Genetically Induced Abnormalities of Epidermal Differentiation and Ultrastructure in Ichthyoses and Epidermolyses: Pathogenesis, Heterogeneity, Fetal Manifestation, and Prenatal Diagnosis

INGRUN ANTON-LAMPRECHT, Sc.D.

Institut fuer Ultrastrukturforschung der Haut, Hautklinik der Ruprecht-Karls-Universität, Heidelberg, Federal Republic of Germany

Comparative ultrastructural investigations on the pathomorphogenesis of inherited ichthyoses and epidermolyses have shown that such heterogeneous skin disorders may serve as model systems for genetic interactions with developmental processes, such as keratinization, or functional systems, such as dermal-epidermal junctional integrity. Most interesting from the morphologic point of view are dominantly inherited skin disorders in the ichthyosis and epidermolysis bullosa groups in which primary structural defects of structural proteins have been demonstrated that seem to be under the direct control of the mutant gene. Such structural abnormalities concern keratohyalin in autosomal-dominant ichthyosis vulgaris, the tonofilament system in hystrix-like ichthyoses, and the anchoring fibrils in dominant dystrophic epidermolyses. Taking bullous congenital ichthyosiform erythroderma (epidermolytic hyperkeratosis) as a central example, we discuss the stability of such structural defects, the heterogeneity in the ultrastructural abnormalities of clinically closely similar entities (ichthyosis hystrix Curth-Macklin, congenital reticulate ichthyosiform erythroderma), and, in the latter keratinization disorder, the presence of an unusual filament system of unknown biochemical composition in the abnormal keratinocytes. Expression of mutant genes during fetal life and fetal manifestation of such abnormalities are a precondition for the prenatal diagnosis of genetic skin disorders (bullous ichthyosiform erythroderma, epidermolysis bullosa dystrophica Hallopeau-Siemens, Herlitz syndrome). Finally, problems related to the differentiation of mutant keratinocytes and of amniotic fluid cells of fetuses at risk of genetic skin disorders under the in vitro conditions of primary cell cultures are briefly discussed.
Biological systems of development or function, such as epidermal keratinization or dermal-epidermal junctional integrity, are controlled by large series of genes that are coding for enzyme proteins, regulatory processes, or structural proteins involved in the respective systems. Mutations that affect these genes therefore induce a large variety of disturbances in the biosynthetic and developmental pathways. In spite of this variety, they finally result in rather uniform replication patterns of the skin, such as hyperkeratoses or blisters, that rarely reflect much of the differences in the underlying pathomechanisms.

Looking at genetic skin disorders more closely, for example, with morphologic or biochemical means, it is no longer astonishing that they turn out to be highly heterogeneous. We are not simply dealing with only one or two clinically similar diseases, but with large groups of closely similar entities sharing the same reaction mechanisms but caused by many different pathogenetic disturbances. Ichthyoses and epidermolyses are examples of such heterogeneous syndromes with more than 15 and 16 genetic types that may be distinguished today.

People looking this way at genetic disorders are often termed splitters; the clinician feels inclined to "jump back over the fence" to the lumpers with his few numbers of entities that are so easy to handle. As long as no causal therapy is known for a genetic skin disorder, this may be sufficient to guarantee an optimal symptomatic treatment. As soon as fundamental problems of genetics and pathogenetics are concerned, as soon as patients and their families ask for the proper mode of inheritance, genetic risk of recurrence, and prognosis, a correct diagnosis of the respective genetic entity is necessary.

In the initial phase of our basic research on ichthyoses and epidermolyses, the main purpose was a better understanding of the underlying pathomechanisms and, in consequence, a better classification of doubtful cases. The large amount of heterogeneity in both groups was one of the unexpected results. It became further apparent that the constant and variable abnormalities found in many of the disorders investigated may be taken as a diagnostic criterion of considerable reliability [1]. Even more important, however, is the fact that such heterogeneous groups of disorders may serve as model systems of genetic interactions and regulatory influences in developmental processes [2]. In the present paper some selected problems related to these genetic interactions shall be discussed.

**STRUCTURAL ABNORMALITIES OF STUCTURAL PROTEINS IN DOMINANT SKIN DISORDERS**

Most interesting findings from the morphologic point of view are related to some dominantly inherited skin disorders. Owing to a genetic concept on the possible molecular basis of dominance or recessivity of mutant genes [3,4], diseases with a recessive mode of inheritance most often are caused by mutations of genes coding for enzyme proteins, whereas dominantly inherited disorders are more likely to concern regulatory processes or structural proteins. Enzyme proteins are normally produced in such an amount that even half of the normal enzyme (in heterozygous carriers of an enzyme deficiency) is still sufficient for the normal metabolic function. A mutation therefore manifests itself only in the homozygous state (recessivity). However, mutations affecting structural proteins, such as keratin or collagen polypeptides, are likely to alter the biophysical properties of the protein so essentially that disturbances are already apparent in the heterozygous state (dominance).

In both ichthyoses and epidermolyses, examples may be given that seem to confirm this concept [5]. Enzyme deficiencies have been demonstrated in several recessive disorders of the skin; X-linked recessive ichthyosis (steroid sulfatase deficiency), Refsum's syndrome (defective alpha oxidation), and probably also in Jögner-Larsson's syndrome (delta-6-desaturase deficiency, [6]). In the epidermolyses, a defective collagenase produced in excessive amounts [7,8] is the basis of blister formation via collagenolysis in the Hallopeau-Siemens type.

Structural proteins of the keratinization process, i.e., tonofilaments (alpha-keratin precursors) and keratohyalin components, are ultrastructurally abnormal in some dominant types of ichthyoses. A hypoplasia of the anchoring fibrils composed of extracellular structural proteins of the dermal-epidermal junction seems to be the primary defect in the dominant dystrophic epidermolyses [5,9].

*Autosomal-dominant ichthyosis vulgaris* is known to have defective keratohyalin that is of abnormal ultrastructure and is formed only in very small amounts as compared with normal skin [10]. This is one of the reasons why the biochemical nature of this defect is still unknown. The ultrastructural appearance of tiny, crumbly granules is very typical and was found in all patients with this type of ichthyosis, not only in hyperkeratotic skin [10], but also in clinically noninvolved skin [11]; in the keratinizing parts of the appendages, such as sweat ducts, hair follicles, and sebaceous ducts [12]; and after treatment [13]. It may be concluded that we deal with a primary genetically induced structural defect of a structural protein, the keratohyalin or one of its polypeptides, that seems to be under direct control of the mutant gene.

A biochemical analysis of this defective protein material with modern molecular biological techniques not only should add some knowledge to the underlying pathomechanism of this ichthyosis type, but also should allow us to draw important clues as to the steps of polypeptide formation during the synthesis of keratohyalin in general. Many other keratinization disturbances react with a reduced synthesis of keratohyalin as well (psoriasis, lamellar ichthyosis, wound healing). In all these instances, however, keratohyalin granules are ultrastructurally normal [14].

Ultrastructural abnormalities of the tonofilament system are most interesting in the context of this Symposium. I shall therefore concentrate on some dominantly inherited disorders in which these structural proteins are primarily affected.

Tonoepidermal clumping (Fig. 1 a) is the basic ultrastructural abnormality in *bullous ichthyosiform erythroderma* (bullous ECI, epidermolytic hyperkeratosis). Whereas the basal cells are quite normal, clumping starts in the first suprabasal layer of the epidermis with the onset of the keratinization process and involves the entire tonofilament material. Abnormalities in one of the polypeptides [15] and increased amounts of filaggrin [16] both have been attributed to this abnormality. The density of aggregation varies to some extent in these clumps. It seems that in less dense aggregations, tonofilaments undergo further keratinization with increased thickness and contrast of the individual filaments, the normal mode of spreading of keratohyalin among tonofilaments is impaired in this material. This basic abnormality of bullous ECI remains unchanged under treatment with oral retinoids [17].

An almost identical clumping of the tonofilaments occurs in another genetic skin disorder: *epidermolyisis bullosa herpetiformis Dowling-Meara* [14,18]. Moreover, in this disorder, clumping is related to the very beginning of the pathogenetic process. However, the clumps are most pronounced in the basal and lowermost suprabasal layers (Fig. 1 b) and occur immediately above the dermal-epidermal junction. They are already discernible by light microscopy of semithin sections. Tonoepidermal aggregations follow the junction in connection with the hemidesmosomes. Ultrastructurally, the clumps are indistinguishable from those of bullous ECI.

Here, however, we deal with an epidermolysis bullosa type in which the blisters predominante and hyperkeratoses occur only transitorily. Clumping of the tonofilaments precedes blister formation in time and spatial relationship in the Dowling-Meara type [18], as it precedes cytolysis in bullous ECI. In the Dowling-Meara type, blisters are deep in the basal layer; in bullous ECI, they are rather superficial. Thus in both, the blister process reflects the site of the major changes of cytoplasmic segregation due to defective keratin proteins. Both, genodermatoses are inherited autosomally dominantly. Predom-
Fig. 1. A, Tonofibrillar clumping in bullous ichthyosiform erythroderma, granular layer. Filament material aggregated into dense clumps (stars) \( (\times 18,000) \). B, Tonofibrillar clumping in epidermolysis bullosa herpetiformis Dowling-Meara, most pronounced in the basal cell layer above the dermal-epidermal junction. Open arrows, tonofibrillar clumps; BL, basal lamina \( (\times 5,000) \).

Fig. 2. Perinuclear shell of tonofilaments (stars) in ichthyosis hystrix Curth-Macklin after treatment with retinoids, prickle cell layer. M, mitochondria; N1 and N2, nuclei in a binuclear cell \( (A: \times 4,000; B: \times 26,000) \).

stance of blisters in the Dowling-Meara type and postnatal development of predominating hyperkeratoses in bullous ECI, accompanied by a decrease in vulnerability of the epithelium, due to possible interrelationship of both processes. Formation of hyperkeratoses in bullous ECI could be understood as a secondary reaction mechanism of the skin to compensate for inferior protective and barrier functions of the mutant keratinocytes.

Further heterogeneity in disorders with a defective tonofilament-alpha-keratin system was demonstrated by electron microscopy in cases that clinically and by histopathology were previously classified as belonging to bullous ECI. Ichthyosis hystrix Curth-Macklin has histopathologic features similar to epidermolysis hyperkeratosis. By electron microscopy, tonofilaments aggregate into concentric perinuclear shells, and no clump formation occurs. Interference of the shells with postmitotic division of daughter cells results in a high percentage of binuclear cell \( [19,20] \). The peripheral tonofilaments in the shells may undergo further keratinization (Fig. 2). New cases of this rare ichthyosis type were recently identified by electron microscopy that reveal identical ultrastructural changes \( [21] \). In addition, in this type the basic defect does not change under oral treatment with retinoids, although patients improve excellently \( (Fig. 2a, b) \). The inheritance again is autosomal-dominant.

A very unusual congenital reticulate ichthyosiform keratinization disorder in a female was recently observed by Marghescu et al. \( [22] \). Based on the light microscopic changes, this case was initially thought to represent a variety of ichthyosis hystrix Curth-Macklin. Perinuclear shells and binuclear cells \( (Fig. 3a) \) are very similar. By a more detailed analysis, however, these shells do not consist of tonofilaments that are attached to their outer surface. Instead, they are formed by a three-dimensional network of fine interdigitating filaments \( (Fig. 3b) \) of unknown biochemical composition. An attempt at typing this material immunologically with antibodies against various types of intermediate filaments \( (Prof. Franke, DKFZ Heidelberg) \) did not yield specific results.

Epidermal cell culture in vitro of an explant of this patient's skin was performed by Drs. Tilgen and Riehl, of the Department of Dermatology, at Heidelberg. They kindly provided material for an ultrastructural control after 14 days of primary culture. Many cells growing in monolayer in the fashion of keratinocytes were present. However, no material corresponding to the unusual filamentous networks of the in vivo material had been synthesized during the short time of in vitro culture. Therefore, at present, the nature of this material, although ultrastructurally similar to glycoproteins, remains unknown. Similarly, in this solitary case, the possible mode of inheritance remains an open question.

FETAL MANIFESTATION AND PRENATAL DIAGNOSIS IN KERATINIZATION DISORDERS

Gynecologists have recently developed techniques to obtain fetal skin biopsies intrauterinely via fetoscopy. This has now opened a rapidly developing new field to apply diagnostic criteria worked out in the postnatal skin to the prenatal diagnosis of many severe skin disorders of genetic origin. Based on normal fetal development \( [23,24] \), we have now the chance to study the manifestation of mutant genes in fetal skin develop-
TABLE 1. Summary of prenatal diagnoses of genetic skin disorders performed at Heidelberg by electron microscopy of fetal skin biopsies

<table>
<thead>
<tr>
<th>Genetic Risk</th>
<th>Number</th>
<th>Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herlitz syndrome</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>
| Epidermolysis bullosa atrophia
era inversa                         | 1      |             |
| Epidermolysis bullosa dystrophica Hallopeau-Siemens | 2      | 1           |
| Bullous congenital ichthyosiform eryth. | 3      | 1           |
| Harlequin fetus                | 2      |             |
| Cutis laxa/Ehlers-Danlos syndrome | 1      |             |
| Incontinentia pigmenti Bloch-Sulzberger | 1      |             |
| Ectodermal dysplasia, X-linked | 1      | 1           |

...and to compare the expression of these genes with that in postnatal life. Of course, a mutant gene will not be expressed unless the respective organ structures have been organized. This is a basic problem in inborn errors of keratinization, since in the normal fetus, keratinization does not start before the twenty-fourth to twenty-sixth week [23]. Again, bullous ECI shall serve as an example.

In the field of prenatal diagnosis we are cooperating with Prof. Rauskob, of Northeim, Dr. Jovanovic, of Giessen, and Docent Gustavii and coworkers, of Lund/Sweden. A summary of all prenatal diagnoses performed until now in our laboratories at Heidelberg is given in Table I. Among these, three pregnancies were at risk of bullous ECI. In two of them, the disorder could be excluded prenatally. In the diagnostic skin samples of the first case (fet. no. 12), obtained under fetoscopy by Dr. Gustavii, the epithelium was completely unkeratinized with periderm cells and a normal basal layer. Positive prenatal diagnosis was based on severe cytolyis throughout the intermediate layers, unilateral detachment of desmosomes, and a few tonofibrillar clumps indicating the basic abnormality of the disorder [25]. After termination, the fetus was proven to be affected by clinical and additional ultrastructural control.* Skin biopsies were provided by Drs. Gedde-Dahl and Molne, of Oslo. A large regional variation was found in the expression of the mutant gene, with lack of keratinization and severe cytolyis (Fig. 4a) on the trunk (buttocks) and prominent precocious hyperkeratoses on the face, palms, and soles. With the onset of keratinization, the amount of tonofilaments increased and pronounced tonofibrillar clumping was demonstrable in such areas with a striking repetitive periodicity (Fig. 4b). No tonofibrillar clumps were present in the periderm cells of nonkeratinized areas [25]. The sweat glands, fully developed in the hyperkeratotic palmar skin, behaved as independent biological units; within the epidermis their ducts were spared from cytolyis with normal ultrastructural appearance and without tonofibrillar clumping.

In comparison with the cases of Golbus et al. [26] and Holbrook et al. [16], considerable differences in gene expression must be taken into account between various risk families, each of them representing independent mutations in the bullous ECI gene. The presence of tonofibrillar clumps in the amniotic fluid cells [16] will, of course, be of valuable diagnostic significance. However, if the clumps are not demonstrable, this does not allow us to exclude the disorder.

Dr. Gedde-Dahl, of Oslo, cultured amniotic fluid cells of our affected fetus (no. 12) during termination of the pregnancy (week 22 MA). He kindly provided the fixed material for an ultrastructural investigation. Two types of cells were growing together in monolayer in vitro: typical fibroblastic cells active in collagen synthesis and secretion, and epithelial cells that in part were incompletely keratinized like periderm cells. The living cells revealed granular and globular inclusions that turned out to be membrane-bound and appeared to be phagolysosomes (Fig. 4c). No signs of specific differentiation were present and no tonofibrillar clumps were demonstrable in this culture.

Amniotic fluid cells in vitro. Cooperating with several human geneticists, especially Prof. Schleiermacher, of Mainz, cultures of amniotic cells and of fetal skin fibroblasts obtained under fetoscopy (Prof. Rauskob and Dr. Jovanovic) are now routinely established in all cases of diagnostic fetoscopies. The types of cells present in the amniotic fluid are studied directly on fresh cell preparations spun down after fixation. Many samples of amniotic fluid cells up to week 20 MA may mainly consist of periderm cells [25]. In a sample of week 21 MA not only were living, regressive, and partly keratinized periderm cells present (Fig. 5a), but also there were nonkeratinocytic cells such as macrophages (Fig. 5b), granulocyte-like cells (Fig. 5c), and even lymphocytes.

A primary cell culture in vitro of the same amniotic fluid sample (Prof. Schleiermacher, of Mainz) revealed cell clones of very homogenous characteristics growing in monolayer in epi-

*To be published in detail.
theloid fashion (Fig. 6a). After 23 days of culture, no signs of keratinization or any other specific differentiation were present. Cell organelles were numerous, including some phagolysosomes (Fig. 6b). Cytoplasmic filaments were concentrated along the cell margins, partly in contact with incomplete desmosome-like junctions (Fig. 6c). Straight, rigid filaments without collagen-like banding patterns accompanied the cell borders, most of them clearly outside the cell; in part, these filaments are in contact with the plasma membrane. Perpendicular sections show a polar distribution; it seems that cells attach to the plastic substrate of the culture bottles with obliquely running filaments and secrete this (proteinaceous?) material themselves into the substrate.

No specific clues can be drawn at present from these small results of the ultrastructural control of fetal cell lines. It should, however, be kept in mind that biological systems need a large complexity of interactions to be able to activate specific mutant genes. It should be a challenge to in vitro cell biologists to find out the factors that are a prerequisite to simulation of in vivo conditions.

FETAL EXPRESSION OF GENES AFFECTING DERMAL-EPIDERMAL JUNCTIONAL INTEGRITY

Prenatal Diagnosis in Epidermolysis Bullosa

The epidermolysis bullosa group represents disorders induced by genes that act on the integrity of the dermal-epidermal junction. The basic structures of the junction area, i.e., hemidesmosomes, basal lamina, and anchoring fibrils, are being organized in fetal skin from the week 12 MA [23] and are well-developed, although still increasing in amount, about the time of prenatal diagnosis, i.e., in the twentieth week of the pregnancy [25]. Expression of genes of the epidermolysis bullosa group is therefore much more likely during early fetal life. It shall be mentioned only briefly at the end of this paper that prenatal diagnosis of recessive epidermolysis bullosa dystrophica of the Hallopeau-Siemens type was possible in week 21 MA in a pregnancy at risk based on the same type of dermolytic (collagenolytic) blister formation below the basal lamina as in postnatal skin; no basic difference in the gene expression was present [27]. Children of this type are often born with severely scarring epithelial defects on the lower legs that must have existed intrauterinently for months. After termination, the fetus showed severe generalized involvement (Prof. Rauskolb).

Herlitz syndrome, like some other epidermolysis bullosa types with junctional blister formation, is caused by hypoplasia of hemidesmosomes [28]. Prenatal diagnosis of this disorder was made at least three times [29,30; fet. no. 51 of our group, in cooperation with Prof. Rauskolb]. Prenatal exclusion of Herlitz syndrome is quite safe on the basis of the normality of the hemidesmosomes about the eighteenth to the twentieth week of the pregnancy [25]. In 6 of 8 pregnancies at risk of Herlitz syndrome, prenatal exclusion was made by us ultrastructurally. Three of the children were born healthy, while in the last cases the pregnancies are still continuing (Prof. Rauskolb and Doc. Gustavii).

Despite increasing experience with prenatal diagnosis of genetic skin disorders, we are still in the very early stages of a fascinating new field that offers many chances of great practical importance and should become a new challenge for a better understanding of genetic interactions in the biology of the skin.

My coworkers in these studies were Dr. Marie-Luise Arnold, Mrs. Barbara Kern, Mrs. Ermelind Schleiermacher, Mrs. Inge Werner, Mrs. Ulrike Michels, Mrs. Elke Bolesta, and Mr. Enrique Boye. I gratefully acknowledge their enthusiasm and skillful scientific and technical work. Thanks are due to Dr. repetitive periodicity within the clumps (×39,000). C, Bullous ichthyosiform erythroderma. Primary culture of amniotic fluid cells (Dr. Gedde-Dahl, of Oslo) with globular and granular inclusions of phagolysosome-like structure. No tonofilaments and no clump formation (×10,800).
Fig. 5. Amniotic fluid cells of fet. no. 46, week 21 MA, in a cell pellet. A, Unkeratinized living periderm cell with nucleus and dead, partly keratinized periderm cell (x9,400). B, Partly keratinized periderm cell and macrophage. PL, phagolysosome; ME, marginal envelope (x10,800). C, Cell with granular inclusion that looks very much like a granulocyte (x10,200).

Tobias Gedde-Dahl, Jr., of Oslo, Professor Engelhardt Schleiermacher, of Mainz, and Drs. Wolfgang Tilgen and Rüdiger Riehl, of Heidelberg, for providing primary cell cultures for electron microscopic investigation. I am most thankful to my colleagues Professor Rüdiger Rauskolb, of Northeim, and Dr. Vanja Jovanovic, of Giessen, and Docent Björn Gustavii, of Lund, and his coworkers for providing control and diagnostic fetal skin biopsies obtained under fetoscopy.
Fig. 6. Amniotic fluid cells of fet. no. 46, week 21 MA, in a primary cell culture after 23 days (Prof. Schleiermacher, of Mainz). Arrows, microtubules; ER, endoplasmic reticulum; G, golgi body; F, peripheral filaments; CJ, cellular junction structures; M, mitochondria; N, nuclei; PL, phagolysosome (A: ×1,750; B: ×23,400; C: ×17,500).
REFERENCES


