

An interaction between $\alpha\beta8$ integrin and Band 4.1B via a highly conserved region of the Band 4.1 C-terminal domain

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$\alpha\beta8$ integrin is highly expressed in the vertebrate CNS, and mice lacking the α or $\beta8$ genes develop cerebral hemorrhage due to defective interactions between blood vessels and $\alpha\beta8$ -expressing neural cells. Although $\alpha\beta8$ binds many of the same extracellular matrix protein ligands as other integrins, very little is known about the intracellular signal transduction events used by $\alpha\beta8$ to regulate CNS development. Here we identify Band 4.1B as an intracellular factor that interacts selectively with the $\beta8$ cytoplasmic tail. The association with $\alpha\beta8$ occurs via the band 4.1B C-terminal domain, a region highly conserved among the various Band 4.1 family members. Indeed, we show that $\beta8$ integrin interacts directly with the C-terminal domains of several Band 4.1 proteins and colocalizes with them in cultured astrocytes and in the brain. These data identify a previously uncharacterized interaction between an integrin and Band 4.1 family members and suggest an important functional role for $\alpha\beta8$ -Band 4.1 interactions in the development and maintenance of the CNS.

central nervous system | extracellular matrix | intracellular signal transduction

The formation of the vertebrate CNS requires the precise regulation of cell adhesion and signaling events, including those involving members of the integrin family of proteins (1–3). Integrins are heterodimeric cell surface receptors consisting of α and β subunits that signal upon binding to extracellular matrix ligands (4). In many cases, integrin signaling involves recruitment of intracellular effectors that contain common 4.1/ezrin/radixin/moesin (FERM) domains (4). The Band 4.1 proteins are members of the FERM domain family and include 4.1B, 4.1G, 4.1N, and 4.1R (5, 6). All Band 4.1 family members are highly expressed in the mammalian CNS; however, rather little is known about their functions during CNS development and maintenance. Functional links between integrins and Band 4.1 proteins have not been analyzed.

Here we have identified Band 4.1B as a protein that interacts selectively with the $\alpha\beta8$ integrin, which plays an important role in the development of the CNS (7–9). The association between $\alpha\beta8$ and Band 4.1B is independent of the Band 4.1B FERM domain and rather occurs via the Band 4.1B C-terminal domain (CTD), a region highly conserved among the Band 4.1 family members. Indeed, we show that the CTDs of multiple Band 4.1 proteins interact directly with the $\beta8$ integrin cytoplasmic domain ($\beta8$ cyto) domain and display overlapping expression patterns with $\alpha\beta8$ *in situ*. These data suggest mechanisms for $\alpha\beta8$ -mediated adhesion and signaling and implicate the $\alpha\beta8$ -Band 4.1 interactions as important for proper CNS development.

Materials and Methods

Plasmids and Antibodies. A cDNA encoding the 54-aa human $\beta8$ cyto was inserted in the yeast two-hybrid (Y2H) plasmid pGBKT7 (Clontech) and used to screen a human fetal brain cDNA library (Clontech). The various Band 4.1 CTD constructs were expressed by using the pGADT7 (Clontech) plasmid (see *Supporting Text*, which is published as supporting information on

the PNAS web site). The full length human $\beta8$ integrin cDNA was inserted into the pcDNA3.1A-V5/His vector (Invitrogen). The human full length or truncated Band 4.1B cDNAs were inserted into pcDNA3.1A-myc/His or pCMV-Myc (Clontech) plasmids.

The rabbit anti-4.1B antiserum was generated by using a synthetic peptide sequence of human Band 4.1B, CTTKGISQTNLITTVTPEKKA. The affinity-purified rabbit anti- $\beta8$ and goat anti-4.1B polyclonal antibodies have been described (6, 9). The following antibodies were purchased: anti-Nestin (Developmental Studies Hybridoma Bank, Iowa City, IA), smooth muscle α actin (Sigma), anti- α (Chemicon), anti-paxillin (BD Transduction Laboratories), anti-4.1N (Protein Express, Chiba, Japan), and Alexa-conjugated secondary antibodies (Molecular Probes).

Transfection. The various constructs (Fig. 3) were transiently expressed in COS or 293T cells by using LF2000 lipofectin (Invitrogen). Cells were lysed in 50 mM Tris, pH 7.4/150 mM NaCl/1% Nonidet P-40/2.5 mM EDTA, and a complete protease inhibitor mixture (Roche, Mannheim, Germany). Detergent-soluble lysates were immunoprecipitated with an anti-myc polyclonal antibody (Santa Cruz Biotechnology). Immunoprecipitates were then resolved by SDS/PAGE, and Western blots were probed with anti-V5 or anti-myc monoclonal antibodies (Invitrogen). In some experiments, detergent-insoluble fractions were washed thoroughly with lysis buffer, and the insoluble pellets were resuspended in boiling 2 \times SDS sample buffer containing protease inhibitors. Detergent-soluble and -insoluble fractions were then resolved by SDS/PAGE. Alternatively, transfected cells were surface-labeled with EZ-Link Sulfo-NHS-LC-Biotin (Pierce). Lysates were immunoprecipitated with antiserum, resolved by SDS/PAGE, and immunoblotted with Streptavidin-horseradish peroxidase (Vector Laboratories).

Immunofluorescence. Adult mouse brains were fresh-frozen in Tissue Tek OCT compound (Miles). Midgestation mouse embryos were dissected, fixed with 4% paraformaldehyde for 1 h, cryopreserved in sucrose, and embedded in OCT compound. Alternatively, astrocytes cultured from neonatal mice as described (9) were plated onto coverslips coated with 10 μ g/ml LAP-TGF β 1 (Research Diagnostics, Flanders, NJ) or laminin (Sigma) for 90 min. Cells were then fixed, permeabilized, and incubated with primary and fluorescent secondary antibodies.

Results

Primary Amino Acid Sequence Comparisons of $\beta8$ cyto. Alignment of the cytoplasmic domains of the five β subunits that pair with α

Abbreviations: $\beta8$ cyto, $\beta8$ integrin cytoplasmic domain; CTD, C-terminal domain; FERM, 4.1/ezrin/radixin/moesin; LAP, latency-associated peptide; Y2H, yeast two-hybrid.

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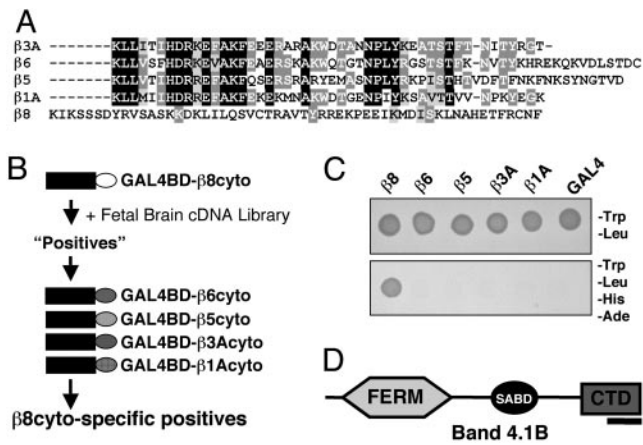


Fig. 1. The β 8 integrin cytoplasmic tail interacts with Band 4.1B. (A) Amino acid sequence alignment of the cytoplasmic domains of β integrin subunits that pair with α v integrin. Identical amino acids are highlighted in black or dark gray and conserved amino acids in light gray. β 8cyto lacks primary amino acid sequence homology with other β subunits and does not contain the NPXY motif or the conserved juxtamembrane sequence. (B) A Y2H screen to identify proteins that interact selectively with β 8cyto. The β 8cyto sequence (white oval) was used to screen a human fetal brain cDNA library. Positive interactors were then screened by using the cytoplasmic tails of other integrins. (C) One example of a β 8cyto-specific interacting positive, as determined by growth on selective media. (D) The β 8cyto-specific positive shown in C encodes Band 4.1B. The portion of Band 4.1B that interacts with β 8cyto is within the C-terminal portion (indicated by black bar).

revealed that the β 8cyto shares little primary amino acid sequence homology with β 1A, β 3A, β 5, and β 6 (Fig. 1A). For example, the juxtamembrane amino acid sequence of β 8 shares no primary amino acid sequence homology with that of other β subunits. This region is involved in a “molecular handshake” with the adjacent integrin α subunit (10), which maintains the integrin extracellular domain in an inactive state (4). The α / β cytoplasmic association is disrupted upon interaction between the β subunit and the intracellular protein talin, leading to “inside-out” integrin activation (11). Talin is recruited to the β cytoplasmic tail via an NPXY tetrapeptide motif found in β 1A, β 2, β 3A, β 5, and β 6. This NPXY sequence is absent from β 8 (Fig. 1A). These results suggest that α v β 8 integrin activation and signal transduction may occur via mechanisms distinct from those used by other integrins.

Band 4.1B Selectively Interacts with β 8 Integrin. We performed a Y2H screen to identify factors that interact selectively with β 8cyto (Fig. 1B). A single clone identified from the Y2H library encoded Band 4.1B (Fig. 1C and D), a member of the Band 4.1 family of proteins (12, 13). Mammalian Band 4.1 proteins contain three distinct functional domains: an N-terminal FERM domain, a central spectrin/actin-binding domain, and a CTD (6, 14, 15). The Band 4.1B cDNA identified from the Y2H screen encoded the last 63 amino acids of the CTD (Figs. 1D and 2A). The CTD amino acid sequences are highly conserved among the mammalian 4.1 family members (15). The last 63 amino acids of the Band 4.1B CTD, encoded exactly by exons 20 and 21, are >75% identical with the other mammalian 4.1 family members (Fig. 2A). This region also contains \approx 60% amino acid sequence homology with a worm Band 4.1 homolog and the fly Band 4.1 protein, coracle (14, 16). We postulated that the CTDs from the other 4.1 family members might also interact with β 8cyto. Indeed, in the Y2H system, we detected an interaction with the isolated CTDs from 4.1G, 4.1N, and 4.1R (Fig. 2C).

Using RNAs isolated from cultured primary astrocytes, embryonic heads, and adult brain and heart, we showed that Band

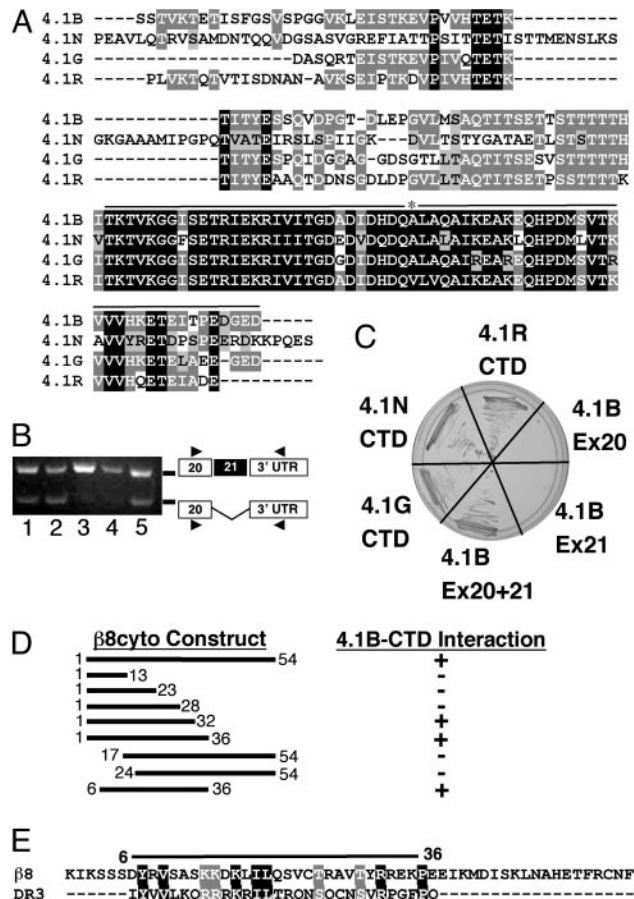


Fig. 2. The β 8 integrin cytoplasmic tail interacts with the region of the Band 4.1B CTD encoded by exons 20 and 21. (A) Alignment of CTD amino acid sequences of human Bands 4.1B, 4.1N, 4.1G, and 4.1R. Identical amino acids are highlighted in black or dark gray and conserved amino acids in light gray. Exons 20 and 21 (black line) encode the portion of Band 4.1B identified by the Y2H screen; asterisk marks boundary between exons 20 and 21. (B) Band 4.1B exon 21 is alternatively spliced. The presence or absence of Band 4.1B exon 21 was determined by using RNA isolated from wild-type mouse astrocytes (lane 1), α v integrin-null astrocytes (lane 2), adult mouse heart (lane 3), adult mouse brain (lane 4), or embryonic day (E)11.5 mouse embryo (lane 5). Exon 21 is included in the adult heart and brain samples but is absent in a portion of the transcripts from astrocytes and E11.5 embryos. (C) The CTDs from Band 4.1G, 4.1N, 4.1R, or 4.1B interact with β 8cyto; however, the Band 4.1B segments encoded by individual exons 20 or 21 do not interact with β 8cyto. (D) Deletion analysis identifies a 31-aa portion of the β 8cyto as necessary for interaction with the Band 4.1B CTD in the Y2H system. (E) The 31-aa sequence of the β 8cyto portion that interacts with the 4.1B CTD shares sequence homology with the second intracellular loop of Dopamine Receptor 3 (DR3), which also binds to multiple 4.1 CTDs.

4.1B exon 21 is differentially spliced in a cell- and tissue-specific manner (Fig. 2B). With this knowledge, we hypothesized that alternative splicing of exon 21 could affect the 4.1B CTD interaction with β 8cyto. Using the Y2H system, no interaction was detected with the Band 4.1B protein fragments encoded by individual exons 20 and 21 (Fig. 2C). These data show that a 63-aa portion of the Band 4.1B CTD, encoded by exons 20 and 21, interacts specifically with the cytoplasmic sequence of β 8 integrin, and that this interaction can be regulated by alternative splicing of Band 4.1B exon 21.

We generated various N- and C-terminal truncations of β 8cyto (Fig. 2D) and tested these constructs in the Y2H system for interactions with the Band 4.1B CTD. We identified a 31-aa portion of the β 8cyto as necessary for interaction with Band 4.1B

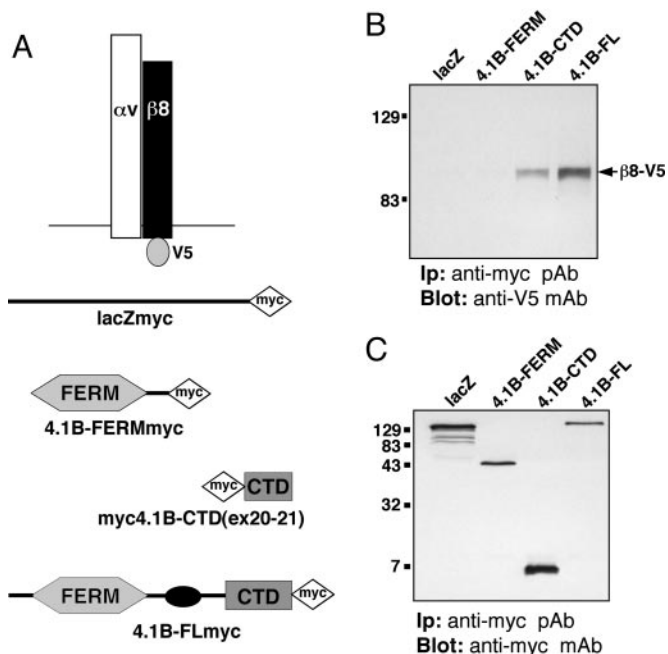


Fig. 3. $\alpha v\beta 8$ integrin and Band 4.1B CTD physically associate in transfected mammalian cells. (A) V5- and myc-tagged constructs used for transfection and coimmunoprecipitation analysis. (B and C) $\beta 8$ -V5 interacts with Band 4.1B via the Band 4.1B CTD encoded by exons 20 and 21. As shown in B, $\alpha v\beta 8$ -V5 coimmunoprecipitates with full length Band 4.1B-myc or with the CTD region encoded by exons 20 and 21 (CTDex20–21-myc). No interaction is detected between $\alpha v\beta 8$ -V5 and the Band 4.1B FERM-myc or the lacZ-myc control. (C) Relative amounts of myc-tagged proteins were immunoprecipitated the various transfected cell lysates.

CTD (Fig. 2D). This region of $\beta 8$ cyto contains significant sequence homology with the second cytoplasmic loop of Dopamine Receptor 3 (DR3-D2L) (Fig. 2E), which also binds to multiple Band 4.1 CTDs (17). Thus, this 31-aa region of $\beta 8$ cyto

contains conserved sequences that may serve as Band 4.1 CTD interaction motifs.

$\alpha v\beta 8$ and the Band 4.1B CTD Interact in Mammalian Cells. We verified the Y2H data by testing for an interaction between Band 4.1B and $\alpha v\beta 8$ integrin using mammalian cells (COS) expressing various epitope-tagged constructs (Fig. 3A). COS cells express detectable levels of endogenous $\alpha v\beta 8$ protein but do not express endogenous Band 4.1B proteins (Fig. 6, which is published as supporting information on the PNAS web site). $\alpha v\beta 8$ coimmunoprecipitated with full length Band 4.1B or a fragment of the Band 4.1B CTD encoded by exons 20 and 21 (Fig. 3B). V5-tagged $\beta 8$ also showed partial colocalization with myc-tagged full length Band 4.1B or the isolated CTD in transiently transfected COS cells (Fig. 7, which is published as supporting information on the PNAS web site). $\beta 8$ integrin also showed partial colocalization with the isolated Band 4.1B FERM domain (Fig. 7); however, coimmunoprecipitation revealed no physical association between $\alpha v\beta 8$ and the Band 4.1B FERM domain or with β -galactosidase (Fig. 3B). We reproducibly detected more detergent-soluble $\alpha v\beta 8$ protein coimmunoprecipitating with full length 4.1B in comparison with the isolated 4.1B CTD fragment (Fig. 3B). This was not due to a lower expression level of the myc-tagged Band 4.1B CTD protein (Fig. 3C) or to significant differences in detergent solubility for $\beta 8$ integrin, the isolated CTD fragment, and full length Band 4.1B (Fig. 6). Previous papers report (17–19) that overexpression of isolated Band 4.1 CTDs reduced the level of associated cell surface receptors. However, the levels of cell surface-expressed $\alpha v\beta 8$ are not altered upon coexpression with Band 4.1B CTD or other Band 4.1B constructs (Fig. 6). Thus, we believe the differential interaction with $\alpha v\beta 8$ can be explained by the fact that the CTD in the context of the entire 4.1B protein is important for a more efficient association with $\alpha v\beta 8$.

$\alpha v\beta 8$ and Band 4.1B Are Expressed in Primary Astrocytes and Colocalize at LAP-TGF $\beta 1$ Adhesion Sites. Astrocytes cultured from neonatal mouse brain expressed several cell surface integrins, including collagen, laminin, and fibronectin receptors (Fig. 4A),

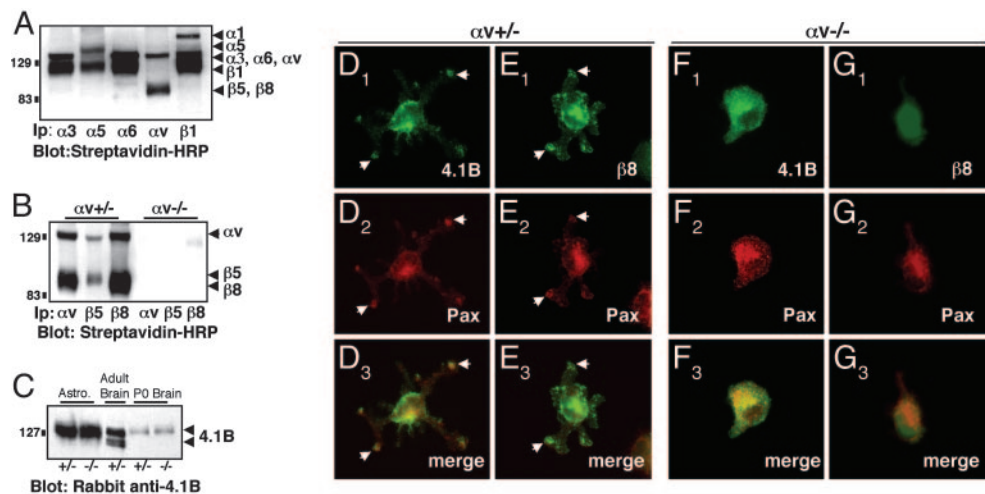


Fig. 4. $\alpha v\beta 8$ integrin and Band 4.1B localize to adhesion sites when astrocytes are plated on the $\alpha v\beta 8$ ligand, LAP-TGF $\beta 1$. (A–C) Primary astrocytes express endogenous $\alpha v\beta 8$ and Band 4.1B. (A) Astrocytes express multiple cell surface integrins as shown by immunoprecipitation with different anti-integrin antibodies. (B) Astrocytes cultured from $\alpha v^{+/+}$ neonates, express $\alpha v\beta 5$ and $\alpha v\beta 8$, whereas $\alpha v\beta 5$ and $\alpha v\beta 8$ are not expressed at the cell surface of $\alpha v^{-/-}$ astrocytes. (C) Protein lysates were prepared from $\alpha v^{+/+}$ or $\alpha v^{-/-}$ astrocytes or from neonatal brains and immunoblotted with anti-4.1B antibody. Similar levels of 4.1B protein are present in $\alpha v^{+/+}$ and $\alpha v^{-/-}$ samples. Adult brain lysates were immunoblotted with anti-4.1B. (D–G) Primary astrocytes cultured from neonatal mice; either $\alpha v^{+/+}$ (D and E) or $\alpha v^{-/-}$ (F and G) were plated on LAP-TGF $\beta 1$. Cells were immunostained with anti-4.1B or $\beta 8$ antibodies in combination with anti-paxillin to visualize adhesion sites. $\alpha v^{+/+}$ astrocytes (D and E) extend multipolar processes when plated on LAP-TGF $\beta 1$. Band 4.1B (D₁) and $\beta 8$ integrin (E₁) colocalize with paxillin (D₂ and E₂) in contact sites (arrows in D_{1–3} and E_{1–3}). $\alpha v^{-/-}$ astrocytes that do adhere to LAP-TGF $\beta 1$ do not spread and form contact sites (F_{1–3} and G_{1–3}).

as well as $\alpha\beta 5$ and $\alpha\beta 8$ integrins (Fig. 4B) (20). In contrast, astrocytes cultured from $\alpha\beta$ -null neonatal mice did not express cell surface $\alpha\beta 5$ or $\alpha\beta 8$ (Fig. 4B). Cultured astrocytes also expressed a 126-kDa Band 4.1B immunoreactive band (Fig. 4C); this molecular weight coincides with the size for Band 4.1B protein described in previous reports (12, 13). The relative amount of Band 4.1B does not change in $\alpha\beta^{-/-}$ astrocytes or neonatal brains (Fig. 4C). A similar immunoreactive band at 126 kDa was detected in the adult mouse brain. Additionally, protein bands were detected at ≈ 112 and 115 kDa (Fig. 4C). Because Band 4.1B mRNA is alternatively spliced, these proteins likely represent adult-specific isoforms of Band 4.1B.

We colocalized Band 4.1B and $\alpha\beta 8$ in cultured astrocytes plated on LAP-TGF β 1. Band 4.1B (Fig. 4D) and $\alpha\beta 8$ (Fig. 4E) protein were present in adhesion sites in some astrocytes, as shown by colocalization with paxillin (Fig. 4D and E). $\alpha\beta^{-/-}$ astrocytes adhered poorly to LAP-TGF β 1, and most cells detached during the immunostaining procedure. $\alpha\beta^{-/-}$ astrocytes that did adhere, however, did not spread and form contact sites (Fig. 4F and G). This was not due to a general adhesion defect, because $\alpha\beta^{-/-}$ astrocytes plated on laminin attached, spread, and formed cell contact sites similar to $\alpha\beta^{+/+}$ astrocytes (Fig. 8, which is published as supporting information on the PNAS web site). Band 4.1B and $\beta 8$ integrin (Fig. 9, which is published as supporting information on the PNAS web site) localization revealed a diffuse pattern on laminin, with no localization to contact sites.

We determined whether expression of full length Band 4.1B or the isolated CTD or FERM domain affected cell adhesion to LAP-TGF β 1. We detected no quantitatively significant differences in the adhesion of transiently transfected COS cells to LAP-TGF β 1 (Fig. 9). Similarly, overexpression of full length Band 4.1B or the isolated CTD fragment in COS cells did not result in significant changes in cell spreading on LAP-TGF β 1 (Fig. 7). In contrast, COS or 293T cells overexpressing the isolated Band 4.1B FERM domain appeared more spread on LAP (Fig. 7), as well as LM (data not shown).

$\alpha\beta 8$ Integrin and Band 4.1 Proteins Colocalize in the Brain. We analyzed the expression patterns of Band 4.1B and $\beta 8$ integrin in the embryonic and adult mouse brain. We previously showed that $\alpha\beta 8$ integrin is expressed on embryonic neuroepithelial and radial glial processes (9). Here, we demonstrate a similar embryonic glial expression pattern for Band 4.1B, as shown by colocalization with nestin (Fig. 5A–C). Additionally, we showed that $\alpha\beta 8$ and Band 4.1B are expressed on some axons in the embryonic hindbrain (Fig. 5D–F).

In the postnatal brain, $\alpha\beta 8$ integrin and Band 4.1B colocalized at the plasma membrane on white-matter axons, particularly in the cerebellum, brainstem, and spinal cord (Fig. 5G–L). We also detected Band 4.1B protein expression on neuronal cell bodies in the cerebellum (Fig. 5J). However, we did not observe a similar expression pattern for $\alpha\beta 8$ (Fig. 5K), suggesting that Band 4.1B may be involved in other neural cell processes.

We also observed a blood vessel-associated expression pattern for $\alpha\beta 8$ protein throughout the adult brain (Fig. 10, which is published as supporting information on the PNAS web site). $\alpha\beta 8$ protein colocalized with smooth muscle α -actin, which is expressed primarily by vascular smooth muscle cells (Fig. 10). Glial endfeet are closely juxtaposed to most blood vessels in the CNS. Indeed, while this manuscript was under review, immunoelectron microscopy localized $\alpha\beta 8$ to perivascular glial endfeet (21). In the adult brain, Band 4.1N, but not Band 4.1B, was also expressed in a perivascular pattern (Fig. 10). These data suggest that $\alpha\beta 8$ adhesion and/or signaling likely involve interactions with distinct Band 4.1 family members and bolster the Y2H results (Fig. 2), which showed that the $\beta 8$ cytoplasmic tail interacts with multiple Band 4.1 protein CTDs.

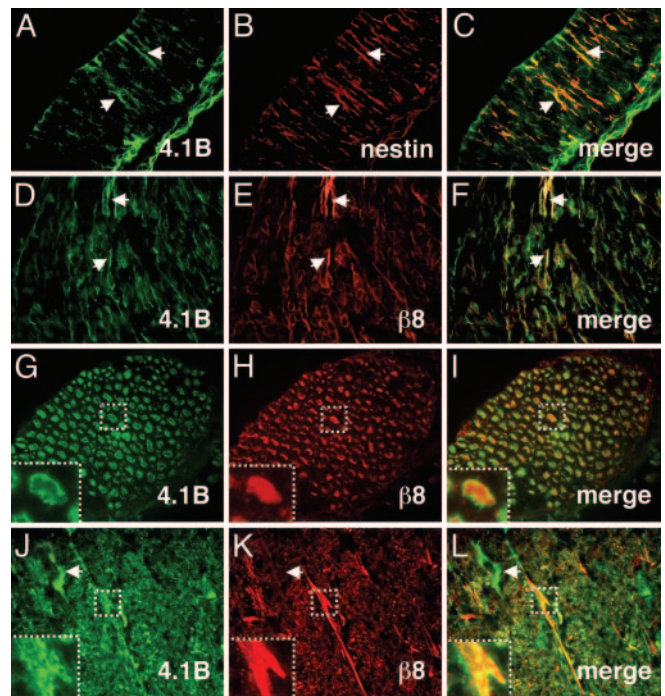


Fig. 5. $\alpha\beta 8$ and Band 4.1B colocalize in neural cells in the embryonic and postnatal central nervous system. (A–C) Band 4.1B is expressed on embryonic neuroepithelial processes (arrows in A), as shown by colocalization with nestin (arrows in B and C). (D–F) Band 4.1B (D) and $\beta 8$ integrin (E) colocalize (F) on neuronal cell bodies and axons in the embryonic hindbrain. Arrows (D–F) show axonal coexpression. (G–I) Band 4.1B (G) and $\beta 8$ (H) are expressed on axons and colocalize (I) at axonal membranes in the adult brainstem. Areas in dashed boxes highlight Band 4.1B and $\beta 8$ colocalization (G–I insets) at higher magnification. (J–L) Band 4.1B (J) and $\beta 8$ integrin (K) colocalize (L) to axons in the adult cerebellum. Band 4.1B is also expressed in neuronal cell bodies (arrows in J and L) where $\beta 8$ integrin is absent (arrow in K). For all colocalization experiments, Band 4.1B was detected with a goat polyclonal antibody (D, G, and J), and $\beta 8$ was detected with a rabbit polyclonal antibody (E, H, and K).

Discussion

A central conclusion of this work is that the highly conserved CTD of Band 4.1B and those of other members of the Band 4.1 family selectively interact with $\alpha\beta 8$ but not with other α -containing integrins. How might Band 4.1 proteins be involved in $\alpha\beta 8$ -mediated function? One possibility is that interaction with Band 4.1 may affect the activation state of $\alpha\beta 8$, thus altering the affinity or avidity for extracellular matrix (ECM) ligands. This function would be similar to the role of talin during inside-out activation of other integrins. Using transient cotransfection analyses, we have not detected significant contributions of full length Band 4.1B or isolated CTD or FERM domains on ECM adhesion. However, it remains to be determined whether Band 4.1B may play subtle roles in regulating the activation state of $\alpha\beta 8$. Second, Band 4.1 interaction with $\alpha\beta 8$ may also be involved in outside-in integrin signaling. Indeed, we show that astrocyte adhesion to LAP-TGF β 1 leads to localization of $\alpha\beta 8$ and Band 4.1B at adhesion sites. Thus, $\alpha\beta 8$ adhesion appears to induce an association with Band 4.1 proteins, which may in turn affect other signaling cascades. Thus, Band 4.1 proteins may serve as a signaling nexus for $\alpha\beta 8$ integrin and probably other cell surface and intracellular proteins.

The functional importance of the Band 4.1 C terminus is highlighted by studies of coracle, the *Drosophila* Band 4.1 protein. Coracle plays an important role in the formation of epithelial septate junctions (22, 23). Many coracle mutant alleles encode truncated proteins that contain the N-terminal FERM

domain but lack large portions of the C terminus, including the CTD. Because the CTDs of coracle and vertebrate Band 4.1 proteins are $\approx 60\%$ identical, it is interesting to speculate that Band 4.1B may be playing a similar role in the regulation of vertebrate cell–cell or cell–extracellular matrix interactions, likely via interactions with $\alpha v\beta 8$ integrin.

Other experimental data support functional links between $\alpha v\beta 8$ integrin and members of the Band 4.1 superfamily. For example, the human Band 4.1B gene product is down-regulated in some lung adenocarcinomas (24). Interestingly, $\alpha v\beta 8$ integrin protein expression is also reduced in some epithelial-derived human lung tumors (25), suggesting that dysregulation of signaling pathways involving Band 4.1B and $\alpha v\beta 8$ may be involved in the progression of lung epithelial tumorigenesis. Last, we have recently generated a mutant mouse model where the αv integrin gene is selectively ablated in the CNS (9). These mice develop neonatal cerebral hemorrhage, leading to spinocerebellar axonal degeneration and a variety of neurological phenotypes. The neurological deficits observed in the αv conditional knockouts

are strikingly similar to symptoms observed in human patients with the hereditary disease cerebral cavernous malformation 1 (CCM1). The causative gene associated with CCM1 onset encodes the FERM domain-containing protein Krit1 (26). It will be interesting to determine whether signaling events involving $\alpha v\beta 8$ integrin, Band 4.1 proteins, and Krit1 are involved in normal cerebrovascular development, and how dysregulation of these events may lead to the pathogenesis of CCM1.

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