Deletion of $\beta 1$ integrins in mice results in inner cell mass failure and peri-implantation lethality

Laurie E. Stephens,^{1,4} Ann E. Sutherland,^{1,5} Irina V. Klimanskaya,¹ Annie Andrieux,^{1,6} Juanito Meneses,² Roger A. Pedersen,^{2,3} and Caroline H. Damsky^{1,3,7}

Departments of ¹Stomatology and ³Anatomy, and the ²Laboratory of Radiobiology, University of California, San Francisco, California 94143-0512 USA

Integrin receptors for extracellular matrix receptors are important effectors of cell adhesion, differentiation, and migration in cultured cells and are believed to be critical effectors of these processes during development. To determine when $\beta 1$ integrins become critical during embryonic development, we generated mutant mice with a targeted disruption of the $\beta 1$ integrin subunit gene. Heterozygous mutant mice were normal. Homozygous loss of $\beta 1$ integrin expression was lethal during early postimplantation development. Homozygous embryos lacking $\beta 1$ integrins formed normal-looking blastocysts and initiated implantation at E4.5. However, the E4.5 $\beta 1$ -null embryos in situ had collapsed blastocoeles, and whereas the trophoblast penetrated the uterine epithelium, extensive invasion of the decidua was not observed. Laminin-positive endoderm cells were detected in the inner cell mass area, but endoderm morphogenesis and migration were defective. By E5.5 $\beta 1$ -null embryos had degenerated extensively. In vitro analysis showed that trophoblast function in $\beta 1$ -null peri-implantation embryos was largely normal, including expression of tissue-specific markers, and outgrowth on fibronectin- and vitronectin-coated, although not on laminin-coated substrates. In contrast, the inner cell mass region of $\beta 1$ -null blastocyst outgrowths, and inner cell masses isolated from $\beta 1$ -null blastocysts, showed highly retarded growth and defective extraembryonic endoderm morphogenesis and migration. These data suggest that $\beta 1$ integrins are required for normal morphogenesis of the inner cell mass and are essential mediators of growth and survival of cells of the inner cell mass. Failure of continued trophoblast development in $\beta 1$ -null embryos after inner cell mass failure could be attributable to either an intrinsic requirement for $\beta 1$ integrins for later stages of trophoblast development, or to the lack of trophic signals from the $\beta 1$ -null inner cell mass.

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Cell-extracellular matrix (ECM) interactions play critical roles in morphogenesis and in the regulation of gene expression (Damsky and Werb 1992; Hynes 1992, 1994; Adams and Watt 1993; Ashkenas et al. 1994; Cross et al. 1994). The integrin family of heterodimeric transmembrane glycoproteins constitutes the major class of receptors mediating cell-ECM interactions. These receptors link the ECM to the internal cytoskeleton and to intracellular signaling pathways. In this role, they mediate cell adhesion and migration, and transduce mechanical and informational signals from the complex extracellular environment, thereby influencing both cytoarchitecture and gene expression.

Integrin heterodimeric receptors for ECM can be classified into two major families: (1) those containing the $\beta 1$ subunit, and, (2) those containing the αV subunit. There is extensive apparent redundancy in the ligand-binding preferences of these integrins. For example, many different integrins bind to fibronectin or to laminin. However, recent studies have shown that unique information is transduced by individual integrin-ECM interactions (e.g., see Huhtala et al. 1995). The most dramatic evidence for this comes from gene targeting of individual α subunits in mice; deletion of $\alpha 5$ or $\alpha 4$, both of which combine with $\beta 1$ to generate fibronectin receptors, results in embryonic lethality with distinct phenotypes (Yang et al. 1993, 1995). Other data comparing the range of ECM ligands recognized by αV and $\beta 1$ integrins suggests that although these families overlap in their abilities to recognize many ECM ligands, including fibronectin, thrombospondin, and tenascin, they also have unique specificities. Thus, intact laminin and collagens

Present addresses: ⁴Chiron Corporation, Emeryville, California 94608-2916 USA; ⁵Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, Virginia 22908 USA; ⁶Department Biologie Moléculaire Structurale (DBMS)/Hématologie CENG 85X 38041, Genoble CEDEX, France.

⁷Corresponding author.

are recognized primarily by $\beta 1$ integrins, whereas vitronectin and fibrinogen are recognized primarily by αV integrins. Finally, although αV - and $\beta 1$ -containing integrins may both function to bind cells to the same ECM ligands (e.g., to fibronectin), they may play distinctive roles in adhesion and signal transduction (Schwartz and Denninghoff 1994).

There is considerable information about the role of ECM receptors in development starting at gastrulation and extending through organogenesis. Until recently, however, very little information has been published about the presence or function of ECM receptors in earlier stages of mammalian development. A significant degree of morphogenesis and lineage determination occurs in these pre- and peri-implantation stages. The process of compaction at the 8- to 16-cell stage is followed by determination of the first differentiated cell type, the trophoblast, and the inner cell mass (ICM). After blastocyst formation, the ICM generates a monolayer of primitive endoderm at its blastocoelic surface, which is the progenitor of parietal endoderm, a migratory cell population, and visceral endoderm, a polarized secretory epithelium. The remaining ICM forms primitive ectoderm (epiblast) that will form ultimately the entire embryo proper. At about the time of initial primitive endoderm formation, the trophoblast differentiates to trophoblast, a poorly understood activation process that results in the conversion of the external surface of the embryo from a quiescent, nonadhesive to a protrusive, adhesive phenotype (Sutherland et al. 1988). The embryo is then capable of attaching to the uterine epithelium in vivo, or to defined substrates in vitro.

Studies on the expression of integrins and ECM ligands have shown that they are regulated both spatially and temporally during the peri-implantation period (Sutherland et al. 1991, 1993; Thorsteinsdottir 1992). In the case of integrins, at least two $\beta 1$ and two αV complexes are expressed from fertilization onward. Additional integrins are turned on at the time of primitive endoderm segregation and development of attachment-competence. By the time implantation is under way [embryonic day 4.5–6 (E4.5–6)], no less than nine integrin heterodimers are expressed in various compartments of the embryo. There are indications that the early lineages present in the peri-implantation embryo (i.e., trophoblast and extraembryonic endoderm) use the αV and $\beta 1$ integrin families differently. For example, antibodies against $\alpha 5$ and $\alpha 6$ block the migration of parietal endoderm cells on fibronectin and laminin, respectively. In contrast, neither antibody interferes with trophoblast outgrowth on these substrates (Sutherland et al. 1993).

To assess directly the functional significance of the regulated expression of integrins in embryonic development, the integrin $\beta 1$ subunit was inactivated by gene targeting in embryonic stem (ES) cells. Heterozygous mice derived from the targeted ES cells were bred to obtain homozygous embryos. These embryos formed normal-looking blastocysts and were able to initiate implantation at E4.5, which suggested that trophoblast formation and initial trophoblast differentiation and

function are not dependent on $\beta 1$ integrins. In contrast, the ICMs of $\beta 1$ -null embryos displayed retarded growth and failed to form normal extraembryonic endoderm in vitro, and degenerated in vivo shortly after implantation. By E5.5, both ICM- and trophoblast-derived portions of the conceptus had degenerated.

Results

Targeted disruption of the $\beta 1$ integrin gene in ES cells

The targeting vector, $\beta 1$ /neo/tk, which spans the first three coding exons of the $\beta 1$ integrin gene (exons 2–4; exon 1 is not translated), was used in gene targeting experiments in F9 cells described previously (Stephens et al. 1993). This vector (Fig. 1A) was introduced into JM-1 ES cells by electroporation and colonies were selected in G418 and FIAU. Of 28 clones tested, 7 clones (25%) were identified by Southern blot analysis to be homologous recombinants, as determined by the presence of a diagnostic 1.2-kb *PvuII* fragment (Fig. 1B). The blots were rehybridized with a probe to the neomycin cassette to ensure that only one insertion event had occurred (data not shown). Three clones with >70% diploid cells (40 chromosomes) were injected into C57BL/6J host blastocysts. Seven chimeric males derived from the three injected clones were bred to Swiss Webster Black (SWB) females, and six of these gave 100% germ-line transmission of the ES cell genotype. The seventh chimera was sterile. Of 118 offspring from male chimera/female SWB crosses, 53% were heterozygote ($\beta 1^{+/-}$) and 47% homozygous wild type ($\beta 1^{+/+}$), as shown by PCR and/or Southern blot analysis of tail DNA samples. Heterozygous animals appeared normal, healthy, and fertile ≤ 8 months after birth.

Homozygous $\beta 1$ -null phenotype results in early postimplantation lethality in utero

Heterozygous mice were then mated and the genotypes of the offspring analyzed by PCR of tail DNA samples (not shown). Of 182 animals tested, 65% were heterozygous, 35% wild type, and 0% homozygous, indicating embryonic lethality resulting from the $\beta 1$ -null mutation (Table 1). To determine the time of death during embryogenesis, embryos from heterozygous crosses were dissected and genotyped (Fig. 2). No homozygous null embryos were detected at E6.5–11.0, although dissection of implantation sites revealed that ~25% of the sites were empty (Table 1). The empty E6.5–8.5 implantation sites themselves appeared normal before dissection, and had decidualized normally. However, after E8.5 the empty sites were smaller than the implantation sites containing embryos, and were less well vascularized. By E10.0 the empty sites showed signs of resorption (not shown). In contrast, only 5% of the implantation sites from heterozygous male and wild-type female crosses (E6.5–10.0) were empty. These data suggest that the $\beta 1$ -null embryos failed before E6.5.

At E6.5, the appropriate numbers of implantation sites

E4.5 embryos were fixed, embedded in either paraffin or diethylene glycol distearate (DGD), sectioned, and stained with antibodies for markers that have distinctive staining patterns in peri-implantation mouse embryos (Sutherland et al. 1991, 1993). About 25% of the E4.5 implantation sites contained embryos that stained poorly with $\beta 1$ antibody and displayed a consistent, ab-

[illegible]

Genotype	Percent			
	live pups (<i>n</i> = 125)	E8.0–11.0 (<i>n</i> = 67)	E6.5–7.5 (<i>n</i> = 68)	blastocysts (<i>n</i> = 195)
+ / +	35	24	28	30
+ / -	65	49	44	47
- / -	0	0	0	23
Empty sites	N.A.	26	28	N.A.

Figure 2. PCR analysis of 7.5-day embryos (*left*) and blastocysts (*right*) resulting from heterozygous crosses. DNA was isolated as described in Materials and methods and primer sets 1 and 2, shown in Fig. 1A, were used to amplify fragments from the wild-type (662 bp) and/or targeted alleles (665 bp) in each sample. For embryos older than 7.5 days, ethidium bromide staining allowed sufficient sensitivity to determine the genotypes. For stages before to E7.5, Southern blot analysis (see Materials and methods) was used to amplify the signal when ethidium bromide staining alone was not adequate.

Figure 3. Hematoxylin/eosin-stained sections of a normal (A) and presumptive $\beta 1$ -null embryo (B) at E5.5. Arrows in A indicate presumptive residual embryonic tissue. Abbreviations in B are (ee) embryonic ectoderm; (ve) visceral endoderm; (epc) ectoplacental cone; (eee) extraembryonic ectoderm. Bar, 50 μ m.

tion to extraembryonic endoderm. However, the laminin-positive cells appeared as a clump, rather than as a monolayer, regardless of the section plane, and no well-defined basement membranes or parietal endoderm cells were evident. In contrast to the case with the normal embryo, clear evidence of trophoblast invasion into the decidua was difficult to detect in the $\beta 1$ -null embryos. The uterine epithelium was penetrated by the trophoblast, as shown by the disruption in the pattern of uterine epithelium nuclei in the DAPI-stained section (Fig. 4E,F). However, only occasional small breaches were observed in the uterine epithelial basement membrane as demonstrated by laminin and syndecan staining (Fig. 4E,H).

$\beta 1$ -null embryos form blastocysts and embryo outgrowths in vitro with normal trophoblast but defective ICM morphogenesis

To examine whether $\beta 1$ -null embryos could form nor-

mal blastocysts, embryos from $\beta 1$ heterozygote crosses were flushed from the oviducts at the two-cell stage (E1.5) and cultured for 5–6 days in vitro until they hatched from the zona pellucida (hatched blastocyst stage). The $\beta 1$ -null embryos were able to divide, compact, form a blastocoele and an inner cell mass, hatch, and expand in synchrony with their wild-type and heterozygous counterparts. In addition, $\beta 1$ wild-type, heterozygous, and homozygous null blastocysts appeared identical by phase contrast microscopy (Fig. 5A,B). Of 195 blastocysts genotyped, 47% (92) were heterozygous, 30% (58) were wild type, and 23% (45) were $\beta 1$ null (Table 1).

To compare the further development and survival of $\beta 1$ -null and $\beta 1$ -containing blastocysts, they were plated individually in drops of medium containing 10% serum and incubated for an additional 5 days (Figs. 6 and 7). At 24 and 48 hr after plating embryos in serum-containing medium, the trophoblast regions of $\beta 1$ nulls were indistinguishable from those of heterozygotes and wild types

Figure 4. Immunofluorescent staining of 1- μ m sections of DGD-embedded normal (A–D) and presumptive $\beta 1$ -null (E–H) E4.5 implantation sites using antibodies against laminin-1 (Ln; A,E), $\beta 1$ integrin subunit (C,G), and syndecan-1 (Synd; H). (B,D,F) Sections stained with DAPI. Note the lack of a blastocoele cavity in the presumptive $\beta 1$ -null embryo. In the null embryo (E–H), a clump of laminin-positive visceral endoderm cells (ve) is present in the ICM (icm) region. Compare with normal (A–D). (pe) Parietal endoderm; (tb) trophoblast; (ue) uterine epithelium. Bar, 50 μ m.

Figure 5. Phase micrographs of a heterozygous (A) and $\beta 1$ -null (B) blastocyst. (icm) Inner cell mass; (te) trophoblast. Bar, 50 μ m.

(Fig. 6A,B). Of 60 embryos plated 19 of 19 wild types, 28 of 28 heterozygotes, and 13 of 13 $\beta 1$ nulls formed trophoblast outgrowths (Fig. 8A). Even after 120 hr of culture in medium with serum, the trophoblast regions of all genotypes appeared equally large and healthy (not shown). Furthermore, all genotypes expressed the trophoblast-specific marker, mouse placental lactogen-1 (mPL-1) by 48 hr (Fig. 6B,D), indicating that initial trophoblast differentiation, as well as morphogenesis, was normal in the $\beta 1$ -null embryos.

In contrast to the trophoblast, the morphogenesis and survival of the ICM in blastocyst outgrowths of $\beta 1$ -null and $\beta 1$ -expressing embryos began to show significant differences by 48 hr. At 24 hr, the ICM regions of the $\beta 1$ -null and $\beta 1$ -containing embryos were indistinguishable (not shown). By 48 hr, the ICM regions of $\beta 1$ -null embryos were smaller and were often disorganized (Fig. 6A,B). In addition, although the ICM regions of $\geq 50\%$ of the wild-type embryos at this stage showed a continuous discrete layer of primitive endoderm (Fig. 6A), and occasionally even migrating parietal endoderm cells, ICM regions of most $\beta 1$ -null embryos did not (Fig. 6B). To quantify differences in ICM size in $\beta 1$ -null, heterozygous, and wild-type embryos, we measured the diameter of the ICM of each outgrowth at 120 hr. Most outgrowths were spherical; however, in cases where the ICM was not spherical, the smallest and largest diameters were measured and the average taken. According to the Tukey-Kramer pair comparison test (Fig. 8B), the difference between the wild type and the $\beta 1$ nulls was statistically significant. Interestingly, the $\beta 1$ heterozygotes were intermediate in size, and did not differ significantly from either of the other two genotypes. In addition to the obvious differences in ICM size, it was also evident that the $\beta 1$ -null ICMs did not undergo further normal morphogenesis. Out of 13 $\beta 1$ -null outgrowths examined at 120 hr, none showed differentiation of a morphologically distinct endodermal rind over the ICM, whereas 9 of 28 heterozygotes (32%) and 10 of 19 (52%) wild types showed a distinct epithelial layer surrounding the remainder of the ICM (Fig. 8A).

Figure 7 shows examples of the most advanced wild-type (Fig. 7A–D) or $\beta 1$ -null (Fig. 7E–H) embryo outgrowths observed at 48 hr of culture in serum-containing medium. Embryos of both genotypes showed vigorous trophoblast outgrowth. The normal embryo had an extensive peripheral monolayer of visceral endoderm and a continuous basement membrane that stained with antibodies against laminin and $\beta 1$ integrins (Fig. 7A–C). In a different normal embryo that produced a parietal endoderm outgrowth, small fusiform cells were present that stained strongly for laminin (Fig. 7D). In contrast, a monolayer of visceral endoderm was not formed in the ICM of the $\beta 1$ -null embryo (Fig. 7E–G). Instead, an irregular cluster of rounded, laminin-positive cells was present (Fig. 7F), indicating that some cells in the $\beta 1$ -null embryo were differentiating to extraembryonic endoderm, but were unable to organize themselves morphologically into a continuous monolayer. There was also no evidence of outmigration of parietal endoderm in $\beta 1$ -null embryo cultures. The irregularly shaped, phase-dense cells evident at the periphery of the ICM in the embryo in Figure 7H, in fact, had the appearance of dying cells.

Figure 6. Phase (A,C) or epifluorescence (B,D) micrographs of 48 hr normal (A,B) and $\beta 1$ -null (C,D) blastocyst outgrowths in serum-containing Eagle Plus medium. Outgrowths were harvested and genotyped by PCR after staining. (B,D) Embryos stained with anti-mouse placental lactogen (mPL): (tb) trophoblast; (ve) visceral endoderm; (icm) inner cell mass; (pe) parietal endoderm. Bars, 50 μ m.

Figure 7. Wild-type (A–D) and $\beta 1$ -null (E–H) 48-hr blastocyst outgrowths. The trophoblast regions of both classes of embryos (tb) show similar outgrowth area. In the ICM region, a visceral endoderm (ve) layer is clearly demarcated in the wild-type embryo (A). The basement membrane (bm) beneath the visceral endoderm stains strongly with anti-Ln and anti- $\beta 1$ integrin (B,C). In a different embryo (D), parietal endoderm cells, which have migrated away from the ICM, stain for laminin. In the $\beta 1$ -null embryo small clusters of cells in the ICM region are stained with anti-laminin, but are unable to form a continuous monolayer. In a different $\beta 1$ -null embryo (H), cells at the periphery of the ICM appear irregular and phase dense, suggesting that they are dying. Bars, 50 μ m.

Endoderm morphogenesis is defective in ICMs isolated from $\beta 1$ -null embryos

A second strategy was used to examine the ability of the ICM region of $\beta 1$ -null embryos to form extraembryonic endoderm *in vitro*. In these experiments, the trophoblast layer of hatched blastocysts was destroyed by immunosurgery (Solter and Knowles 1975), allowing isolation of an intact ICM virtually free of trophoblast. The ICM at this stage consists of two cell types, primitive ectoderm and primitive endoderm. When cultured in serum or presented with a substrate such as fibronectin, ICMs isolated from normal embryos attached and the primitive endoderm migrated and differentiated further to visceral and parietal endoderm (Fig. 9A). Parietal endoderm cells with their rounded, spindle-shaped morphology (Behrendtsen et al. 1995), or patches of visceral endoderm

cells with their epithelial morphology were present in ~100% of the ICMs isolated from $\beta 1$ wild-type and heterozygous blastocysts, whether they were cultured on a fibronectin substrate in serum-free medium or on plastic in serum-containing medium (Figs. 8C and 9A).

Under the same conditions, ICMs isolated from $\beta 1$ -null embryos attached, spread, and differentiated poorly (Figs. 8C and 9B), both in the presence and absence of serum. Particularly striking was the inability of cells to migrate out from the $\beta 1$ -null inner cell masses. In the presence of serum, none of the $\beta 1$ -null ICMs examined (0/7) formed endoderm of either type by morphological criteria. When $\beta 1$ -null ICMs were plated on fibronectin in the absence of serum (Fig. 8C), only 3 of 17 showed morphogenesis of endoderm, and the extent was limited to only a few isolated cells. In those few cases, the ICMs continued to expand in size for several days.

Figure 8. (A) Graphic representation of the percentage of embryos of different genotypes that formed outgrowths of spread trophoblast (solid bars) or formed a discrete visceral endoderm layer as detected morphologically (shaded bars). (B) Graph of ICM size (y-axis units are $\mu\text{m} \times 10^{-1}$) in embryos of different genotype after 120 hr of blastocyst outgrowth in the presence of serum. Data analyzed by a Tukey–Kramer pair comparison test indicate that the average size of ICMs formed by $\beta 1$ -null outgrowths was statistically smaller than that formed by wild-type embryos. Heterozygous ($\beta 1 + / -$) embryos formed ICMs of intermediate size. (C) Graphic representation of the percentage of embryos of different genotypes that formed endoderm outgrowths from isolated ICM cultured on a fibronectin substrate.

Differential role for αV and $\beta 1$ integrins in trophoblast and endoderm morphogenesis

The results described thus far suggest that the loss of $\beta 1$ integrins had a more drastic effect on the ICM-derived than on the trophoblast-derived portions of the peri-implantation conceptus, both in vivo and in vitro. Our data suggest that ICM morphogenesis requires $\beta 1$ integrins, whereas trophoblast interactions with at least some ECM substrates are not $\beta 1$ integrin-dependent, and instead may use αV family integrins. To test this, normal blastocysts were plated on defined substrates in serum-free medium in the presence or absence of antibodies against the $\beta 1$ or αV integrin families (Fig. 10). In the presence of nonimmune antibody, 100% (15 of 15) of the blastocysts formed outgrowths on fibronectin (Fig. 10A). In the presence of anti- $\alpha V\beta 3$ only 36% (9 of 25) of the blastocysts formed outgrowths on fibronectin (Fig. 10C). Anti- $\beta 1$ antibody by itself did not affect the percentage of blastocysts forming outgrowths on fibronectin (Fig. 10B), although the extent of outgrowth was more limited in the anti- $\beta 1$ -containing cultures, suggesting a very lim-

ited role for $\beta 1$ integrins in mediating trophoblast outgrowth on fibronectin. Including anti- $\beta 1$ along with anti- $\alpha V\beta 3$ in the assay did not enhance its blocking effect significantly; 3 of 10 (30%) formed outgrowths on fibronectin when both antibodies were present. Similar results were obtained for vitronectin (not shown). The opposite result was obtained for outgrowth on laminin; 21 of 23 (91%) of the blastocysts formed outgrowths in the presence of $\alpha V\beta 3$, whereas no blastocysts (0/14) formed outgrowths in the presence of anti- $\beta 1$ (Fig. 10, cf. D and F with E).

Experiments comparing trophoblast outgrowth of $\beta 1$ -containing and $\beta 1$ -null blastocysts on fibronectin and laminin reinforced the antibody data. The $\beta 1$ -containing and $\beta 1$ -null embryos grew out equally well on fibronectin (Fig. 11A,B,E) as is evident in the degree of spreading and migration of the large flat trophoblasts. Similar results were obtained on vitronectin substrates (not shown). However, when laminin was provided as a substrate (Fig. 11C,D) only 1 of 14 $\beta 1$ -null embryos showed any outgrowth, whereas 37 of 40 (92.5%) of the heterozygotes and 23 of 23 (100%) of the wild types formed well-spread trophoblast cells on laminin (Fig. 11E). Taken together, these studies not only indicate that early stages of trophoblast function are $\beta 1$ independent but also that trophoblast–laminin interactions are not critical for initial trophoblast function in vivo.

Discussion

We sought to determine when the $\beta 1$ integrin family becomes critical in development by targeting the $\beta 1$ integrin subunit and therefore, eliminating function of the entire $\beta 1$ integrin family. Our results demonstrate clearly that $\beta 1$ integrins are crucial for those cell–ECM interactions required for the development of the embryo proper, and the endoderm-derived extraembryonic lineages during peri-implantation mouse development. Nevertheless, $\beta 1$ -null embryos formed normal-looking blastocysts. Therefore, the cleavage stages, compaction, differentiation of trophoblast, and blastocoel formation can occur independently of $\beta 1$ integrins. These observations are consistent with our previous studies showing that a broad-spectrum antiserum that recognizes both $\beta 1$ and αV integrins does not interfere with preimplantation mouse development to the hatched blastocyst stage, although an anti-E-cadherin antibody did block compaction and blastocyst formation (Richa et al. 1985; see also Larue et al. 1994). $\beta 1$ -Null embryos also initiated implantation in vivo at E4.5, and stimulated a normal decidual response. However, even at this early stage the ICM region of $\beta 1$ -null embryos appeared abnormal and by E5.5, both trophoblast- and ICM-derived regions of $\beta 1$ -null embryos had deteriorated.

Peri-implantation trophoblast function is $\beta 1$ integrin independent

$\beta 1$ -Null blastocysts cultured in serum-containing medium attached to tissue culture plastic in vitro and

Figure 9. Phase micrographs of outgrowths from isolated ICMs of heterozygous (A) or $\beta 1$ -null (B) embryos. The ICMs were isolated by immunosurgery as described in Materials and methods and cultured for 4 days on fibronectin in serum-free medium. Visceral endoderm (ve) cells form a sheet of cells with an epithelial morphology, and parietal endoderm (pe) cells are small and spindle-shaped and do not adhere to one another. Bar, 50 μ m.

formed normal trophoblast outgrowths in which the trophoblast-specific antigen mPL-1 was expressed. Thus, the transition from trophoctoderm to trophoblast occurred, and early trophoblast differentiation markers were induced in the absence of $\beta 1$ integrins. Only when blastocysts were cultured in serum-free medium on defined ECM substrates did differences in trophoblast function in $\beta 1$ -null and normal embryos become apparent. $\beta 1$ -null embryos could grow out on fibronectin-coated or vitronectin-coated substrates, but not on a substrate coated with laminin. This last result indicates that residual maternal $\beta 1$ integrin is not responsible for the otherwise relatively normal trophoblast function in $\beta 1$ -null peri-implantation embryos.

In wild-type embryos, at least some $\beta 1$ and αV integrins are present on the apical surface of attachment-competent blastocysts, and therefore, are in a position to play a role in initial blastocyst-uterine interactions (Sutherland et al. 1993; A. Sutherland, unpubl.). However, the present study shows that an anti- $\alpha V\beta 3$ antiserum, but not anti- $\beta 1$ -specific antibodies, blocked trophoblast outgrowth on fibronectin and vitronectin. In contrast, anti- $\beta 1$, but not anti- $\alpha V\beta 3$, antibodies blocked trophoblast outgrowth on laminin. Taken together with our data that $\beta 1$ -null embryos can initiate implantation, these results suggest that trophoblast interactions with laminin are not critical for either blastocyst formation or early stages of implantation (i.e., attachment and penetration of the uterine epithelium). Because the trophoblast region as well as the ICM of $\beta 1$ -null embryos *in vivo* deteriorated very soon after initial implantation, we could not determine whether trophoblast-laminin interactions might be important for further trophoblast invasion (as suggested by studies of human cytotrophoblasts; Damsky et al. 1993, 1994) or for successful interaction of trophoblasts with maternal decidual cells or endothelium.

Our studies with the $\beta 1$ -null embryos and with integrin-perturbing antibodies suggest that αV integrins and/or other classes of receptors must play the dominant role in whatever cell-ECM interactions are important for early trophoblast function and initial implantation. A role for αV integrins in initial implantation is suggested by several observations (for review, see Cross et al. 1994). First, the basement membrane proteoglycan perlecan, a

Figure 10. Phase micrographs of normal blastocyst outgrowths on fibronectin (Fn) (A–C) or laminin (Ln) (D–F) in the presence of normal IgG (A,D), anti- $\beta 1$ monoclonal IgG (B,E), or anti- $\alpha V\beta 3$ polyclonal IgG (C,F). Anti- $\alpha V\beta 3$ blocks outgrowth on Fn, but not Ln, whereas anti- $\beta 1$ antibody has the opposite effect. White arrowheads mark the extent of outgrowth of the flat trophoblast cell sheet. Bar, 100 μ m.

Figure 11. Phase micrographs of trophoblast outgrowth from heterozygous (A,C) and $\beta 1$ -null (B,D) blastocysts cultured 4 days in serum-free medium on fibronectin (A,B) or laminin (C,D). Bar graphs (E) depict the percent of blastocysts of each genotype that showed outgrowth of trophoblast cells on fibronectin or laminin in serum-free medium. Bar, 50 μ m.

ligand for $\alpha V\beta 3$, is expressed on the attachment competent mouse embryo (Carson et al. 1993) and proteoglycans have been shown to play a role in trophoblast outgrowth in vitro (Farach et al. 1987). In addition, αV integrins are expressed on the apical surface of glandular uterine epithelium in humans. The staining patterns of the $\beta 3$ and $\beta 6$ subunits are particularly intriguing, as they are detected on the apical surface of the uterine epithelium only during the window of receptivity in the human menstrual cycle (Lessey et al. 1992, 1994, 1995; Breuss et al. 1993). Thus, it is possible that αV integrin interaction with proteoglycans or other ligands could promote initial blastocyst-uterine epithelial interactions, or stabilize a primary interaction mediated by other molecules (for a review of other possible mediators of initial trophoblast-uterine interaction, see Cross et al. 1994).

Given the data summarized above, one might predict that αV -null embryos will not be able to implant. Alternatively, $\beta 1$ integrins, although perhaps not the preferred ECM receptors for early trophoblast function, might be able to support these events well enough to allow the embryo to implant even if αV integrins are absent. This possibility is supported by the finding that at the time trophoblast cells in normal embryos start migrating in vitro, they express at least nine different integrin complexes (three αV - and six $\beta 1$ -containing integrin complexes; Sutherland et al. 1993; A. Sutherland, unpubl.). Thus, it is possible that αV -null embryos, like $\beta 1$ -null embryos, will have apparently normal preimplantation development and be able to initiate implantation.

ICM morphogenesis is $\beta 1$ integrin dependent

In contrast to the results for trophoblast, development of the ICM region of the blastocyst in cultured $\beta 1$ -null embryos was highly abnormal. By 48 hr, the ICMs of $\beta 1$ -null blastocyst outgrowths were frequently smaller than normal ICMs. They also appeared more disorganized and did not show the continuous monolayer of endoderm present in >50% of the $\beta 1$ -containing ICM. By 120 hr of outgrowth there was a clear difference in ICM size, and even minimal endoderm morphogenesis was visible in

only a few $\beta 1$ -null ICMs. These results indicate that ICM growth and endoderm morphogenesis are $\beta 1$ integrin dependent.

The extent to which tissue-specific endoderm differentiation is also affected has been more difficult to evaluate, as the ICM region of $\beta 1$ -null embryos begins to fail shortly after the point at which it would normally start turning on biochemical markers of differentiation. Staining of E4.5 implantation sites and 48 hr embryo outgrowths with antibodies against laminin or SSEA-1 suggests that individual cells and small groups of cells can turn on enhanced laminin expression (Fig. 7) and down-regulate SSEA-1 (not shown) properties of primitive endoderm. However, the pattern of laminin staining is not well polarized, and appears to surround the positive cells. This result suggests that biochemical differentiation of the endoderm is initiated in the absence of $\beta 1$ integrins, although morphogenesis, in particular cell migration and epithelium formation, is abnormal. These results relating to morphogenesis and differentiation, are similar to those found in $\beta 1$ -null F9 embryonal carcinoma cells, which model extraembryonic endoderm differentiation in many respects. In those studies, we show that $\beta 1$ -null F9 cells turn on the gene expression programs for both parietal and visceral endoderm, but migration of parietal endoderm and epithelialization of visceral endoderm in that system are both defective (Stephens et al. 1993). Any contribution of $\beta 1$ integrins to the regulation of normal cell growth or survival cannot be assessed in the F9 system, because these cells are transformed. Both our present studies and results in the F9 system are also in agreement with the data obtained by Fassler and Meyer (this issue), in which $\beta 1$ -null ES cells with differentiated phenotypes are found in many different tissues in chimeric embryos, at least in small numbers, although when present above a certain threshold, tissue formation and embryo survival are highly abnormal.

Are $\beta 1$ integrins required for ICM survival?

The early postimplantation death of the embryo and the failure of the $\beta 1$ -null ICMs to continue to increase in

size in vitro suggest that cell replication is retarded or that cell death is accelerated. Either could result from not receiving an appropriate anchorage signal. $\beta 1$ Integrins have been shown to be able to supply an anchorage requirement for progression of anchorage-dependent cells through the G_1/S checkpoint (Guadagno et al. 1993). Furthermore, some cells can be rescued from apoptosis by integrin-ECM interactions, or by anti-integrin antibody-induced clustering of cell-surface integrins (Meredith et al. 1993; Re et al. 1994; for review, see Ruoslahti and Reed 1994; Schwartz and Ingber 1994). Studies to evaluate effects of $\beta 1$ deletion on survival or on cell replication in $\beta 1$ -null peri-implantation embryos are in progress.

Even if ICM deterioration can be explained by the need for a $\beta 1$ anchorage or survival signal, the question remains as to why the trophoblast region also deteriorates so rapidly after initial implantation in vivo, although initial trophoblast differentiation and outgrowth in vitro appear quite normal. The trophoblast region may fail because of a direct impairment of a postimplantation, $\beta 1$ -dependent step in trophoblast morphogenesis or differentiation. Alternatively, trophoblast growth and development could fail indirectly because of the absence of signals from the ICM that are required for its continued propagation. Although their biochemical nature is still unknown, such signals have been shown to be required (Gardner and Papaioannou 1975; Rossant and Tamura-Lis 1981). Studies in which chimeric embryos are formed from tetraploid wild-type embryos and $\beta 1$ -null ES cells (Wood et al. 1993; Guillemot et al. 1994) should determine whether a $\beta 1$ -null ICM is competent to signal continued postimplantation growth and morphogenesis of $\beta 1$ -positive trophoblast.

Fässler and Meyer (this issue) also report that homozygous disruption of $\beta 1$ integrin gene leads to peri-implantation lethality of mouse embryos in vivo. Therefore, it is intriguing that these investigators were able to isolate $\beta 1$ -null ES cells, which occurred as a result of successful targeting of both $\beta 1$ alleles at the time of initial ES cell transfection with the $\beta 1$ -*geo* targeting vector (Fässler et al. 1995). Because these $\beta 1$ -null ES cells are derived ostensibly from the ICM, and ICM growth and/or survival in $\beta 1$ -null embryos are severely affected by the loss of the $\beta 1$ gene, it would not have been surprising if such cells could not be isolated. Although these $\beta 1$ -ES cells are viable when maintained in the undifferentiated state, extensive cell death occurs when they are cultured in the absence of lymphocyte inhibitory factor (LIF) under standard methods for inducing ES embryoid body formation and differentiation (R. Fässler, unpubl.). This reinforces the suggestion that $\beta 1$ integrins contribute important survival signals to the peri-implantation embryo. Nevertheless, when introduced into normal blastocysts, $\beta 1^{-/-}$ ES cells can survive and contribute, although at low levels (2%–20%), to many differentiated tissues in viable chimeric offspring (Fässler and Meyer, this issue). This provides clear evidence that the absence of $\beta 1$ integrins does not interfere with tissue-specific differentiation or survival at the single cell, or small cell group

level as long as the surrounding cells are normal. However, when the contribution of $\beta 1$ -null cells in chimeras exceeds a certain threshold (~25% overall as evaluated at E9.5), the embryos are highly abnormal and do not develop to term. Thus, the lack of $\beta 1$ integrins does affect morphogenesis and survival at the tissue and embryo level when larger groups of $\beta 1$ -null cells try to execute normal differentiation processes.

The $\beta 1$ -null phenotype and other peri-implantation lethal mutations

The $\beta 1$ -null phenotype in vivo has features in common with those of certain other genes that have been targeted in mice, in particular FGF-4. Like $\beta 1$ -null embryos, FGF-4-null embryos initiate implantation but are highly abnormal by E5.5. Trophoblast outgrowth of FGF-4-null embryos in culture is normal, but ICM growth and endoderm differentiation are defective (Feldman et al. 1995). Furthermore, it has been shown that FGF-4 is produced by the ICM in the peri-implantation embryo, and promotes the differentiation and outgrowth of parietal endoderm (Rappolee et al. 1994). The extensive similarity in the $\beta 1$ - and FGF-4-null phenotypes suggests that these molecules might regulate similar processes, perhaps cooperatively. This is reminiscent of experiments showing that the effects of neurotrophins on the survival and differentiation of neurons depends on the presence of an appropriate ECM (in that case, laminin; Kalcheim et al. 1987; Ernsberger et al. 1989). Addition of exogenous FGF-4 to $\beta 1$ -null embryos to compensate either for the absence of appropriate $\beta 1$ -mediated ECM signals or possibly for reduced expression of FGF-4, may allow further development of these embryos. This may permit a more comprehensive assessment of the differentiative capacity of ICM and endoderm in $\beta 1$ -null embryos. The *Evx-1*- (evenskipped-1; Spyropoulos and Cappechi 1994) and *HNF-4*- (hepatocyte nuclear factor-1) null embryos die slightly later, because of the failure of the epiblast to survive. *HNF-4* is expressed in visceral endoderm, and thus must enable the visceral endoderm to signal the epiblast to continue function. Deletion of this gene delays and disrupts gastrulation (Chen et al. 1994). Thus, recently several genes have been shown to affect ICM survival or visceral endoderm function.

In summary, this and the Fässler and Meyer study (this issue) show that $\beta 1$ integrins are critical for the survival and morphogenesis of the ICM in the peri-implantation mouse embryo. However, these studies, as well as those of Stephens et al. (1993), show that $\beta 1$ integrins are not required for tissue specific differentiation in many, although perhaps not all, lineages. Together these studies support the idea that when placed in a normal context, small groups of $\beta 1$ -null cells can survive, differentiate, and participate in normal morphogenesis (e.g., migration), perhaps by passive means (discussed in Fässler and Meyer, this issue). Without cues from surrounding normal tissues, however, larger groups of $\beta 1$ -null cells in chimeric embryos, as well as intact β -null embryos, cannot execute normal morphogenesis, and exhibit poor survival.

Materials and methods

Targeting vector

The β 1 integrin replacement targeting vector pBS β 1/neo/tk (Fig. 1), described previously (Stephens et al. 1993), was used to disrupt the function of one β 1 integrin allele in ES cells. This vector contains 6 kb of the 5' region (spanning exons 2–4, the first three coding exons) of the β 1 gene, and carries both a neomycin (promotorless) and herpes simplex virus–thymidine kinase (HSV–tk) cassette to allow for a positive/negative selection scheme (Mansour et al. 1988).

Culture, transfection, and selection of ES cells

JM-1 ES cells were derived in this laboratory from morulae stage embryos obtained from matings of 129/SvJ mice (agouti chinchilla) as described previously (Robertson 1987; M. Qiu, A. Bulfone, S. Martinez, J.J. Meneses, K. Shinamura, R.A. Pedersen, and R. Rubenstein, in prep.). ES cell lines were maintained on feeder layers of STO fibroblasts or SNL76/7 cells transfected with *neo^r* and LIF (McMahon and Bradley 1990) and cultured in 10% newborn calf serum and 10% heat-inactivated fetal calf serum, supplemented with 5%–10% conditioned medium from CHO–LIF cells (Genetics Institute, Cambridge, MA). JM-1 cells were electroporated with 25 μ g/ml of pBS β 1/neo/tk DNA in PBS at 240 mV and 500 μ F in a BRL electroporator (GIBCO–BRL, Gaithersburg, MD). Transfected cells were selected in 150 μ g/ml of active G418 (GIBCO–BRL) and 0.2 μ M FIAU (gift from Bristol-Myers Squibb) for 7–10 days. Resistant colonies were picked, expanded, and screened by Southern blot analysis as described previously (Stephens et al. 1993) to identify homologous recombinants.

Generation and breeding of chimeras

Chimeras were generated as described by Bradley (1987), by injecting five to eight ES cells into C57BL/6J blastocysts at E3.5. Embryos were maintained in culture for an additional 1–2 hr to allow re-expansion, then 10–15 embryos were reimplanted into each pseudopregnant CD-1 female (Charles River Laboratories, Wilmington, MA) at 2.5 days postcoitum (E2.5). The degree of chimerism was estimated visually according to the contribution of chinchilla/agouti hair to the coat. Male offspring that were ~50%–100% chimeric were bred to SWB females to generate heterozygous mice. Genotyping of the offspring from these matings was carried out by PCR on tail DNA using the PCR conditions described below. Heterozygous males and females were mated to each other to produce homozygous offspring.

Genotyping of animals and embryos by PCR

Offspring were routinely weaned at 3 weeks, and tail DNA was isolated by digesting cut tails in 0.5% SDS, 0.1 M NaCl, 50 mM Tris (pH 8.0), 4 mM EDTA, and 100 μ g/ml of proteinase K overnight at 55°C. Samples were extracted in 0.4 ml of phenol/chloroform/isoamyl alcohol (25:24:1), vortexed, and transferred to Insta-Mini-Prep phase separator tubes (5prime-3prime, Inc, Boulder CO) for centrifugation for 10 min at 4°C in a microcentrifuge. The aqueous phase was precipitated with sodium acetate and ethanol, washed in 70% ethanol, and resuspended in TE (10 mM Tris, 1 mM EDTA) containing 40 μ g/ml of RNase A (Sigma, St. Louis, MO). DNA samples were then analyzed by Southern blot analysis as described previously (Stephens et al. 1993) or by PCR to determine the genotype of the animal. For PCR analysis, DNA of animals resulting from the chimera/SWB matings were subject to amplifications with primer set 2 (Fig.

1A), consisting of a 5' primer residing in the β 1 gene (5'-TCACCTCCTAACCCCTGAGAT-3') and a 3' primer in the *neo^r* gene (5'-GCAATCCATCTTGTTC AATG-3'). This reaction yields a 665-bp fragment that is amplified only from the targeted allele. Reaction conditions were optimized using the Invitrogen optimizer kit (Invitrogen Corp. San Diego, CA) and carried out in buffer J according to the manufacturer's instructions. Cycle times and temperatures were as follows: 35 cycles of 94°C, for 1 min; 60°C for 1 min, 72°C for 1 min.

Offspring of heterozygote crosses (whole embryos, outgrowths, or tail DNAs) were subject to PCR analysis using two sets of primers: one to amplify only the targeted allele, as just described, and the other, primer set 1 (Fig. 1A), to amplify only the wild-type allele by using a 3' primer (5'-CAATCCAGGAAACCAGTTGC-3') from the genomic β 1 DNA that had been deleted in the targeting vector. Primer set 1 yields a 662-bp fragment. Reaction conditions were the same as described above. PCR products from each reaction were run separately on 1.2% agarose gels and, for tail DNAs and for embryos older than E7.5, the products were visualized by ethidium bromide staining of the agarose gels. PCR products from embryos younger than E7.5 were visualized by Southern blot analysis as described previously (Stephens et al. 1993), except the blots were hybridized with a random-primed 330-bp *Eco*R1–*Bst*E1 fragment from the β 1 integrin gene, allowing visualization of both the targeted and wild-type bands.

DNA from embryos older than E9.0 was prepared exactly as described for tail DNA. However, embryos younger than stage E9.0 (as well as cultured outgrowths; see below) were prepared as follows: each embryo (or each outgrowth scraped off the substrate with a drawn glass pipet) was frozen in 20–50 μ l of water (depending on the stage of the embryo), heated to 94°C, treated with 1 μ l of 2 mg/ml proteinase K for 15 min to overnight at 55°C, heated again to 94°C to inactivate the proteinase K, then subjected to amplification using 1–5 μ l of the solubilized tissue per reaction.

Embryo recovery and culture

Superovulated females were caged overnight with males and checked for plugs early the following morning. Fertilization was assumed to occur at midnight and embryos were staged accordingly (noon on day 1 of plugging equals E0.5). Embryos at the two- to four-cell stage were flushed from oviducts of superovulated females at E1.5 and cultured for 4 days under oil in drops of embryo culture medium as described (Sutherland et al. 1988).

Trophoblast outgrowth assays

Blastocyst-stage embryos obtained as described above were cultured for an additional 24 hr in serum-free Eagle's medium supplemented with 2 $\times$ amino acids, bovine serum albumin (BSA; 4 mg/ml), and Mito Plus (Collaborative Research, Bedford, MA, 0.1%; Sutherland et al. 1988, 1993; this medium is referred to in this study as Eagle Plus). Individual blastocysts were then transferred either to drops on substrates coated with 25 μ g/ml of rat fibronectin or mouse laminin-1 (laminin) and cultured in serum-free Eagle Plus medium, or to drops of Eagle Plus medium containing 15% serum on uncoated tissue culture plastic substrates. They were then cultured for an additional 3–5 days, at which time they were photographed and harvested for PCR analysis as described above.

Endoderm outgrowth assays

Blastocysts were flushed from the oviducts of superovulated females at E3.5 and cultured overnight in the serum-free Eagle Plus medium described above for the trophoblast outgrowths.

Hatched blastocysts were then subject to immunosurgery (Solter and Knowles 1975) to isolate the ICM free of trophoblast cells. Briefly, expanded blastocysts (day 4.5) were transferred to a drop of rabbit anti-mouse erythrocyte antibody diluted 1:4 in Eagle Plus medium, and incubated for 10 min at 37°C. The blastocysts were washed through seven drops of Eagle Plus medium with 10% FBS and the dead trophoblast or zona pellucida, or a combination of both, and were removed from the ICMs by passage through small bore pipets. Finally, the ICMs were washed through three more drops of serum-free Eagle Plus medium before being plated under oil in serum-free Eagle Plus medium on a substrate coated with 100 µg/ml of fibronectin or in serum-containing Eagle Plus medium on tissue culture plastic. All cultures were grown for 4–5 days, then photographed and harvested for PCR analysis as described above.

Antibodies

Antibodies for immunofluorescence were obtained from the following sources: anti-β1 cytoplasmic domain antibody from Chemicon; hamster anti-rat monoclonal anti-β1 antibody, Ha2/11, which cross-reacts with mouse cells from Dr. Donna Mendrick (Department of Pathology, Harvard Medical School, Boston, MA); rat monoclonal anti-mouse syndecan-1 (281-2), from Dr. Merton Bernfield (Joint Program in Neonatology, Harvard University Cambridge, MA); anti-mouse placental lactogen-1 (mPL-1) from Dr. Frank Talamantes (University of California at Santa Cruz, CA); anti-β3 from Pharmingen Inc. (San Diego, CA); polyclonal anti-αVβ3 from Dr. Erkki Ruoslahti (La Jolla Cancer Research Foundation, CA) and Dr. James Gailit (Department of Dermatology, State University of New York, Stony Brook); anti-EHS laminin from E-Y Labs (San Mateo, CA); and anti-SSEA-1, Developmental Studies Hybridoma Bank, NICHD (Baltimore, MD). Secondary antibodies against mouse, rat, hamster, and rabbit IgG, conjugated to fluorescein or rhodamine, were obtained from Jackson ImmunoResearch (West Grove, PA).

Histology and immunocytochemistry

Implantation sites at 4.5–5.5 days of gestation (E4.5–E5.5) were fixed either in Carnoy's solution (6:3:1 ethanol/chloroform/glacial acetic acid) or 4% buffered paraformaldehyde for 15–30 min, dehydrated, and embedded in paraffin or in DGD (Sutherland et al. 1991). Embryos destined for paraffin were taken through xylene, whereas those for DGD were dehydrated through butanol. Sections were cut at 5 µm from the paraffin-embedded blocks and at 1 µm from the DGD-embedded samples and mounted on poly-L-lysine-coated slides. Paraffin or DGD was removed with xylene or butanol and the sections were rehydrated.

Blastocysts were plated individually in 10 µl drops (Eagle Plus medium containing 10% serum), under oil, on 60-mm plastic petri dishes for 48 hr. Each blastocyst outgrowth was numbered and photographed so that each could be genotyped unambiguously by PCR after analysis of the immunostaining. After removing the oil, the plates containing the blastocyst outgrowths were rinsed twice with PBS, fixed and permeabilized with methanol (–20°C, 3–5 min), and rinsed twice with PBS.

Sections of implantation sites and plates containing intact blastocyst outgrowths were blocked with PBS containing 1% BSA and 10% normal goat serum before staining with antibodies. Primary antibodies were used at a dilution of 1:100 for 1 hr (except for mPL-I which was used at 1:3000), followed by three washes in 0.5% Tween 20/PBS. Samples were incubated for 1 hr with fluorescein or rhodamine-conjugated secondary antibodies at 1:100, washed in Tween 20/PBS, incubated with DAPI stain (4,6 diamidino-2-phenylindole, 2.5 mM in PBS; Sigma Chemical

Co., St. Louis, MO) for 15 min and then washed again in PBS before mounting in Vectashield (Vector Labs, Burlingame, CA) to prevent quenching. Samples were viewed under a Zeiss Axiopt microscope using phase and epifluorescence optics. After photography, the blastocyst outgrowths were individually scraped off the substrate and genotyped by PCR as described above.

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References

- Adams, J.C. and F.M. Watt. 1993. Regulation of development and differentiation by the extracellular matrix. *Development* **117**: 1183–1198.
- Ashkenas, J., C.H. Damsky, M.J. Bissell, and Z. Werb. 1994. Integrins, signaling and the remodeling of extracellular matrix. In *The integrins* (ed. D. Cheresh and R. Mecham), pp. 79–103. Academic Press, New York.
- Behrendtsen, O., C.M. Alexander, and Z. Werb. 1995. Cooperative interactions between extracellular matrix, integrins and parathyroid hormone-related peptide regulate parietal endoderm differentiation. *Development* (in press).
- Bradley, A. 1987. Production and analysis of chimaeric mice. In *Teratocarcinomas and embryonic stem cells: A practical approach* (ed. E. J. Robertson), pp. 113–151. IRL Press, Oxford, UK.
- Breuss, J.M., N. Gillett, L. Lu, D. Sheppard, and R. Pytela. 1993. Restricted distribution of integrin beta 6 mRNA in primate epithelial tissues. *J. Histochem. Cytochem.* **41**: 1521–1527.
- Carson, D.D., J.P. Tang, and J. Julian. 1993. Heparan sulfate proteoglycan (perlecan) expression by mouse embryos during acquisition of attachment competence. *Dev. Biol.* **155**: 97–106.
- Chen, W.S., K. Manova, D.C. Weinstein, S.A. Duncan, A.S. Plump, V.R. Prezioso, R.F. Bachvarova, and J.E. Darnell Jr. 1994. Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes & Dev.* **8**: 2466–2477.
- Cross, J.C., Z. Werb, and S.J. Fisher. 1994. Implantation and the placenta: Key pieces of the development puzzle. *Science* **266**: 1508–1518.
- Damsky, C.H. and Z. Werb. 1992. Signal transduction by integrin receptors for extracellular matrix: Cooperative processing of extracellular information. *Curr. Opin. Cell. Biol.* **4**: 772–781.
- Damsky, C., A. Sutherland, and S. Fisher. 1993. Extracellular matrix 5: Adhesive interactions in early mammalian embryogenesis, implantation, and placentation. *FASEB J.* **7**: 1320–1329.

- Damsky, C.H., C. Librach, K.-H. Lim, M.L. Fitzgerald, M. Master, M. Janatpour, Y. Zhou, S. Logan, and S.J. Fisher. 1994. Integrin switching regulates normal trophoblast invasion. *Development* **120**: 3657–3666.
- Ernsberger, U., D. Edgar, and H. Rohrer. 1989. The survival of early chick sympathetic neurons in vitro is dependent on a suitable substrate but independent of NGF. *Dev. Biol.* **135**: 250–262.
- Farach, M.C., J.-P. Tang, G.L. Decker, and D.D. Carson. 1987. Heparin/heparan sulfate is involved in attachment and spreading of mouse embryos in vitro. *Dev. Biol.* **123**: 401–410.
- Fassler, R. and M. Meyer. 1995. Consequences of lack of beta-1 integrin gene expression in mice. *Genes & Dev.* (this issue).
- Fassler, R., P.N.J. Schnegelsberg, J. Dausman, T. Shinya, Y. Muragaki, M.T. McCarthy, B.R. Olsen, and R. Jaenisch. 1995. The lack of beta-1 integrin gene in embryonic stem cells affects cell morphology, migration and adhesion but not integration into the inner cell mass of blastocysts. *J. Cell Biol.* **128**: 979–988.
- Feldman, B., W. Poueymirou, V.E. Papaioannou, T.M. DeChiara, and M. Goldfarb. 1995. Requirement of FGF-4 for post implantation mouse development. *Science* **267**: 246–249.
- Gardner, R.L. and V.E. Papaioannou. 1975. Differentiation in the trophoctoderm and inner cell mass. In *The early development of mammals* (ed. M. Balls and A.E. Wild), pp. 107–133. Cambridge University Press, Cambridge, UK.
- Guadagno, T.M., M. Ohtsubo, J.M. Roberts, and R.K. Assoian. 1993. A link between cyclin A expression and adhesion-dependent cell cycle progression. *Science* **262**: 1572–1575.
- Guillemot, F., A. Nagy, A. Auerbach, J. Rossant, and A.L. Joyner. 1994. Essential role of Mash-2 in extraembryonic development. *Nature* **371**: 333–336.
- Huhtala, P., M. Humphries, J. McCarthy, P. Tremble, Z. Werb, and C.H. Damsky. 1995. Cooperative signaling by alpha-5 beta-1 and alpha-4 beta-1 regulates metalloproteinase gene expression on fibronectin. *J. Cell Biol.* **129**: 867–879.
- Hynes, R.O. 1992. Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* **69**: 11–25.
- . 1994. Genetic analyses of cell-matrix interactions in development. *Curr. Opin. in Genet. Dev.* **4**: 569–574.
- Kalcheim, C., Y.-A. Barde, H. Thoenen, and N.M. LeDouarin. 1987. In vivo effect of brain-derived neurotrophic factor on the survival of developing dorsal root ganglion cells. *EMBO J.* **6**: 2871–2873.
- Larue, L., M. Ohsugi, J. Hirchenhain, and R. Kemler. 1994. E-cadherin null mutant embryos fail to form a trophoctoderm epithelium. *Proc. Natl. Acad. Sci.* **91**: 8263–8267.
- Lessey, B.A., L. Damjanovich, C. Coutifaris, A. Castelbaum, S.M. Albelda, and C.A. Buck. 1992. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *J. Clin. Invest.* **90**: 188–195.
- Lessey, B.A., A.J. Castelbaum, C.A. Buck, Y. Lei, C.W. Yowell, and J. Sun. 1994. Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. *Fertil. Steril.* **62**: 497–506.
- Lessey, B.A., A.J. Castelbaum, S.W. Sawin, C.A. Buck, R. Schinlar, W. Bilker, and B.L. Strom. 1995. Aberrant integrin expression in the endometrium of women with endometriosis. *J. Clin. Endocrinol. Metab.* **79**: 643–649.
- Mansour, S.L., K.R. Thomas, and M.R. Capecchi. 1988. Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: A general strategy for targeting mutations to non-selectable genes. *Nature* **336**: 348–352.
- McMahon, A.P. and A. Bradley. 1990. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* **62**: 1073–1085.
- Meredith, J.E., B. Fazeli, and M.A. Schwartz. 1993. The extracellular matrix as a cell survival factor. *Mol. Biol. Cell.* **4**: 953–961.
- Rappolee, D.A., D. Basilico, Y. Patel, and Z. Werb. 1994. Expression and function of FGH-4 in peri-implantation development in mouse embryos. *Development* **120**: 2259–2269.
- Re, F., A. Zanetti, M. Sironi, N. Polentarutti, L. Lanfranccone, E. Dejana, and F. Colotta. 1994. Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.* **127**: 537–546.
- Richa, J., C.H. Damsky, C.A. Buck, and B.B. Knowles. 1985. Cell surface glycoproteins mediate compaction, trophoblast attachment, and endoderm formation during early mouse development. *Dev. Biol.* **108**: 513–521.
- Robertson, E., ed. 1987. Embryo-derived stem cells. In *Teratocarcinomas and embryonic stem cells: A practical approach*, pp. 71–112. IRL Press, Oxford, UK.
- Rossant, J. and W. Tamura-Lis. 1981. Effect of culture conditions on diploid to giant-cell transformation in postimplantation mouse trophoblast. *J. Embryol. Exp. Morphol.* **62**: 217–227.
- Ruoslahti, E. and J.C. Reed. 1994. Anchorage dependence, integrins, and apoptosis. *Cell* **77**: 477–478.
- Schwartz, M.A. and K. Denninghoff. 1994. Alpha v integrins mediate the rise in intracellular calcium in endothelial cells on fibronectin even though they play a minor role in adhesion. *J. Biol. Chem.* **269**: 11133–11137.
- Schwartz, M.A. and D.E. Ingber. 1994. Integrating with integrins. *Mol. Biol. Cell.* **5**: 389–393.
- Solter, D. and B.B. Knowles. 1975. Immunosurgery of mouse blastocyst. *Proc. Natl. Acad. Sci.* **72**: 5099–5102.
- Spyropoulos, D.D. and M.R. Capecchi. 1994. Targeted disruption of the even-skipped gene, *evx1*, causes early postimplantation lethality of the mouse conceptus. *Genes & Dev.* **8**: 1949–1961.
- Stephens, L.E., J.E. Sonne, M.L. Fitzgerald, and C.H. Damsky. 1993. Targeted deletion of beta 1 integrins in F9 embryonal carcinoma cells affects morphological differentiation but not tissue-specific gene expression. *J. Cell Biol.* **123**: 1607–1620.
- Sutherland, A.E., P.G. Calarco, and C.H. Damsky. 1988. Expression and function of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. *J. Cell Biol.* **106**: 1331–1348.
- . 1993. Developmental regulation of integrin expression at the time of implantation in the mouse embryo. *Development* **119**: 1175–1186.
- Sutherland, A.E., R.D. Sanderson, M. Mayes, M. Seibert, P.G. Calarco, M. Bernfield, and C.H. Damsky. 1991. Expression of syndecan, a putative low affinity fibroblast growth factor receptor, in the early mouse embryo. *Development* **113**: 339–351.
- Thorsteinsdottir, S. 1992. Basement membrane and fibronectin matrix are distinct entities in the developing mouse blastocyst. *Anat. Rec.* **232**: 141–149.
- Wood, S.A., W.S. Pascoe, C. Schmidt, R. Kemler, M.J. Evans, and N.D. Allen. 1993. Simple and efficient production of embryonic stem cell-embryo chimeras by coculture. *Proc. Natl. Acad. Sci.* **90**: 4582–4585.
- Yang, J.T., H. Rayburn, and R.O. Hynes. 1993. Embryonic mesodermal defects in alpha-5 integrin-deficient mice. *Development* **119**: 1093–1105.
- . 1995. Cell adhesion events mediated by alpha-4 integrins are essential in placental and cardiac development. *Development* **121**: 549–580.

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L E Stephens, A E Sutherland, I V Klimanskaya, et al.

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