
Research Article

Discrimination of *Peromyscus maniculatus* Leukocytes by Flow Cytometry

Julie Vaughn and Tony Schountz*

Department of Biological Sciences. Mesa State College. Grand Junction, CO 81501

Abstract. Deer mice (*Peromyscus maniculatus*) have been identified as the principal host species for Sin Nombre hantavirus. Assessment of deer mouse immune responses to this agent has been difficult because of a lack of reagents with defined specificity for the discrimination of leukocyte populations. The purpose of this work was to identify currently available immunological reagents directed at house mouse (*Mus musculus*) leukocyte cell surface antigens that cross-react with deer mouse cells. A panel of 19 monoclonal antibodies was screened for reactivity by flow cytometric analysis. Seven antibodies exhibited binding to deer mouse splenocytes at varying levels compared to house mouse controls. The panel detected cellular phenotypes that are associated with natural and adaptive immune responses, including T cells, B cells, macrophages, dendritic cells, neutrophils and granulocytes. This panel should facilitate the further examination of deer mouse immune responses to infectious agents.

Introduction

Members of the genus *Peromyscus* host several human pathogens, including the agents of granulocytic ehrlichiosis, Lyme disease, babesiosis, cryptosporidia, bartonellosis and hantavirus cardiopulmonary syndrome (HCPS) (Childs et al., 1994; Elliott et al., 1994; Richter et al., 1998; Hofmeister et al., 1999; Stafford et al., 1999; Welch et al., 1999; Breitschwerdt and Kordick, 2000; Perz and Le Blancq, 2001; Ravyn et al., 2001). Deer mice (*Peromyscus maniculatus*) are the principal host of Sin Nombre hantavirus (SNV), which has

caused over 100 deaths in the United States since its discovery in 1993. Understanding maintenance of these pathogens in peromyscine host populations is critical to understanding transmission to humans (Mills and Childs, 1998).

Little is known about how deer mice contain infection with SNV. The virus is found in many tissues of infected deer mice, including the lungs (Green et al., 1998), but with little or no pathology (Botten et al., 2000) and pulmonary function is not compromised (O'Connor et al., 1997). Infected deer mice appear to remain persistently infected for the remainder of their lives (Botten et al., 2000). In HCPS, however, a pronounced pulmonary immune response occurs without viral cytopathology that is characterized by the presence of mononuclear cells producing proinflammatory cytokines that are thought to cause the clinical manifestations of disease (Zaki et al., 1995; Mori et al., 1999). Serological evidence in-

*Correspondence to: Tony Schountz, Ph.D. Department of Biology, Mesa State College, 1100 North Ave., Grand Junction, CO 81501. Phone: (970) 248-1936; FAX: (970) 248-1700; e-mail: tschount@mesastate.edu.

dicates that humans and deer mice produce strong antibody responses to the same region of the viral nucleocapsid antigen (Jenison et al., 1994; Yamada et al., 1995).

Virtually no immunological reagents of defined specificity have been developed for evaluation of immune responsiveness in deer mice. However, many monoclonal antibodies (Mab) specific for cluster of differentiation (CD) proteins and other cell surface molecules are available for laboratory-derived strains of house mice (*Mus musculus*). The presence or absence of these molecules on the cell surface permits the phenotyping of leukocyte subpopulations. We report here that some of these Mab are cross-reactive with deer mouse leukocytes by flow cytometry. These antibodies will be useful for identifying cellular subpopulations during deer mouse immune responses in naturally and experimentally infected animals.

Materials and Methods

Mice

All mice were obtained from breeding colonies at Mesa State College. Animals were used at 6–12 weeks of age and the institutional Animal Care and Use Committee approved all experimental animal procedures. The *Peromyscus maniculatus bairdii* colony was established with mice purchased from the University of South Carolina Peromyscus Stock Center and were maintained under the provisions of the Animal Welfare Act. The house mouse strain used was BALB/c.

Antibodies

Fluorochrome-conjugated monoclonal antibodies (kindly provided by D. Grantham, Pharmingen) directed against house mouse CD3 ϵ (clone 145–2C11), CD4 (RM4–4 and GK1.5), CD8 α (53–6.7), CD11a (M17/4), CD11b (M1/70), CD11c (HL3), CD19 (1D3), CD21/35 (7G6), CD24 (M1/69), CD43 (S7), CD45R-B220 (RA3–6B2), CD61 (2C9.G2), CD62L (MEL-14), CD80 (16–10A1), CD86 (GL1), immunoglobulin- κ chain (Ig κ) (R8–

140), IgD^a (AMS 9.1), and T cell receptor- β chain (TCR β) (H57–597) were used for these experiments.

Flow cytometry

Cells were prepared and stained with antibodies as previously described (Schountz et al., 1996). Spleens were removed and made into single-cell suspensions and red blood cells and fibroblasts were removed by centrifugation over Lymphocyte Separation Medium (Cappel/Organon-Teknika). Cells were washed twice in 5% fetal bovine serum in phosphate buffered saline (FBS-PBS) (pH 7.3) and 5×10^5 cells were stained with monoclonal antibody for 1 hr on ice. After repeated washing in 5% FBS-PBS, cells were fixed in 1% paraformaldehyde-PBS (pH 7.3) and 10,000 events collected on a FACScan flow cytometer (Becton-Dickinson Immunocytometry Systems). Leukocytes were gated (Figure 1A) and analyzed with Cell Quest software (BDIS). To compare cellular distributions between house mice and deer mice, an event ratio (ER) for each antibody was calculated by dividing the number of deer mouse cells occurring in marker 2 (M2) of histograms by the number of house mouse cells occurring in marker 2 (Table 1; flow data not shown). Event ratios of greater than 1 indicated that more deer mouse cells expressed the cell surface protein than do house mouse cells.

T Cell Proliferation Assay

Monoclonal antibody 145–2C11 was coated onto 96-well tissue culture plates in PBS (pH 7.4) overnight at 4° C. The next day, unbound antibody was removed and 5×10^5 freshly isolated splenocytes in 5% FBS-Clicks medium were added to wells of the plate. The cells were incubated 48 hours in a humidified 5% CO₂ incubator and proliferation was assessed using the CellTiter 96 Aqueous MTS proliferation kit (Promega) according to the manufacturer's directions. A proliferation index was determined by the maximal proliferation of cells incubated with 5 μ g/ml of concanavalin-A (arbitrarily set to 10).

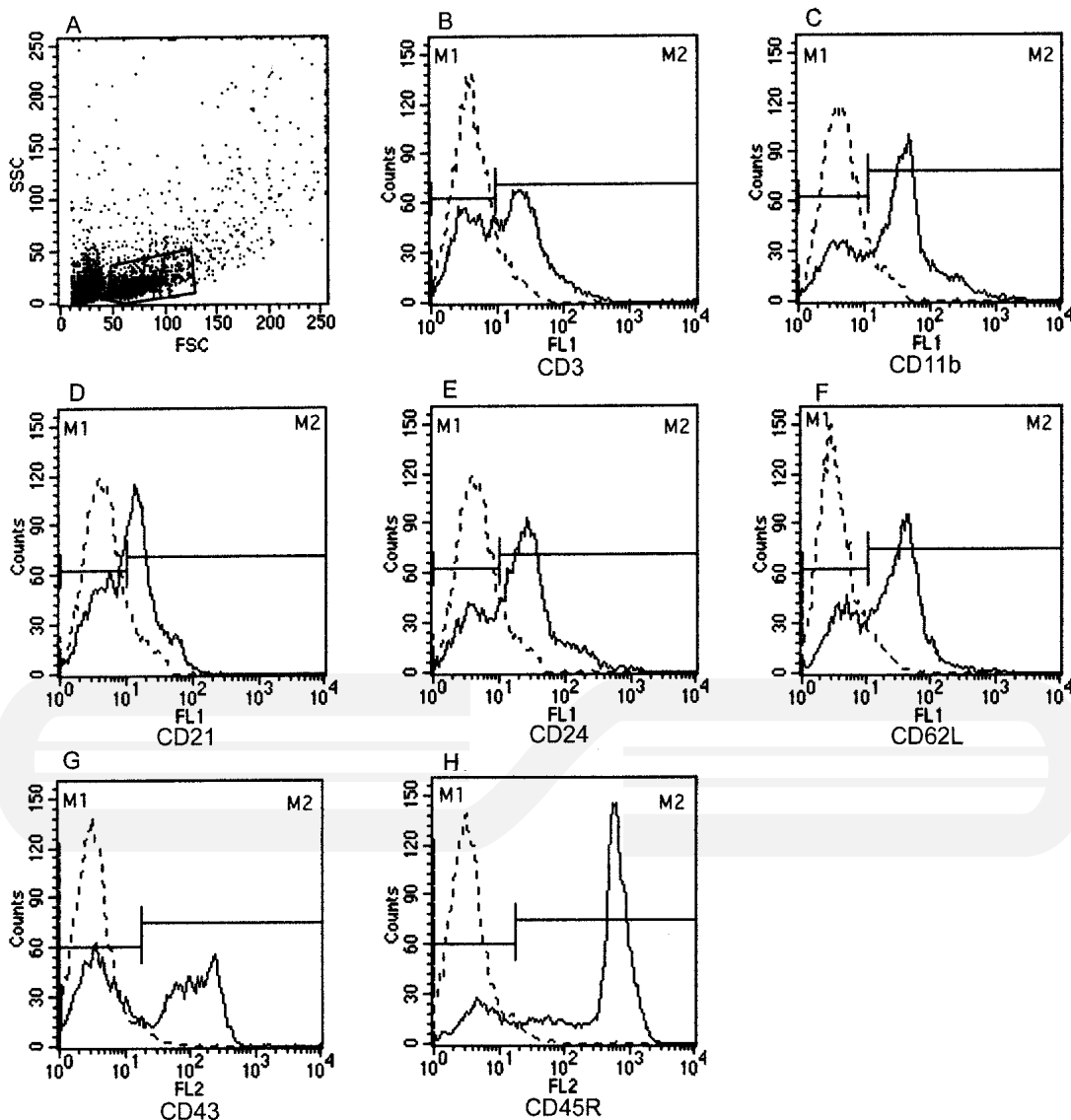


Figure 1. Binding characteristics of antibodies directed to house mouse leukocyte cell surface antigens on deer mouse splenocytes. Five hundred thousand spleen cells were stained with antibodies directed against house mouse cell surface antigens. Cells were washed and fixed in 1% paraformaldehyde-PBS (pH 7.4) and 10,000 events were collected on a FACScan flow cytometer. A gate was established around leukocytes for analysis. Panels: A, Forward/side scatter of unstained cells with gate. Markers for FL1 and FL2 were established with nonbinding isotype control antibodies. B. CD3 (145-2C11); C. CD11b (M1/70); D. CD21/35 (7G6); E. CD24 (M1/69); F. CD62L (MEL-14); G. CD43 (S7); H. CD45R-B220/B220 (RA3-6B2). Solid lines, CD-specific antibody; hatched lines, isotype control antibody (B, G=clone HL3, hamster IgG[FITC]; C, D, E=RM4-4, rat IgG2b[FITC]; F=53-6.7, rat IgG2a[FITC]; H, I=1D3, rat IgG2a[PE]). The data are from a single deer mouse and are representative of five animals.

Results

Splenocytes were evaluated for the presence of cells in marker 2 (M2), which was suggestive of antibody binding. Binding to CD4, CD8 α , CD11a, CD19, CD80 CD86, Ig κ , IgD^a and

TCR β on deer mouse leukocytes was not detected since the histograms lacked a distinct peak in M2 (data not shown) and had a low ER (<0.30; Table 1). CD11c staining was inconclusive because no discernable peak was detected in staining of either deer mouse or house mouse spleno-

cytes (data not shown) and the ER was moderate (0.51). Notably, each tested Ig isotype exhibited at least one Mab that failed to bind to deer mouse cells, suggesting that none of the isotypes bind to deer mouse Fc receptors (Table 1).

We detected moderate staining of CD3 on deer mouse splenocytes compared to house mouse controls (Figure 1B). Although more cells from deer mice were CD3⁺ (ER=1.25, Table 1), staining was less bright as determined by mean channel fluorescence (MCF) (Table 1) suggesting that antibody affinity for the epitope is lower in deer mice or that deer mouse cells express fewer CD3 molecules. To verify that Mab 145-2C11 binds CD3, we induced T cell proliferation with plate-bound antibody (Figure 2). The kinetics of proliferation were similar, but less in deer mouse cells, supporting the contention that the antibody binds with less affinity or fewer CD3 molecules exist on the surface of deer mouse T cells. Maximal proliferation was observed at 1 μg/ml for both species, while 10 μg/ml induced an unresponsive state.

Binding to CD11b revealed a relatively homogenous population of cells in deer mice (Figure 1C). Again, more deer mouse cells were CD11b⁺ (ER=1.89) but antibody binding was less. As with CD3, binding to CD21/CD35 was

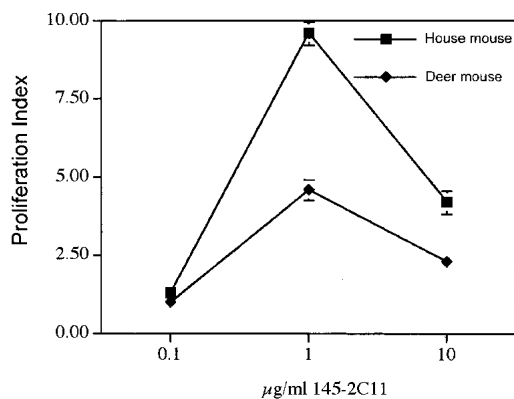


Figure 2. Proliferation of deer mouse splenocytes to plate-bound anti-CD3 monoclonal antibody 145-2C11. Plates were coated overnight with antibody diluted in PBS at the noted concentrations. 5×10^5 deer mouse or house mouse splenocytes were added to each well in 5% FBS-Click's medium and incubated 48 hours. Proliferation was measured by MTS assay and a proliferation index was determined relative to maximal proliferation (arbitrarily assigned as 10) of splenocytes incubated with 5 μg/ml of Con A.

present, although at less fluorescent intensity (Figure 1D). The frequency of CD21/CD35⁺ cells was nearly identical in deer mice and house mice (ER=0.96). Anti-CD24 also exhibited similar binding pattern as CD3, with less fluorescence but more deer mouse cells expressed the protein (Figure 1E; ER=1.41).

CD62L binding produced a slightly less fluorescent peak and positive cells were fewer in deer mice than in house mice (Figure 1F; ER=0.86). CD43 expression was nearly identical in deer mouse and house mouse splenocytes, but more house mouse cells expressed the protein (Figure 1G; ER=0.77). Staining of CD45R-B220 produced nearly congruent histograms with house mouse (compare MCF in Table 1) and the frequency of cells expressing the protein was virtually identical (Figure 1H; ER=0.99).

Discussion

Contemporary techniques for assessing host-parasite relationships in deer mice have not been well-developed. One such deficiency is a lack of immunological reagents specific for cell surface antigens found on the surfaces of leukocytes. We have addressed this issue by evaluating a panel of monoclonal antibodies directed against house mouse cell surface antigens for cross-reactivity on deer mouse leukocytes. Seven of 19 Mab screened by flow cytometry exhibited binding to deer mouse cells. The cellular distribution of these molecules is known in house mice (Table 2), but unknown in deer mice. The availability of these antibodies will be useful in determining the distribution of the markers in deer mouse leukocyte subpopulations.

The antibodies that did not stain deer mouse cells eliminated the possibility of nonspecific binding by Fc receptors. All isotypes were represented by nonbinding antibodies from each species (Table 1). These included RM4-4 (rat IgG2b), 53-6.7 (rat IgG2a), R8-140 (rat IgG1), AMS 9.1 (mouse IgG2b) and H57-597 (hamster IgG). These antibodies provided important controls for assessing binding.

Several antibodies detected the presence of molecules associated with lymphocyte signaling and activation. CD3 is found on T lymphocytes

and activated NK cells. It is composed of three homologous noncovalently-linked transmembrane proteins, γ , δ and ϵ , which exist as $\epsilon\gamma$ or $\epsilon\delta$ dimers (Blumberg et al., 1990) and is noncovalently associated with the T cell receptor (TCR) (Clevers et al., 1988). The molecule provides TCR-proximal signal transduction events leading to T cell activation and clonal expansion (O'Rourke et al., 1990). Mab 145-2C11 binds to the ϵ chain and it will permit assessment of T cell-mediated immune functions in deer mice.

CD45R-B220 is a phosphatase found on all B cells that participates in surface Ig signaling events by regulating *src*-family tyrosine kinase activity (Hathcock et al., 1992) and is first detected on pro-B cells (Hardy, et al., 1991). It is also found on activated NK cells (Ballas and Rasmussen, 1993).

Many complement proteins facilitate phagocytosis by receptor-mediated endocytosis. CD11b forms dimers with other polypeptides to mediate binding to the complement fragment opsonin C3bi and intercellular adhesion by CD54, facilitating neutrophil and macrophage adherence to the endothelium and phagocytosis of iC3b- or IgG-coated particles (Springer et al., 1979; Lub et al., 1996). Its distribution includes macrophages, granulocytes, NK cells, activated dendritic cells and B cells (Springer et al., 1979; Ault and Springer, 1981; Hamilton, Lehen and Kearney, 1994; Kantor et al., 1992; Vremec et al. 1992).

CD21 and CD35 are alternative splice variants of the *Cr2* gene and are found on activated granulocytes, B cells and follicular dendritic cells (Kinoshita et al., 1988). CD21 is the receptor for complement protein C3d that is involved in antigen trapping in lymphoid tissues. Zaki et al. (1995) have reported that cells resembling follicular dendritic cells in HCPS patients react with antisera specific for SNV. However, it is unclear whether these cells are infected by virus or if antibodies are binding to CD21/CD35-ligated immune complexes containing viral antigens.

Recruitment of leukocytes to sites of infection is mediated by a large family of adhesion molecules. CD62L (L-selectin) is expressed by a variety of leukocytes and plays an early role in at-

tachment to activated vascular endothelium (Arbones et al., 1994). Chemotactic factors synthesized by traumatized tissues influence the expression of CD62L, thus targeting leukocytes into the tissue by extravasation.

CD43 is found on NK cells, T cells, B cells, macrophages, granulocytes, megakaryocytes, and platelets. It is an anti-adhesion molecule and negatively regulates CD62L-mediated tethering, rolling and adherence to capillary endothelial cells (Stockton et al., 1998).

CD24 is expressed by erythrocytes, granulocytes, monocytes and lymphocytes (Ledbetter and Herzenberg, 1979; Stall and Wells, 1997) and has been detected on splenic dendritic cells (Vremec et al., 1992). Its precise role in the immune response has remained elusive, however B cells expressing high levels of CD24 have been associated with primary IgM responses (Klinman, 1997).

There are currently more than 150 Mab available for the detection of house mouse cell surface proteins. We assessed 19 of these and have demonstrated that several stain deer mouse leukocytes by flow cytometry and it is likely that many of the remaining antibodies will also recognize deer mouse proteins.

Most of the antibodies that are reactive with deer mouse cells are not suitable for use with paraffin-embedded tissues (according to manufacturer's data sheets). However, they can be utilized for localization of proteins in acetone-fixed cryopreserved tissue sections, and it is likely that they can also be used with cryopreserved deer mouse tissues. Moreover, this panel of antibodies should facilitate rapid identification of the biochemical functions of deer mouse CD molecules by the various immunochemical methods currently available, such as immunoprecipitation and western blotting. Many of these proteins are temporally regulated during leukocyte development and this panel should permit examination of immunological maturation in the bone marrow and thymus of deer mice. We believe these antibodies will also recognize proteins from other *Peromyscus* species as well, such as white-footed mice (*P. leucopus*).

We also have developed assays for detecting

cytokine and chemokine expression in deer mice (Herbst et al., 2002; in preparation). The combination of these assays and the antibodies described in this work will provide important tools for examining immune responses in peromyscine rodents.

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Literature Cited

- Arbones, M. L., D. C. Ord, K. Ley, H. Ratech, C. Maynard-Curry, G. Otten, D. J. Capon and T. F. Tedder. 1994. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity*. **1**:247–260.
- Ault, K. A., and T. A. Springer. 1981. Cross-reaction of a rat anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. *Journal of Immunol.* **126**:359–364.
- Ballas, Z. K., and W. Rasmussen. 1993. Lymphokine-activated killer cells. VII. IL-4 induces an NK1.1⁺CD8 α ⁺ β ⁻ TCR- $\alpha\beta$ B220⁺ lymphokine-activated killer subset. *Journal of Immunol.* **150**:17–130.
- Blumberg, R., S. Ley, J. Sancho, N. Lonberg, E. Lacy, F. McDermott, V. Schad, J. L. Greenstein and C. Terhorst. 1990. Structure of the T cell antigen receptor: Evidence for two CD3 ϵ subunits in the T cell receptor-CD3 complex. *Proc Natl Acad Sci U S A.* **87**:7220–7224.
- Botten, J., K. Mirowsky, D. Kusewitt, M. Bharadwaj, J. Yee, R. Ricci, R. M. Feddersen, and B. Hjelle. 2000. Experimental infection model for Sin Nombre hantavirus in the deer mouse (*Peromyscus maniculatus*). *Proc Natl Acad Sci U S A.* **97**:10578–83.
- Breitschwerdt, E. B., and D.L. Kordick. 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clin Microbiol Rev.* **13**:428–38.
- Childs, J. E., T. G. Ksiazik, C. F. Spiropoulou, J. W. Krebs, S. Morzunov, G. O. Maupin, K. L. Gage, P. E. Rollin, J. Sarrisky, R. E. Enscore, J. K. Frey, C. J. Peters and S. T. Nichol. 1994. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *J Infect Dis.* **169**:1271–1280.
- Clevers, H., B. Alarcon, T. Willeman and C. Terhorst. 1988. The T cell receptor/CD3 complex: A dynamic protein ensemble. *Ann Review of Immunol.* **6**:629–662.
- Elliott, L. H., T. G. Ksiazek, P. E. Rollin, C. F. Spiropoulou, S. Morzunov, M. Monroe, C. S. Goldsmith, C. D. Humphrey, S. R. Zaki, J. W. Krebs, G. Maupin, K. Gage, J. E. Childs, S. T. Nichol and C. J. Peters. 1994. Isolation of the causative agent of hantavirus pulmonary syndrome. *Am J Trop Med Hygiene.* **51**:102–109.
- Green, W., R. Feddersen, O. Yousef, M. Behr, K. Smith, J. Nestler, S. Jenison, T. Yamada, and B. Hjelle. 1998. Tissue distribution of hantavirus antigen in naturally infected humans and deer mice. *J Infect Dis.* **177**:1696–700.
- Hamilton, A. M. A. Lehuen and J. F. Kearney. 1994. Immunofluorescence analysis of B-1 cell ontogeny in the mouse. *International Immunol.* **6**:355–361.
- Hardy, R. R., C. E. Carmack, S. A. Shinton, J. D. Kemp, and K. Hayakawa. 1991. Resolution and characterization of a pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J Exp Med.* **173**:1213–1225.
- Hathcock, K. S., H. Hirano, S. Murakami and R. J. Hodes. 1992. CD45 Expression by B cells. Expression of different CD45 isoforms by subpopulations of activated B cells. *J Immunol.* **149**:2286–2294.
- Herbst, M. M., J. Prescott, A. D. Palmer, and T. Schountz. 2002. Sequence and expression analysis of deer mouse interferon- γ , interleukin-10, tumor necrosis factor, and lymphotoxin- α . *Cytokine.* **17**:202–213.
- Hofmeister, E.K., G. E. Glass, J. E. Childs, and D. H. Persing. 1999. Population dynamics of a naturally occurring heterogeneous mixture of *Borrelia burgdorferi* clones. *Infect Immun.* **67**:5709–16.
- Jenison, S., T. Yamada, C. Morris, B. Anderson, N. Torrez-Martinez, N. Keller and B. Hjelle. 1994. Characterization of human antibody responses to Four Corners hantavirus infections among patients with hantavirus pulmonary syndrome. *J Virol.* **68**:3000–3006.
- Kantor, A. B., A. M. Stall, S. Adams, L. A. Herzenberg and L. A. Herzenberg. 1992. Differential development of progenitor activity for three B cell lineages. *Proc Natl Acad Sci U S A.* **89**:3320–3324.
- Kinoshita, T., J. Takeda, K. Hong, H. Kozono, H. Sakai and K. Inoue. 1988. Monoclonal antibodies to mouse complement receptor type 1 (CR1). Their use in distribution study showing that mouse erythrocytes and platelets are CR1-negative. *J Immunol.* **140**:3066–3072.
- Klinman, N. R. 1997. The cellular origins of memory in B cells. *Sem Immunol.* **9**:241–247.
- Ledbetter, J. A., and L.A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol Rev.* **47**:63–90.
- Lub, M., Y. van Kooyk and C. G. Figdor. 1996. Competition between lymphocyte function-associated antigen 1 (CD11a/CD18) and Mac-1 (CD11b/CD18) for binding to intercellular adhesion molecule-1 (CD54). *J Leukocyte Biol.* **59**:648–655.
- Mills, J. N., and J. E. Childs. 1998. Ecologic studies of rodent reservoirs: Their relevance for human health. *Emerging Infect Dis.* **4**:529–537.
- Mori, M., A. L. Rothman, I. Kurane, J. M. Montoya, K. B. Nolte, J. E. Norman, D. C. Waite, F. T. Koster and F. A. Ennis. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. 1999. *J Infect Dis.* **179**:295–302.
- O’Conner, C. S., J. P. Hayes and S. C. St. Jeor. 1997. Sin Nombre virus does not impair respiratory function of wild deer mice. *J Mammalogy.* **78**:661–668.
- O’Rourke, A. M., J. Rogers and M. F. Mescher. 1990. Activated CD8 binding to class I protein mediated by the T cell receptor results in signaling. *Nature.* **346**:187–189.
- Perz, J.F. and S.M. Le Blancq. 2001. *Cryptosporidium parvum* infection involving novel genotypes in wildlife from lower New York State. *Appl Environ Microbiol.* **67**:1154–62.

- Ravyn, M.D., C. B. Kodner, S. E. Carter, J. L. Jarnefeld, and R. C. Johnson. 2001. Isolation of the etiologic agent of human granulocytic ehrlichiosis from the white-footed mouse (*Peromyscus leucopus*). *J Clin Microbiol.* 39(1): p. 335–8.
- Richter, D., A. Spielman, and F.R. Matuschka. 1998. Effect of prior exposure to noninfected ticks on susceptibility of mice to Lyme disease spirochetes. *Appl Environ Microbiol.* 64:4596–9.
- Schountz, T., J. P. Kasselmann, F. A. Martinson, L. A. Brown and J. S. Murray. 1996. MHC genotype controls the capacity of ligand density to switch T helper (Th)-1/Th-2 priming *in vivo*. *J Immunol.* 157:3893–3901.
- Springer, T., G. Galfre, D. S. Secher and C. Milstein. 1979. Mac-1: A macrophage differentiation antigen identified by monoclonal antibody. *Eur J Immunol.* 9:301–306.
- Stafford, K.C., R. F. Massung, L. A. Magnarelli, J. W. Ijdo, and J. F. Anderson. 1999. Infection with agents of human granulocytic ehrlichiosis, lyme disease, and babesiosis in wild white-footed mice (*Peromyscus leucopus*) in Connecticut. *J Clin Microbiol.* 37:2887–92.
- Stall, A. M., and S. M. Wells. 1997. FACS analysis of murine B cell populations. In “Weir’s Handbook of Experimental Immunology,” 5th Ed. L. A. Herzenberg, D. M. Weir and C. Blackwell, Eds. Blackwell Science Publishers. pp. 63.1–63.17.
- Stockton, B. M., G. Cheng, N. Manjunath, B. Ardman and U. H. von Andrian. 1998. Negative regulation of T cell Homing by CD43. *Immunity.* 8:373–381.
- Vremec, D., M. Zorbas, R. Scollay, D. J. Saunders, C. F. Ardavin, L. Wu and K. Shortman. 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen: Investigation of the CD8 expression by a subpopulation of dendritic cells. *J Exp Med.* 176:47–58.
- Welch, D.F., K. C. Carroll, E. K. Hofmeister, D. H. Persing, D. A. Robison, A. G. Steigerwalt, and D. J. Brenner. 1999. Isolation of a new subspecies, *Bartonella vinsonii* subsp. arupensis, from a cattle rancher: identity with isolates found in conjunction with *Borrelia burgdorferi* and *Babesia microti* among naturally infected mice. *J Clin Microbiol.* 37:2598–601.
- Yamada, T., B. Hjelle, R. Lanzi, C. Morris, B. Anderson and S. Jenison. 1995. Antibody responses to Four Corners hantavirus infections in the deer mouse (*Peromyscus maniculatus*): Identification of an immunodominant region of the viral nucleocapsid protein. *J Virol.* 69:1939–1943.
- Zaki, S. R., P. W. Greer, L. M. Coffield, C. S. Goldsmith, K. B. Nolte, K. Foucar, R. M. Feddersen, R. E. Zumwalt, G. L. Miller, A. S. Khan, P. E. Rollin, T. G. Ksiazek, S. T. Nichol, B. W. J. Mahy and C. J. Peters. 1995. Hantavirus pulmonary syndrome: Pathogenesis of an emerging infectious disease. *Am. J. Path.* 146:552–579.

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