CD2AP expression in podocytes rescues lethality of CD2AP deficiency
James A. Grunkemeyer¹, Christopher Kwoh², Tobias B. Huber¹ and Andrey S. Shaw¹,*
From the Department of Pathology and Immunology, Box 8118, Department of Medicine, Renal Division, Box 8126, Washington University School of Medicine
660 South Euclid, Saint Louis, MO 63110
Running title: Rescue of CD2AP lethality
Address correspondence to: Andrey S. Shaw, Department of Pathology, Box 8118, Washington University School of Medicine, 660 South Euclid, Saint Louis, MO 63110, 314-362-4614, E-mail: shaw@pathbox.wustl.edu

Mice born without CD2AP develop renal failure and nephrotic syndrome about 4 weeks after birth and die around 6 weeks of age. Although CD2AP is widely expressed, the severity of the renal failure precludes a clear determination of the role of CD2AP in other tissues. Here we generated transgenic mice expressing CD2AP using a podocyte specific promoter. Podocyte specific expression of CD2AP prevented the development of proteinuria demonstrating that the renal failure is solely due to loss of CD2AP in podocytes and not in other renal or in immune cells. CD2AP deficient mice are long lived and appear phenotypically normal. Histological analysis demonstrated testicular abnormalities that were age-related. CIN85, a paralog of CD2AP, is poorly expressed in both the podocyte and the basal seminiferous tubule, suggesting that the loss of CD2AP in specific may be compensated for by CIN85.

CD2 associated protein (CD2AP) was originally cloned as a protein that interacts with the cytoplasmic domain of CD2, a T lymphocyte and NK specific membrane protein (1). Although originally cloned from a lymphocyte library, CD2AP is broadly expressed and found in all tissues except brain. Recently, a gene related to CD2AP was cloned. The protein encoded by this gene, CIN85 is also widely expressed and found in most tissues including brain (2-5). Both molecules contain three amino terminal SH3 domains followed by a proline rich region and a coiled coil domain at the carboxy terminus. CIN85 was originally cloned as an interactor of c-cbl (3) and the p85-alpha subunit of PI-3 kinase (4).

The broad expression of CD2AP in most tissues suggested that CD2AP would have functions in cells outside of the immune system. The phenotype of CD2AP deficient mice, which develop massive proteinuria around 3 weeks of age, develop nephrotic syndrome around 4 weeks of age and ultimately die from renal failure at 6-10 weeks of age, suggested an important role for CD2AP in the kidney (6). In the kidney, CD2AP is expressed mainly by podocytes, proximal tubule and collecting duct (7). Because the first detectable defect was podocyte foot process fusion, we postulated that podocyte dysfunction was the underlying cause of the renal failure even though the major histological feature of the lesion is significant mesangial expansion (6). In addition, whether CD2AP plays important roles in other organs was potentially masked by the severe renal failure in CD2AP deficient animals.

Here we report that transgenic mice expressing CD2AP in podocytes are completely rescued from the renal phenotype of CD2AP deficiency. No proteinuria is detected and the mice appear to have a life-span similar to wild-type animals. This demonstrates that CD2AP’s most important functions are in the kidney. Interestingly, transgenic/CD2AP deficient male animals are infertile and this is supported by histological studies. This suggests that CD2AP also plays an important role in the development of sperm in the testes.

METHODS

Generation of transgenic mice and genotyping
A cDNA encoding myc-tagged CD2AP was ligated into a vector containing the nephrin promoter (kind gift of S. Quaggin) (8). The transgenic construct was purified away from vector backbone, and used for microinjection into mouse pro-nuclei in the Department of Pathology Transgenic Core Facility at Washington University Medical School. Founders were
identified using a PCR-based strategy, and were bred to animals heterozygous for the CD2AP null allele to establish a colony of breeders positive for the transgene and heterozygous at the CD2AP locus. Transgene genotyping PCR was performed with primers (FWD: 5'-GGCACCAAAGAATGTCTCGG-3' and REV; 5'-CATCCTTGGTCTGCTCTCG-3') that amplify the region of the transgene joining the mouse nephrin promoter to the CD2AP cDNA and yield a 750bp product. PCR was performed using standard conditions. Animals selected for experiments were euthanized according to approved protocols.

**Immunoblotting.** Immortalized podocytes were obtained as a generous gift from Peter Mundel (9). Whole kidneys, organs, or isolated podocytes were rinsed in PBS and homogenized in lysis buffer containing 100mM Tris, pH7.5; 150mM NaCl; 1mM EDTA; 1% NP-40; 5mM aprotonin and 5mM leupeptin. Protein concentrations were estimated using the BCA method (Pierce), and equivalent amounts were fractionated by denaturing SDS-PAGE. Protein was then transferred to nitrocellulose membrane and probed with rabbit anti-CD2AP and rabbit anti-Erk2 (Santa Cruz) or rabbit anti-CD2AP or mouse anti-synaptopodin (generous gift of Peter Mundel) followed by HRP-conjugated donkey anti-rabbit secondary antibodies (Jackson Immunoresearch).

**Histology.** After dissection, kidneys and testes were rinsed in PBS and fixed overnight in 4% paraformaldehyde (Sigma) at 4°C. Tissues were then rinsed in PBS and taken to the Histopathology Laboratory at Washington University Medical School for paraffin-embedding, sectioning and staining with eosin and hematoxylin.

**Immunohistochemistry.** After dissection, kidneys and testes were washed with PBS and immediately frozen in tissue molds containing OCT freezing medium (Tissue Tek). Sections were collected onto fibronectin-coated slides, and stained with rabbit anti-CD2AP and rat anti-entactin or goat anti-vimentin (C-20 Santa Cruz) antibodies or rabbit anti-CIN85 and rat anti-entactin antibodies followed by Cy3-conjugated donkey anti-rabbit (Jackson Immunoresearch), FITC-conjugated goat anti-rat (Jackson Immunoresearch), or FITC-conjugated donkey anti-goat (Jackson Immunoresearch) secondary antibodies. Lymphocytes were collected on poly-L-lysine coated slides and stained with FITC conjugated anti-mouse TCR-β monoclonal antibody (BD Biosciences).

**RESULTS**

**Generation of podocyte-specific CD2AP transgenic animals.** The CD2AP cDNA was appended with sequences encoding a myc epitope and ligated into an expressing vector containing the mouse nephrin promoter that directs expression specifically in podocytes (8). Transgenic mice were generated using standard microinjection techniques, and two founder animals (21 and 396) were identified. Both founders were bred to CD2AP heterozygous mice to generate transgenic, CD2AP deficient mice (6). These mice were grossly normal, and have life-spans that are not noticeably different from wild-type animals with survival at least to 15 months.

Immunoblot analysis of whole kidney lysate with a rabbit anti-CD2AP serum confirmed transgene expression in both founder lines (Figure 1, top immunoblot, lanes 3 and 4). Immunoblots of various organs revealed no ectopic expression of the the transgene except in muscle (Figure 1, bottom immunoblot). Transgene expression levels in the kidney appeared to be considerably lower than the levels detected in a CD2AP heterozygous control mouse (Fig. 1, compare lanes 3 and 4 with lane 1) but CD2AP is expressed in tubules and collecting duct in addition to its expression in podocytes (7). Immunofluorescence staining was therefore used to assess the specificity of transgene expression as well as relative levels of CD2AP compared to heterozygous controls. In control CD2AP heterozygous animals, CD2AP antibodies stained glomeruli in the typical, ribbon-like pattern consistent with podocyte expression (Fig. 2, panels A and B). CD2AP expression was also clearly detectable in tubular epithelia. CD2AP knockout animals were used as negative controls (Fig. 2, panels C and D). Staining of transgenic KO mice from lines 21 (Fig. 2, panels E and F) and 396 (panels G and H) demonstrated podocyte specific expression of the CD2AP without detectable transgene expression in tubular epithelium. The staining intensity of CD2AP suggests that podocyte expression levels of the
transgene were comparable to levels observed in heterozygous controls. (Fig. 2, compare panels E and G with panel A). Transgenic expression of CD2AP was not seen in T-cells (Figure 2 panel I-L). Immunoblots of lysates from multiple tissues, including brain, intestine, and liver, were negative for myc-tagged CD2AP (data not shown).

CD2AP is enriched in the podocyte (Fig. 3 panels A-C). However it’s paralog, CIN85, is more strongly expressed in the mesangial cell rather than the podocyte (Fig. 3 panels D-F). The intraglomerular staining of CIN85 did not change in the CD2AP KO (Fig. 3 panels G-I). In immunoblots from lysates of immortalized podocyte cell lines, however, there is weak expression of CIN85 (Fig. 3 panel J).

Phenotypic characterization of transgenic, CD2AP deficient mice. Transgenic CD2AP deficient animals were tested for proteinuria. While CD2AP deficient animals develop proteinuria around 3 weeks of age, transgenic/CD2AP deficient animals did not exhibit proteinuria at any time, followed up to 14 months. In addition, serum chemistries were found to be within normal ranges and comparable to wild type mice as tested out to 10 months, including BUN (KO 22 mg/dL, st dev 2.0, WT 19 mg/dL st dev 3.3), creatinine (KO 0.6 mg/dL st dev 0, WT 0.6 3 mg/dL st dev 0.05), and glucose (103 mg/dL st dev 12). Hematoxylin and eosin staining of formalin fixed kidney sections showed no apparent differences between wild-type and transgenic CD2AP deficient mice (Figure 4). Thus, podocyte specific expression of CD2AP completely ameliorates the renal defects of the CD2AP deficient animals.

Since CD2AP and CIN85 are proposed to be involved in the downregulation of growth factor receptors (10) (11) (12), we analyzed the propensity of transgenic/CD2AP deficient mice to develop tumors. Histological analysis of multiple organs from 10.5-month-old transgenic/CD2AP deficient animals did not reveal any obvious neoplastic processes (data not shown). Furthermore, breeding onto a tumorigenic background (p53 heterozygosity) did not increase the incidence of tumors compared to p53 single-null animals (data not shown).

Since CD2AP has also been implicated in downregulation of TCR signal transduction, we also looked for evidence of autoimmunity (12). There was no histological evidence of any chronic inflammatory process up to 10 months of age. FACS analysis of splenocytes from wild-type and transgene-positive, CD2AP deficient mice demonstrated normal ratios of B cells to T cells without elevation of CD44+ T cells in transgenic, KO mice indicating that the basal state of T lymphocyte activation is normal in these animals.

**Infertility in transgenic/CD2AP deficient mice.** During the course of establishing and expanding both transgenic lines, we found that transgene positive, CD2AP deficient male mice were infertile. Histological analysis on testes from KO and wild-type mice showed decreased numbers of mature spermatids in transgenic, CD2AP KO mice as compared to littermate controls (Figure 5). Although there were some hypcellular seminiferous tubules at 5 weeks of age, the majority of the seminiferous tubules were histologically normal (Fig. 5, panels A and D). The hypcellularity and sperm loss became more apparent and more widespread as the mice aged (Fig. 5, panels B, C, E, F). To confirm that CD2AP is expressed in testes, we stained sections of testes with CD2AP antibodies. We found that CD2AP is specifically expressed in vascular endothelial cells, Leydig cells, and in the basal portion of the seminiferous tubule (Fig. 6 panel A). Co-staining with anti-vimentin antibody, which in the seminiferous tubule is a marker for Sertoli cells but not for germ cells, revealed partial co-localization of CD2AP with Sertoli cells (Fig. 6 panel D-F). In Sertoli cells, CD2AP was localized primarily to the basal-lateral surface. Interestingly, when we stained with antibodies to the CD2AP paralog, CIN85, we found a distinct non-overlapping pattern of expression with staining restricted to areas adjacent to and within the lumens of the seminiferous tubules (Fig. 6 panel C). The staining pattern for CIN85 in the wild type testes was identical in CD2AP KO animals (data not shown). The immunofluorescence findings suggest non-redundant patterns of expression of CD2AP and CIN85 in testis and suggests that CD2AP plays an important role in the supporting cells of the seminiferous tubule and also potentially in immature spermatocytes and spermatogonia.

**DISCUSSION**
Although CD2AP is widely expressed, CD2AP deficient animals die of kidney failure at 6-8 weeks of age (6). While the pathology of the kidney is most notable for mesangial changes with the deposition of significant amounts of mesangial matrix by four weeks of age, the specific expression of CD2AP in podocytes, proximal tubule and collecting duct led us to propose that the absence of CD2AP in podocytes was responsible for the massive renal failure. Because the mice begin developing proteinuria around 3 weeks of age and die around 6 weeks of age (6), it was not clear whether other tissues might be affected by the absence of CD2AP. To answer these questions, we generated a transgenic mouse expressing CD2AP on a podocyte promoter (8). When bred to the CD2AP knockout, we found that the transgene could completely rescue the kidney phenotype of the CD2AP deficient mouse.

We found some ectopic expression of transgenic CD2AP in muscle, however this should impact neither on the kidney phenotype nor on the immune phenotype. Importantly, there has been no report of nephrin expression in skeletal muscle in mouse (13) or human (14). However, despite expression of nephrin in the mouse hindbrain (13), there is no evidence of brain transgene brain expression by immunoblot.

Surprisingly, given the wide expression of CD2AP, the CD2AP deficient, transgenic animals have a normal life span and did not exhibit any obvious abnormalities. This suggests that the place where CD2AP is most important is in the podocyte, or that other related molecules can compensate for CD2AP in other tissues. There is one CD2AP paralog, CIN85, which is also broadly expressed in multiple tissues (15) (2).

We have now identified two areas, the podocyte and the basal seminiferous tubule, where CD2AP is highly expressed while CIN85 is weak or absent. In the seminiferous tubules, antibodies to CD2AP reacted mainly with the seminiferous epithelium, which is composed of Sertoli cells and immature spermatocytes. Antibodies to CIN85, in contrast, only stained the more apical portions of the seminiferous tubule, where mature spermatocytes exist. This suggests that the loss of CD2AP is having a specific effect on the seminiferous epithelium.

These two tissues with a mutually exclusive pattern of expression of CD2AP and CIN85, the glomerulus and testes, also develop the most severe phenotype in the setting of CD2AP deficiency, suggesting that these two proteins may be somewhat redundant and loss of one protein may be compensated for by the presence of the paralog. It will be interesting to evaluate the phenotype of a true CIN85 knockout animal and eventually an animal deficient in both of these proteins.

The Sertoli cells form the blood-testis barrier and provide an environment that supports the development of spermatocytes. Junctional complexes between Sertoli cells prevent the egress of large proteins from the serum into the luminal space of the tubule. This is proposed to function to protect immature spermatocytes from the immune system as they develop as well as to optimize the microenvironment for proper sperm maturation (Reviewed in (16)). The stem cells, the spermatogonia, sit in a position at the base of the epithelium and are outside of the blood-testis barrier. As spermatocytes develop (and begin to express new antigens), the spermatocytes move towards the lumen by traversing the space between the Sertoli cells. To do this, they must break and reform junctional complexes to reach the lumen of the tubule (16). Similar to its postulated role in the kidney, CD2AP may play an important role in the biogenesis or stability of junctional complexes in the testis.

Since the testes look histologically normal in young animals, CD2AP is not required to form a normal testis architecture. We suspect that the absence of CD2AP either leads to Sertoli cell loss or dysfunction with a concomitant breakdown of the blood-testis barrier as the mice age. In this way, the phenotype of the testis is similar to the kidney phenotype of CD2AP deficient mice. The kidneys of CD2AP deficient animals appear histologically normal until around 2-3 weeks of age when proteinuria is first detected (6). We have postulated a role for CD2AP in the function of the slit diaphragm and suggested a break down in this specialized junctional complex as blood begins to flow through the glomerular capillaries at high pressure soon after the mice are born. Breakdown of the junctional complexes could result in both podocyte and Sertoli cell loss as well as access of the luminal contents to self-reactive antibodies.

Because CD2AP deficient T cells show hyper-reactive responses (12), we were interested
to see if CD2AP deficient animals might have an increased incidence of autoimmunity or cancer. Work from several labs has suggested that CD2AP and CIN85 are involved in downregulation of tyrosine kinase based receptors like EGFR, HGFR and the T cell receptor (10-12). This is supported by reported associations of CD2AP and CIN85 with endophilin, synaptojanin and PI3 kinase (17) (4,10,11). Our own work suggests that the defect is not on internalization but rather is related to multi-vesicular body formation (18). Surprisingly, we found no evidence of auto-immunity or cancer in animals examined up to one year of age. This could be related to increased levels of apoptosis (19) or could be due to differences of responses in vivo. Nevertheless, we are currently testing to see whether these transgenic, CD2AP deficient animals are more susceptible to the induction of autoimmune diseases.

Lastly, given the hyperproliferative phenotype of CD2AP deficient T cells and the deposition of immunoglobulin into the basement membranes of CD2AP heterozygous mice, it was possible that the kidney disease was related to immunologic damage rather than primary podocyte dysfunction. The successful rescue of the kidney phenotype of CD2AP deficient animals with podocyte specific expression of CD2AP convincingly demonstrates that the kidney dysfunction is primary to the podocyte and is not mediated by an immunological process. Supporting this, CD2AP deficient RAG deficient mice, which lack T and B cells, develop kidney failure at a rate that is similar to CD2AP deficient mice alone (P. Allen, unpublished observations). Thus, our mice demonstrate that podocyte dysfunction is solely responsible for the kidney failure and death of CD2AP deficient animals. The additional phenotype of infertility and testicular damage suggest potential roles of CD2AP in other specialized junctional complexes.

REFERENCES

1. Dustin, M. L., Olszowy, M. W., Holdorf, A. D., Li, J., Bromley, S., Desai, N., Widder, P., Rosenberger, F., van der Merwe, P. A., Allen, P. M., and Shaw, A. S. (1998) Cell 94(5), 667-677
2. Tibaldi, E. V., and Reinherz, E. L. (2003) Int Immunol 15(3), 313-329
3. Take, H., Watanabe, S., Takeda, K., Yu, Z. X., Iwata, N., and Kajigaya, S. (2000) Biochem Biophys Res Commun 268(2), 321-328
4. Gout, I., Middleton, G., Adu, J., Ninkina, N. N., Drobot, L. B., Filonenko, V., Matsuka, G., Davies, A. M., Waterfield, M., and Buchanan, V. L. (2000) Embo J 19(15), 4015-4025
5. Bogler, O., Furnari, F. B., Kindler-Roehrborn, A., Sykes, V. W., Yung, R., Huang, H. J., and Cavenee, W. K. (2000) Neuro-oncol 2(1), 6-15
6. Shih, N. Y., Li, J., Karpitskii, V., Nguyen, A., Dustin, M. L., Kangawa, O., Miner, J. H., and Shaw, A. S. (1999) Science 286(5438), 312-315
7. Li, C., Ruotsalainen, V., Tryggvason, K., Shaw, A. S., and Miner, J. H. (2000) Am J Physiol Renal Physiol 279(4), F785-792
8. Wong, M. A., Cui, S., and Quaggin, S. E. (2000) Am J Physiol Renal Physiol 279(6), F1027-1032
9. Mundel, P., Reiser, J., Zuniga Mejia Borja, A., Ravenstadt, H., Davidson, G. R., Kriz, W., and Zeller, R. (1997) Exp Cell Res 236(1), 248-258
10. Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W. Y., and Dikic, I. (2002) Nature 416(6877), 183-187.
11. Petrelli, A., Gilestro, G. F., Lanzardo, S., Comoglio, P. M., Migone, N., and Giordano, S. (2002) Nature 416(6877), 187-190.
12. Lee, K. H., Dinner, A. R., Tu, C., Campi, G., Raychaudhuri, S., Varma, R., Sims, T. N., Burack, W. R., Wu, H., Wang, J., Kangawa, O., Markiewicz, M., Allen, P. M., Dustin, M. L., Chakraborty, A. K., and Shaw, A. S. (2003) Science 302(5648), 1218-1222
13. Pataala, H., Sainio, K., Sariola, H., and Tryggvason, K. (2000) J Am Soc Nephrol 11(6), 991-1001
14. Kuusniemi, A. M., Kestila, M., Patrakka, J., Lahdenkari, A. T., Ruotsalainen, V., Holmberg, C., Karikoski, R., Salonen, R., Tryggvason, K., and Jalanko, H. (2004) Pediatr Res 55(5), 774-781
15. Dikic, I. (2002) *FEBS Lett* **529**(1), 110-115
16. Mruk, D. D., and Cheng, C. Y. (2004) *Endocr Rev* **25**(5), 747-806
17. Kowanetz, K., Husnjak, K., Holler, D., Kowanetz, M., Soubeyran, P., Hirsch, D., Schmidt, M. H., Pavelic, K., De Camilli, P., Randazzo, P. A., and Dikic, I. (2004) *Mol Biol Cell* **15**(7), 3155-3166
18. Kim, J. M., Wu, H., Green, G., Winkler, C. A., Kopp, J. B., Miner, J. H., Unanue, E. R., and Shaw, A. S. (2003) *Science* **300**(5623), 1298-1300
19. Huber, T. B., Hartleben, B., Kim, J., Schmidts, M., Schermer, B., Keil, A., Egger, L., Lecha, R. L., Borner, C., Pavenstadt, H., Shaw, A. S., Walz, G., and Benzing, T. (2003) *Mol Cell Biol* **23**(14), 4917-4928
**FIGURE LEGENDS**

**Figure 1. Expression of CD2AP transgene in various tissues.** Two different transgenic founder lines (line 21 and line 396) were bred to CD2AP deficient background. Whole kidney cell lysates (30 mg/lane) from wild-type (lane 1), CD2AP-deficient (lane 2), or from transgenic, CD2AP-deficient mice from line 21 (lane 3) and line 396 (lane 4) were immunoblotted with rabbit polyclonal antibodies against CD2AP. Whole organ lysates from wild type (lanes 1-6) and transgene positive CD2AP deficient mice (lanes 7-12) were immunoblotted with rabbit polyclonal anti-CD2AP antibodies revealing transgene expression in the kidney and in the muscle.

**Figure 2. Transgene is expressed in mouse glomeruli but not tubules or T lymphocytes.** Cortical sections (panels A, C, E and G) or medullary sections (panels B, D, F and H) of kidney were stained with rabbit anti-CD2AP antibody. Panels A and B—in CD2AP haploinsufficient animals, the CD2AP staining pattern indicates expression in glomeruli (podocytes) as well as in tubular epithelial cells. In CD2AP deficient animals, no CD2AP expression is observed. Panels E through H—in both transgenic lines, CD2AP expression is observed in glomeruli but not in tubular regions of the kidney. Peripheral T lymphocytes were collected from wild type (I and J) and transgene-positive CD2AP KO mice (K and L) and identified with anti-mouse TCR-β antibody (J and L). Anti-CD2AP antibody stained wild type T-cells, however there was no expression on transgene-positive CD2AP KO lymphocytes.

**Figure 3. CD2AP and CIN85 are enriched in different glomerular regions.** Glomeruli of wild type (A-F) and CD2AP KO mice (G-I) were stained with either rabbit anti-CD2AP (A) or rabbit anti-CIN85 antibody (D and G) and co-stained with mouse anti-synaptopodin antibody (B, E, and H, and I) which is specific for podocytes and does not stain mesangium. The merge is shown in panels C, F, and I. By immunofluorescence, CD2AP is enriched in the podocyte while CIN85 is enriched in the mesangium. Panel J shows an immunoblot of equally loaded lysates of immortalized wild type (panel J lane 1) and CD2AP knockout podocytes (panel J lane 2). Positive controls (panel J lane 3) include 293 cells transfected with FLAG-tagged CD2AP or mouse epithelial fibroblasts. The immortalized podocytes express low levels of CIN85 with no change in expression levels with CD2AP deficiency.

**Figure 4. Kidneys from transgenic, CD2AP deficient mice are histologically normal.** H + E stains of kidney sections from 11 week old wild-type mouse (panel A), 7 week old CD2AP deficient mouse (panel B), a 16 week old transgenic, CD2AP deficient mouse from line 21 (panel C), and an 11 week old transgenic, CD2AP deficient mouse from line 396 (panel D).

**Figure 5. Decreased mature spermatid production in transgenic, CD2AP deficient mouse seminiferous tubules.** H+E stains of wild-type (A-C) and CD2AP deficient (D-F) testes. Testes were harvested from 5 week old (A and D), 16 week old (B and E) or 10.5 month old (C and F) mice. Note the relative hypocellularity and the reduction in mature (tail bearing) spermatids in the lumen of seminiferous tubules from older CD2AP deficient mice.

**Figure 6. CD2AP and CIN85 are differentially expressed in wild-type mouse testis.** Sections of mouse testis were stained with rabbit anti-CD2AP on wild type testes (A, oblique cut) with background staining on CD2AP knockout testes used as a negative control (B). Anti-CIN85 antibodies (red in C) were co-stained with anti-vimentin, which is used as a marker for Sertoli cells, but not germ cells (green in C). Anti-CD2AP (D) was co-stained with anti-vimentin (E). Merge (F) reveals that CD2AP partially co-localizes with vimentin at the basal portion of the seminiferous tubules with apparent staining of both Sertoli cells and spermatogonia. CIN85, in contrast, localizes within and adjacent to the lumen of the seminiferous tubules (panel C).
Wild Type Transgene CD2AP KO

CD2ap → myc-CD2ap → Erk2

Wild Type

Transgene CD2AP KO

Brain Lung Heart Kidney Muscle Liver

Brain Lung Heart Kidney Muscle Liver
Wild Type Glomeruli

CD2AP Knockout Glomerulus

Expression in murine differentiated podocytes
CD2AP expression in podocytes rescues lethality of CD2AP deficiency
James A. Grunkemeyer, Christopher Kwoh, Tobias B. Huber and Andrey S. Shaw

J. Biol. Chem. published online June 10, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M504004200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts