA* **84:** 1374–1378
41. Ledbetter J.A., Rouse R.V., Micklem H.S., Herzenberg L.A. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J. Exp. Med.* **152:** 280–295
42. Ledbetter J.A., Herzenberg L.A. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* **47:** 63–90
43. Coffman R.L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol. Rev.* **69:** 5–23
44. Springer T. (ed.) 1980. *Cell-surface differentiation in the mouse*. Plenum Press, New York
45. Bhattacharya A., Dorf M.E., Springer T.A. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J. Immunol.* **127:** 2488–2495
46. Ikuta K., Kina T., MacNeil I., Uchida N., Peault B., Chien Y.H., Weissman I.L. 1990. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell* **62:** 863–874
47. Stall A.M., Adams S., Herzenberg L.A., Kantor A.B. 1992. Characteristics and development of the murine B-1b (Ly-1 B sister) cell population. *Ann. NY Acad. Sci.* **651:** 33–43
48. Stall A.M., Wells S.M., Lam K.P. 1996. B-1 cells: unique origins and functions. *Sem. Immunol.* **8:** 45–59
49. Amoura Z., Chabre H., Koutouzov S., Lotton C., Cabrespines A., Bach J.F., Jacob L. 1994. Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum.* **37:** 1684–1688
50. Zouali M., Stollar B.D. 1986. A rapid ELISA for measurement of antibodies to nucleic acid antigens using UV-treated polystyrene microplates. *J. Immunol. Methods* **90:** 105–110
51. Yang W., Mckenna S.D., Jiao H., Tabrizi M., Lynes M. A., Shultz L.D., Yi T. 1998. SHP-1 deficiency in B-lineage cells is associated with heightened lyn protein expression and increased lyn kinase activity. *Exp. Hematol.* **26:** 1126–1132
52. Yang W., Tabrizi M., Berrada K., Yi T. 1998. SHP-1 phosphatase C-terminus interacts with novel substrates p32/p30 during erythropoietin and interleukin-3 mitogenic responses. *Blood* **91:** 3746–3755
53. Murray A.B., Luz A. 1990. Acidophilic macrophage pneumonia in laboratory mice. *Vet. Pathol.* **27:** 274–281
54. Ward J.M. 1978. Pulmonary pathology of the motheaten mouse. *Vet. Pathol.* **15:** 170–178
55. Pani G., Kozlowski M., Cambier J. C., Mills G.B., Siminovitch K.A. 1995. Identification of the tyrosine phosphatase PTP1C as a B cell antigen receptor-associated protein involved in the regulation of B cell signaling [see comments]. *J. Exp. Med.* **181:** 2077–2084
56. Takahashi K., Miyakawa K., Wynn A. A., Nakayama K.I., Myint Y.Y., Naito M., Shultz L.D., Tominaga A., Takatsu K. 1998. Effects of granulocyte/macrophage colony-stimulating factor on the development and differentiation of CD5-positive macrophages and their potential derivation from a CD5-positive B-cell lineage in mice. *Am. J. Path.* **152:** 445–456
57. Yu C.C., Tsui H.W., Ngan B.Y., Shulman M.J., Wu G.E., Tsui F.W. 1996. B and T cells are not required for the viable motheaten phenotype. *J. Exp. Med.* **183:** 371–380
58. Katoh S., Tominaga A., Migita M., Kudo A., Takatsu K. 1990. Conversion of normal Ly-1-positive B-lineage cells into Ly-1-positive macrophages in long-term bone marrow cultures. *Dev. Immunol.* **1:** 113–125
59. Davidson W.F., Pierce J.H., Holmes K.L. 1992. Evidence for a developmental relationship between CD5+ B-lineage cells and macrophages. *Ann. NY Acad. Sci.* **651:** 112–129
60. Cumano A., Paige C.J., Iscove N.N., Brady G. 1992. Bipotential precursors of B cells and macrophages in murine fetal liver. *Nature* **356:** 612–615
61. Borrello M.A., Phipps R.P. 1996. The B/macrophage cell: an elusive link between CD5+ B lymphocytes and macrophages [see comments]. *Immunol. Today* **17:** 471–475
62. Graf B.A., Nazarenko D.A., Borrello M.A., Roberts L.J., Morrow J.D., Palis J., Phipps R.P. 1999. Biphenotypic B/macrophage cells express COX-1 and up-regulate COX-2 expression and prostaglandin E(2) production in response to pro-inflammatory signals. *Eur. J. Immunol.* **29:** 3793–3803
63. Martin M., Strasser A., Baumgarth N., Cicuttini F.M., Welch K., Salvaris E., Boyd A.W. 1993. A novel cellular model (SPGM 1) of switching between the pre-B cell and myelomonocytic lineages. *J. Immunol.* **150:** 4395–4406
64. Borrello M.A., Phipps R.P. 1999. Fibroblast-secreted macrophage colony-stimulating factor is responsible for generation of biphenotypic B/macrophage cells from a subset of mouse B lymphocytes. *J. Immunol.* **163:** 3605–3611
65. Tanaka T., Wu G.E., Paige C.J. 1994. Characterization of the B cell-macrophage lineage transition in 70Z/3 cells. *Eur. J. Immunol.* **24:** 1544–1548
66. Hara H., Sam M., Maki R.A., Wu G.E., Paige C.J. 1990. Characterization of a 70Z/3 pre-B cell derived macrophage clone. Differential expression of Hox family genes. *Int. Immunol.* **2:** 691–696
67. Hanecak R., Zovich D.C., Pattengale P.K., Fan H. 1989. Differentiation in vitro of a leukemia virus-induced B-cell lymphoma into macrophages. *Mol. Cell Biol.* **9:** 2264–2268

68. Boyd A.W., Schrader J.W. 1982. Derivation of macrophage-like lines from the pre-B lymphoma ABLS 8.1 using 5-azacytidine. *Nature* **297:** 691–693
69. Luo W., Van de Velde H., von Hoegen I., Parnes J.R., Thielemans K. 1992. Ly-1 (CD5), a membrane glycoprotein of mouse T lymphocytes and a subset of B cells, is a natural ligand of the B cell surface protein Lyb-2 (CD72). *J. Immunol.* **148:** 1630–1634
70. Van de Velde H., von Hoegen I., Luo W., Parnes J.R., Thielemans K. 1991. The B-cell surface protein CD72/Lyb-2 is the ligand for CD5 [see comments]. *Nature* **351:** 662–665
71. Biancone L., Bowen M.A., Lim A., Aruffo A., Andres G., Stamenkovic I. 1996. Identification of a novel inducible cell-surface ligand of CD5 on activated lymphocytes. *J. Exp. Med.* **184:** 811–819
72. Bikah G., Lynd F.M., Aruffo A.A., Ledbetter J.A., Bondada S. 1998. A role for CD5 in cognate interactions between T cells and B cells, and identification of a novel ligand for CD5. *Inter. Immunol.* **10:** 1185–1196
73. Pospisil R., Fitts M.G., Mage R.G. 1996. CD5 is a potential selecting ligand for B cell surface immunoglobulin framework region sequences. *J. Exp. Med.* **184:** 1279–1284
74. Agostini C., Trentin L., Perin A., Facco M., Siviero M., Piazza F., Basso U., Adami F., Zambello R., Semenzato G. 1999. Regulation of alveolar macrophage-T cell interactions during Th1-type sarcoid inflammatory process. *Am. J. Physiol.* **277:** L240–L250
75. Calvo J., Places L., Padilla O., Vila J.M., Vives J., Bowen M.A., Lozano F. 1999. Interaction of recombinant and natural soluble CD5 forms with an alternative cell surface ligand. *Eur. J. Immunol.* **29:** 2119–2129
76. Aruffo A., Melnick M.B., Linsley P.S., Seed B. 1991. The lymphocyte glycoprotein CD6 contains a repeated domain structure characteristic of a new family of cell surface and secreted proteins. *J. Exp. Med.* **174:** 949–952
77. Aruffo A., Bowen M.A., Patel D.D., Haynes B.F., Starling G.C., Gebe J.A., Bajorath J. 1997. CD6-ligand interactions: a paradigm for SRCR domain function? *Immunol. Today* **18:** 498–504
78. Bowen M.A., Aruffo A. 1999. Adhesion molecules, their receptors, and their regulation: analysis of CD6-activated leukocyte cell adhesion molecule (ALCAM/CD166) interactions. *Transplant Proc.* **31:** 795–796
79. Klingmuller U., Lorenz U., Cantley L.C., Neel B.G., Lodish H.F. 1995. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell* **80:** 729–738
80. Bikah G., Carey J., Ciallella J.R., Tarakhovsky A., Bondada S. 1996. CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. *Science* **274:** 1906–1909
81. Imboden J.B., June C.H., McCutcheon M.A., Ledbetter J.A. 1990. Stimulation of CD5 enhances signal transduction by the T cell antigen receptor. *J. Clin. Invest.* **85:** 130–134
82. Ceuppens J.L., Baroja M.L. 1986. Monoclonal antibodies to the CD5 antigen can provide the necessary second signal for activation of isolated resting T cells by solid-phase-bound OKT3. *J. Immunol.* **137:** 1816–1821
83. Verwilghen J., Vandesande R., Vandenberghe P., Ceuppens J.L. 1990. Crosslinking of the CD5 antigen on human T cells induces functional IL2 receptors. *Cell Immunol.* **131:** 109–119
84. Simarro M., Calvo J., Vilà J.M., Places L., Padilla O., Alberola-Ila J., Vives J., Lozano F. 1999. Signaling through CD5 involves acidic sphingomyelinase, protein kinase C-zeta, mitogen-activated protein kinase kinase, and c-Jun NH2-terminal kinase. *J. Immunol.* **162:** 5149–5155
85. O'Keefe T.L., Williams G.T., Davies S.L., Neuberger M.S. 1998. Mice carrying a CD20 gene disruption. *Immunogenetics* **48:** 125–132