The Appearance of Four Basement Membrane Zone Antigens in Developing Human Fetal Skin


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In order to study the ontogeny of various structural and antigenic components of the basement membrane zone of human skin, we have examined skin specimens from 20 aborted fetuses ranging in gestational ages from 6 to 25 weeks, utilizing light microscopy, transmission electron microscopy, and indirect immunofluorescence with antibodies to bullous pemphigoid antigen, laminin, type IV collagen, and to the antigen defined by KF-1 monoclonal antibody. Both laminin and type IV collagen were detectable as early as 6 weeks of gestational age. In contrast, bullous pemphigoid antigen and the antigen defined by KF-1 monoclonal antibody were not detectable before 10 weeks and 16 weeks, respectively. The appearance of bullous pemphigoid antigen correlated with stratification of the epidermis and the formation of hemidesmosomes and anchoring fibrils at the basement membrane zone. KF-1 antigen is first expressed when the epidermis is further stratified, hemidesmosomes and anchoring fibrils are present in greater numbers and with increased frequency at the dermal-epidermal junction, and hair follicles have begun to bud downward from the basal layer of the epidermis. Our findings suggest an orderly sequence to the appearance of these basement membrane zone components within human skin.

Recent studies have defined the localization of various biochemical components of the basement membrane zone (BMZ) of adult human skin. Bullous pemphigoid antigen (BPA), a protein with a molecular weight of approximately 220,000, is present within the lamina lucida [1-3]. Laminin, a noncollagenous glycoprotein composed of disulfide-linked chains of 220,000 and 440,000 daltons, is also localized to the lamina lucida [4]. Type IV collagen [5] and the antigen defined by KF-1 monoclonal antibody [6], the latter being a noncollagenous component of the BMZ of stratified squamous epithelium, are both found within the lamina densa. Although both the lamina lucida and the lamina densa are well-defined structural regions of the BMZ in all samples of human fetal skin, including those examined from embryos as early as 36 days gestation, little is known about the ontogeny of the various biochemical components of the BMZ or whether the expression of any of them corresponds with the development of structures of the BMZ such as hemidesmosomes or anchoring fibrils.

We have therefore evaluated 20 specimens of human fetal skin of 15 different gestational ages (6-25 weeks) for binding by antibodies directed against BPA, laminin, type IV collagen, and the antigen defined by the KF-1 monoclonal antibody. In addition, we evaluated these fetal skins for binding by antibodies to fibronectin, a basement membrane-associated protein [7]. The data were correlated with the presence of various structural components of the BMZ, the stage of epidermal differentiation, and the stage of development of epidermal appendages which form from the basal epidermal cell layer.

MATERIALS AND METHODS

Skin Specimens

Skin specimens were sampled from the shoulder or thigh of 20 presumed normal, aborted human embryos and fetuses ranging in ages from 6-25 weeks. All material was obtained through the courtesy of the Central Laboratory for Human Embryology at the University of Washington and from the Maternité Universitaire, Liège, Belgium. One sample from each abortus was embedded directly in O.C.T. compound and snap-frozen in liquid nitrogen, and stored at -70°C until ready for sectioning. A sample from the same region of each embryo/fetus was immersed in fixative and processed further for light and electron microscopy. Three-millimeter punch biopsies from each of 3 healthy adult volunteers and 2 human neonatal foreskins were frozen in O.C.T. to serve as normal controls for the immunofluorescence experiments.

Immunofluorescence Studies

Six micron-thick cryostat sections of each of the specimens of fetal skin, adult human skin, and neonatal foreskin were stained by indirect immunofluorescence using various dilutions of bullous pemphigoid serum, the murine monoclonal antibody KF-1, and sheep serum containing polyclonal antibodies directed against laminin and type IV collagen [4,5] or by direct immunofluorescence using a fluorescein-conjugated IgG fraction of goat antihuman fibronectin (Cappel Labs., West Chester, Pennsylvania). In some experiments, affinity-purified antibodies to laminin and type IV collagen were also used. All dilutions were made in phosphate-buffered saline (PBS), pH 7.4. Bullous pemphigoid serum was used at 1:10 and 1:20 dilutions, antilaminin and anti-type IV collagen at 1:40-1:1280 dilutions, and the antifibronectin at 1:10 dilution. When affinity-purified antilaminin and anti-type IV collagen were used, concentrations of 8-16 μg/ml (anti-type IV) and 50-100 μg/ml (antilaminin) were employed. Normal human serum, normal sheep serum, and normal mouse ascitic fluid (the latter produced in BALB/c mice by intraperitoneal injection of pristane and nonimmunoglobulin-producing SP 2/0-Ag 14 murine myeloma cells) were used as negative controls. After incubation of antibody or serum with tissue at room temperature for 30 min in a moist chamber, the tissues were rinsed in PBS. Dependent upon the species in which the first-step antibody was raised, fluoresceinconjugated goat antihuman IgG (1:40 dilution), rabbit antihorse IgG (1:20 dilution), or rabbit antirabbit IgG (1:40 dilution) was used as the second step. Following 30-min incubation and subsequent rinsing in PBS, each tissue section was covered with 50% glycerin in PBS and examined by fluorescence microscopy.

Control tissue (either adult human skin or neonatal foreskin) was always tested simultaneously with fetal skin. Each specimen was evaluated for the presence, intensity and distribution of specific fluorescent staining.

Light and Electron Microscopy

Samples of tissue were fixed for 2–4 h in half-strength Karnovsky’s fixative [8] in the cold, washed in 0.1 M sodium cacodylate buffer, and postfixed in 1% OsO₄ in distilled water for an additional hour. Potas-

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Abbreviations:
BMZ: basement membrane zone
BPA: bullous pemphigoid antigen
EB: epidermolysis bullosa
PBS: phosphate-buffered saline
sium ferrocyanide (1-5%) in 0.1 M cacodylate, pH 7.4 was added to the second fixative to enhance membrane contrast and stain intracellular glycogen [9]. Samples then were dehydrated through a graded series of alcohols and embedded in Epon by conventional methods [10]. Samples were flat-embedded and oriented so that a full-thickness section through the skin could be taken for light and transmission electron microscopy (TEM). One-micron sections were stained with Richardson's stain [11]. Thin sections were stained with uranyl acetate and Reynolds's lead citrate [12] and examined with a Philips 201 transmission electron microscope. The TEM data were used to document the development of structural components of the BMZ. The data are summarized here in chart form and are from Smith, Riddle, and Holbrook (unpublished data).

**RESULTS**

Table I summarizes the data on antibody binding to each of the 4 BMZ antigens and fibronectin in fetal skin. In the earliest fetal skin examined, aged 6 weeks and thereafter, both laminin and type IV collagen were detectable along the BMZ of the dermal-epidermal junction and dermal vasculature (Figs 1-3). Fibronectin was also detectable in all skin specimens. In some, it was seen throughout the dermis while in others it was observed as a somewhat thickened band with accentuation at the dermal-epidermal junction and within dermal vessels. As antifibronectin binds mainly to an area below the lamina densa [13] it should not be considered a true BMZ constituent.

Bullous pemphigoid antigen was first detectable focally along the BMZ of skin at approximately 10-11 weeks gestational age (Figs 1, 2). Antibody binding was not regularly uniform until approximately the 17th gestational week. At the age when BPA first appears, the epidermis stratifies from a two- to a three-layered epithelium and the first hemidesmosomes and anchoring fibrils of the BMZ structural complex are already observed.

The antigen defined by KF-1 monoclonal antibody was not detectable in fetal skin until approximately the 16th week, at which time it could be seen focally along the dermal-epidermal junction. This antigen was detectable in all older fetal specimens examined (Fig 3) although homogeneous linear staining of the BMZ was not seen until on or after week 21.

At the older fetal ages, the epidermis was further stratified by the addition of 1-2 more intermediate cells. Hemidesmosomes and anchoring fibrils were increased in number and distributed with greater frequency along the BMZ.

Hair germ were seen in tissues studied at 12 weeks gestation and by 14 weeks, hair pegs and bulbous hair pegs projected deeply into the dermis. Keratinization of the follicle inner sheaths and hair were evident in 16-week specimens.

**DISCUSSION**

With the exception of BPA, little is known of the biochemical composition of the BMZ of human embryonic and fetal skin at progressive stages of development. Since different structures of the BMZ develop progressively with gestation [14], sampling of this tissue at various time intervals during development provides an excellent opportunity to correlate the appearance and distribution of BMZ antigens with specific structural features of the tissue. In the present study, we evaluated the expression of 4 BMZ antigens in the context of BMZ morphology, epidermal stratification, and the formation and differentiation of an epidermal appendage, the hair follicle.

Muller et al [15] examined 29 human fetuses by indirect immunofluorescence and were able to detect BPA focally as early as 12 weeks. Linear distribution of this antigen was noted only after 22 weeks of gestation. In another study of 70 fetal skin specimens [16], ranging in gestational age from 9-38 weeks, BPA was undetectable by indirect immunofluorescence in any specimen younger than 16 weeks and tended to be linear in older specimens. Our results agree that a focal staining pattern is characteristic of the antigen in younger-aged fetal skin, but we have detected BPA within the BMZ as early as 10 weeks. Uniform linear staining was not regularly seen until 17 weeks gestational age.

Although laminin and type IV collagen have been evaluated in skin, kidney, and ovary of fetal mice [17-21], there are no previous studies regarding the appearance of these antigens within human fetal skin. We have been able to detect both laminin and type IV collagen in all specimens of human fetal skin examined, the earliest being 6 weeks old. This is consistent with the finding by electron microscopy of an intact lamina lucida and lamina densa in all samples of embryonic and fetal skin examined, as early as weeks 5-6 of gestational life [14,22]. In the youngest specimens (6 weeks) examined in the present study, BMZ staining was more prominent along the dermal-epidermal junction than adjacent dermal blood vessels. In addition, marked focal thickening of the dermal-epidermal junction was noted in several of the specimens aged 57-74 days. This may correspond with the early formation and matrix deposition in human fetal skin [23] and immunoelectron microscopic studies using type III and type IV antibodies both show staining of this region.

The monoclonal antibody, KF-1, defines a noncollagenous constituent of the BMZ of adult human skin [6]. By immunoelectron microscopy, this antigen has been shown to be present within the lamina densa, an area previously known to contain primarily type IV collagen. Recently, clinically uninvolved skin from patients with dystrophic epidermolysis bullosa (EB) has been shown to be deficient in the antigen defined by KF-1 [24]. The skin of patients with the usually more clinically severe form of this disease, recessively inherited dystrophic EB, lack or have markedly diminished amounts of this antigen while patients with the usually less severe form, dominantly

### Table 1. Expression of BMZ antigens in human fetal skin correlated with structural properties of the BMZ and epidermal differentiation

<table>
<thead>
<tr>
<th>Age (trimester)</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
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<tbody>
<tr>
<td>Weeks</td>
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<tr>
<td>6-7</td>
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<td></td>
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</tr>
<tr>
<td>8-9</td>
<td>n = 5</td>
<td></td>
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<tr>
<td>10-11</td>
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<tr>
<td>12-14</td>
<td>n = 2</td>
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<tr>
<td>16-17</td>
<td>n = 1</td>
<td></td>
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<tr>
<td>21</td>
<td>n = 1</td>
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</tr>
<tr>
<td>25</td>
<td>n = 1</td>
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**Notes:**
- The antigen was not detected in 3 of the youngest (all 67-day) specimens in the group and was focal in 2 older specimens (70 and 74 days).
- The BPA antigen was not detected in 1 of 3 specimens.
- Distribution of the antigen focal in 1 of 2 specimens.
- Distribution of the antigen focal in 2 of 3 specimens.
- Distribution of the antigen focal in 3 of 5 specimens.
- Distribution of the antigen focal in 4 of 5 specimens.

- Distribution of the antigen focal in 5 of 5 specimens.

**BMZ structures**

<table>
<thead>
<tr>
<th>BMZ structures</th>
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<tbody>
<tr>
<td>Lamina lucida and anchoring filaments</td>
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<td></td>
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</tr>
<tr>
<td>Lamina densa</td>
<td>+</td>
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<tr>
<td>Hemidesmosomes</td>
<td>-</td>
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<tr>
<td>Anchoring fibrils</td>
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**Antibody Binding:**

- BPA
- Laminin
- Type IV collagen
- KF-1
- Fibronectin
inherited dystrophic EB, have reduced amounts of this antigen. As this antigen can be detected within normal fetal skin as early as the 16th week of gestation, it may be possible to use this antibody as another means of in utero diagnosis of this particular form of EB [25]. Moreover, further study of the appearance and distribution of KF-1 in the fetal skin lamina densa at ages corresponding to the appearance and denser distribution of anchoring fibrils may allow us to determine whether KF-1 is found at sites (within the lamina densa) related to the origin of anchoring fibrils.

On the basis of our findings we conclude that these 4 BMZ antigens are not all present at the same stage of embryologic development of human skin but rather appear in a well-defined sequence that corresponds with particular stages in the formation of BMZ structure and epidermal differentiation. Lamminin and type IV collagen are detectable as early as 6 weeks of..."
gestation, whereas BPA and the antigen defined by KF-1 antibody appear sequentially at 10 and 16 weeks, respectively. This pattern is different from that observed in experimentally induced wounds [36]; in the latter situation, BPA could be detected throughout the BMZ of the entire healing wound while laminin and type IV collagen were undetectable from the undersurface of the more distal migrating epidermis. The variable distribution of BMZ components has been interpreted in the wound as evidence for the importance of BPA in the early interaction between epidermal cells and adhesion to the wound bed. The lack of detectable BPA and the antigen defined by KF-1 during early embryonic development of human skin suggests that neither of these is necessary for the maintenance of dermal-epidermal adhesion in early fetal life before any significant stratification of the epidermis. Although wound healing and ontogenetic development are usually considered as similar events in which it is theoretically possible to study the formation and differentiation of structural, biochemical, and functional properties, the two situations are quite different in terms of the potential epithelial-matrix interactions. In one situation, a fibrin wound bed interacts with a migrating epithelium while in the other, a glycosaminoglycan-rich, collagenous matrix serves as the epithelial substrate. It is probable that the induction of the expression of structural properties of the BMZ is highly dependent on specific signals.

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REFERENCES