Purinergic Receptors Are Part of a Signaling System for Keratinocyte Proliferation, Differentiation, and Apoptosis in Human Fetal Epidermis

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We have investigated the expression of $P2X_5$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptor subtypes in 8- to 11-wk-old human fetal epidermis in relation to markers of proliferation (proliferating cell nuclear antigen (PCNA) and Ki-67), keratinocyte differentiation (cytokeratin K10 and involucrin), and markers of apoptosis (TdT-mediated dUTP nick end labeling (TUNEL) and anti-caspase-3). Immunohistochemistry showed that each of the four receptors was expressed in spatially distinct zones of the developing epidermis: $P2Y_1$ receptors were found in the basal layer, $P2X_5$ receptors were predominantly in the basal and intermediate layers, and both $P2Y_2$ and $P2X_7$ receptors were in the periderm. Colocalization experiments suggested different functional roles for these receptors. $P2Y_1$ receptors were found in fetal keratinocytes positive for PCNA and Ki-67, suggesting a role in proliferation. $P2X_5$ receptors double labeled with differentiated fetal keratinocytes that were positive for cytokeratin K10, suggesting a role in differentiation. $P2X_7$ receptors colocalized with anti-caspase-3 antibody and were also expressed in periderm cells positive for TUNEL, suggesting a role in periderm cell apoptosis. $P2Y_2$ receptors were found only in periderm cells and may have a role in chloride and fluid secretion into the amniotic fluid. ***Key words: apoptosis cell differentiation/proliferation receptors/periderm/purinergic P2.*** ***J Invest Dermatol 121:1145–1149, 2003***

Adenosine 5′-triphosphate (ATP) is now recognized as an important messenger molecule for cell–cell communication, with ATP binding specifically to purinergic receptors (Ralevic and Burnstock, 1998). There is increasing evidence that purinergic signaling can have long-term, trophic effects in embryonic development, cell proliferation, differentiation, and death (Abbracchio and Burnstock, 1998; Burnstock, 2001).

Purinergic receptors are classified into two groups: P1 receptors are selective for adenosine and P2 receptors are selective for ATP and adenosine 5′-diphosphate, which act as extracellular signaling molecules (Burnstock, 1978). P2 receptors are divided into two main families: P2X (ligand-gated ion channels) and P2Y (G-protein-coupled receptors), based on molecular structure, transduction mechanisms, and pharmacologic properties (Abbracchio and Burnstock, 1994). Eight subtypes of P2Y receptors have been described, and seven subtypes of P2X receptors are recognized (Burnstock, 2003).

ATP is likely to be an important local messenger in the epidermis. Both $P2X_5$ and $P2X_7$ receptors are expressed on adult rat cutaneous keratinocytes and functional roles in the regulation of proliferation, differentiation, and cell death have been proposed (Gröschel-Stewart *et al*, 1999). $P2Y_2$ receptors, found in the basal layer of normal adult epidermis, are claimed to be involved in keratinocyte proliferation (Dixon *et al*, 1999). Studies on adult human epidermis and primary keratinocyte cultures have suggested that $P2Y_1$ and $P2Y_2$ receptors are involved in keratinocyte proliferation, that $P2X_5$ receptors are likely to be involved in keratinocyte differentiation, and that $P2X_7$ receptors are likely to be part of the machinery of end stage terminal differentiation/apoptosis of keratinocytes (Greig *et al*, 2003).

Human fetal skin is unique in structure and function and is in a continuum of change until birth. The transition from fetal to mature epidermis of the neonate and adult involves formation of new epidermal layers, invagination and remodeling of epidermal appendages, and finally loss of fetal-specific cell types and onset of adult-type differentiation (see Holbrook and Hoff, 1984, for a review). The primitive epidermis is established at 7 to 8 d when ectoderm and endoderm are defined in the inner cell mass of the implanted blastocyst. At this stage the epidermis is a single-layered "indifferent ectoderm." A second epidermal layer forms at the end of the fourth week. The outermost of the two layers is the periderm. The periderm is the transient, protective covering of the epidermis that is sloughed into the amniotic fluid as soon as differentiation of the underlying epidermal layers is complete at 20 to 24 wk. Beneath the periderm, basal keratinocytes comprise the single layer of the "epidermis proper." At the end of the ninth week, the embryonic–fetal transition, basal cells divide to give rise to daughter cells that move vertically to form the first intermediate cell layer that characterizes the fetal epidermis. Two or three more layers of intermediate cells are added to the epidermis in the second trimester (12–24 wk). Once a granular layer is established in the sixth month, the intermediate cells become known as spinous cells; thus each layer of the epidermis assumes

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Abbreviations: ATP, adenosine 5′-triphosphate; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; TUNEL, TdT-mediated dUTP nick end labeling; UTP, uridine 5′-triphosphate.

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the adult nomenclature as the tissue assumes the adult characteristics. The first stratum corneum develops at the end of the second trimester (24 wk), consisting of only a few cell layers, which increase in thickness during the third trimester and, at birth, it is approximately equivalent to that of the adult.

This study demonstrates the distribution of P2X and P2Y receptors in human fetal epidermis. We propose that these receptors are part of the normal mechanisms controlling fetal epidermal development in relation to keratinocyte proliferation, differentiation, and apoptosis.

MATERIALS AND METHODS

Tissues Twenty-five samples of normal human fetal skin were examined from nine fetuses at 8 to 11 wk estimated gestational age, which was determined by fetal measurements after pregnancy termination. Samples were obtained from fully informed, consenting patients undergoing elective terminations of pregnancy. Ethics Committee approval was obtained to harvest human fetal samples. Tissue was frozen in isopentane precooled in liquid nitrogen. Blocks were sectioned at 10 μm on a cryostat (Reichert Jung CM1800), collected on gelatin-coated slides, and air-dried at room temperature. The slides were stored at −20°C.

Antibodies The immunogens used for production of polyclonal $P2X_5$ and $P2X_7$ antibodies were synthetic peptides corresponding to 15 receptor-type-specific amino acids in the intracellular C-termini of the cloned rat and human P2X receptors, as described previously (Gröschel-Stewart *et al*, 1999; Oglesby *et al*, 1999). $P2X_5$ and $P2X_7$ antibodies (provided by Roche Bioscience, Palo Alto, CA) were kept frozen at a stock concentration of 1 mg per mL. Polyclonal anti-$P2Y_1$ and $P2Y_2$ antibodies were obtained from Alomone Laboratories (Jerusalem, Israel) and corresponded to the third extracellular loop of the $P2Y_1$ (amino acids 242–258) and $P2Y_2$ receptor (amino acids 227–244). Antibodies were kept frozen at a stock concentration of 0.6 mg per mL ($P2Y_1$, $P2Y_2$). Proliferating cell nuclear antigen (PCNA) is a marker for proliferation in normal adult human keratinocytes (Miyagawa *et al*, 1989). Cytokeratin K10 and involucrin are markers for keratinocyte differentiation (Eckert *et al*, 1997). PCNA (monoclonal anti-PCNA, clone PC10, raised in mouse ascites fluid; Sigma Chemical Co., Poole, UK), cytokeratin K10 (BioGenex, San Ramon, CA), and involucrin (Sigma Chemical Co.) antibodies were raised in mouse. Ki-67 antigen is a marker for cell proliferation in normal human keratinocytes (Tucci *et al*, 1998). Active caspase-3 is part of the apoptotic machinery of the cell and is expressed in terminally differentiating keratinocytes (Weil *et al*, 1999). Ki-67 (Dako, Denmark) and active caspase-3 (Abcam, Cambridge, UK) antibodies were both raised in rabbit.

Immunohistochemistry: double-labeling techniques. Double labeling of P2X or P2Y receptor antibodies with PCNA, cytokeratin K10, or involucrin For immunostaining of cryostat sections, the avidin–biotin technique was used according to a revised protocol (Llewellyn-Smith *et al*, 1992). Air-dried sections were fixed for 2 min in 4% formaldehyde in 0.1 M phosphate buffer, containing 0.2% of a saturated solution of picric acid (pH 7.4). Endogenous peroxidase was blocked for 10 min with 50% methanol containing 0.4% hydrogen peroxide. Nonspecific binding sites were blocked by a 20-min preincubation in 10% normal horse serum in 0.1 M phosphate buffer, containing 0.05% merthiolate (Sigma Chemical Co.), followed by incubation with the primary antibodies diluted to 1:100 or 1:200 in antibody diluent (10% normal horse serum in phosphate-buffered saline (PBS) + 2.5% sodium chloride at 4°C overnight.

After a wash step, biotinylated donkey anti-rabbit IgG antibody, diluted 1:500 in 1% normal horse serum in PBS, was then applied for 1 h followed by streptavidin–Texas red (Amersham International plc., UK), diluted 1:200 in PBS–merthiolate for 1 h at room temperature. Sections were preincubated for 30 min with 10% normal goat serum diluted in 0.1 M phosphate buffer, containing 0.05% merthiolate (Sigma Chemical Co.). They were then incubated for 2 h at room temperature with one of the following antibodies: PCNA antibody (Sigma Chemical Co.) diluted 1:1000; mouse anti-human cytokeratin K10 (BioGenex) diluted 1:50; mouse monoclonal anti-involucrin (Sigma Chemical Co.) diluted 1:50. After a wash step, the directly labeled secondary antibody goat anti-mouse FITC (Nordic Immunological Laboratories, Tilburg, the Netherlands) was applied at a dilution of 1:200 for 1 h, and then sections were washed and mounted in Citifluor (Citifluor Ltd, Leicester, UK). P2X or P2Y receptor immunostaining appeared red and PCNA, cytokeratin K10, and involucrin stained green.

Double-labeling of P2X or P2Y receptor antibodies with either Ki-67 antigen or antihuman caspase-3 Sections were fixed and incubated with P2X or P2Y antibodies overnight, as described above. After being washed, sections were incubated with biotinylated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratory) diluted to 1:500 in 1% normal horse serum in PBS for 1 h, followed by ExtrAvidin peroxidase conjugate (Sigma Chemical Co.) diluted to 1:1500 in PBS for 1 h, tyramide amplification for 8 min (tyramide amplification kit, NEN Life Science Products, Boston, MA) and then streptavidin–Texas red (Amersham International plc.), diluted 1:200 in PBS–merthiolate for 10 min. Sections were washed three times in PBS after each of the above steps. Sections were preincubated for 20 min in 10% normal goat serum and then incubated at room temperature for 2 h with one of the following antibodies: rabbit anti-human Ki-67 antigen (Dako) 1:50 or rabbit anti-human active caspase-3 (Abcam). Sections were then washed and incubated with the directly labeled secondary antibody Oregon-green-labeled goat anti-rabbit IgG (Jackson ImmunoResearch Laboratory), diluted 1:100 for 45 min. Sections were then washed and mounted in Citifluor (Citifluor Ltd). P2X or P2Y receptor immunostaining appeared red and Ki-67 and caspase-3 stained green.

Double labeling of $P2X_7$ receptor antibodies with TdT-mediated dUTP nick end labeling (TUNEL) TUNEL identifies cells undergoing apoptosis by labeling nuclear DNA fragments that have been cleaved during apoptosis (Gavrieli *et al*, 1992). TUNEL labeling was performed using a kit (Boehringer Mannheim, Germany). After overnight incubation with $P2X_7$ receptor antibody diluted to 1:200 as above, sections were washed in PBS and then incubated with the TUNEL reaction mixture for 1 h at 37°C. As a negative control, sections were incubated with the TUNEL solution only. After additional washes in PBS, sections were incubated for 1 h with biotinylated donkey anti-rabbit antibody at a concentration of 1:500. Sections were washed in PBS and then incubated for 1 h with streptavidin–Texas red (Amersham International plc.) at a concentration of 1:200. Sections were washed in PBS and mounted in Citifluor (Citifluor Ltd). $P2X_7$ receptor immunostaining appeared red and TUNEL labeling was green.

Controls Control experiments were carried out with primary antibodies omitted from the staining procedure or the primary antibodies preabsorbed with the corresponding peptides.

Photography The results were photographed using a Zeiss Axioplan, high-definition light microscope (Oberkochen, Germany) mounted with a Leica DC 200 digital camera (Heerbrugg, Switzerland).

RESULTS

$P2X_5$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptor expression in human fetal epidermis $P2X_5$ and $P2X_7$ receptor immunoreactivity was observed in the epidermal keratinocytes of all human fetal skin samples (**Fig 1*a*,*b***). $P2X_5$ receptor immunoreactivity was found mainly in the basal layer and to a lesser extent in the intermediate cell layer (**Fig 1*c***). $P2X_5$ receptors were found in the cell membranes and sometimes in the cell cytoplasm, but not in the nucleus. The $P2X_5$ receptor staining on keratinocytes in the stratum basale appeared to be polarized, being concentrated on the basal aspects of cells associated with the basement membrane (**Fig 1*c***). In the epidermis, $P2X_7$ receptor immunoreactivity was associated with periderm cells only (**Fig 1*d***); $P2X_7$ receptor immunostaining also labeled fetal blood cells. $P2Y_1$ receptors were found in the basal layer of the epidermis (**Fig 1*e***) and $P2Y_2$ receptors were expressed only in the periderm (**Fig 1*f***).

Double labeling of $P2Y_1$ and $P2Y_2$ receptors with markers for cellular proliferation Fetal skin was double-stained for Ki-67 and $P2Y_1$ receptors (**Fig 1*g***) and PCNA and $P2Y_2$ receptors (**Fig 1*h***). The proliferation markers identified a proliferating subpopulation of basal keratinocytes. Cells positive for these two markers were also positive for $P2Y_1$ but not for $P2Y_2$ receptors, which were only found in periderm cells.

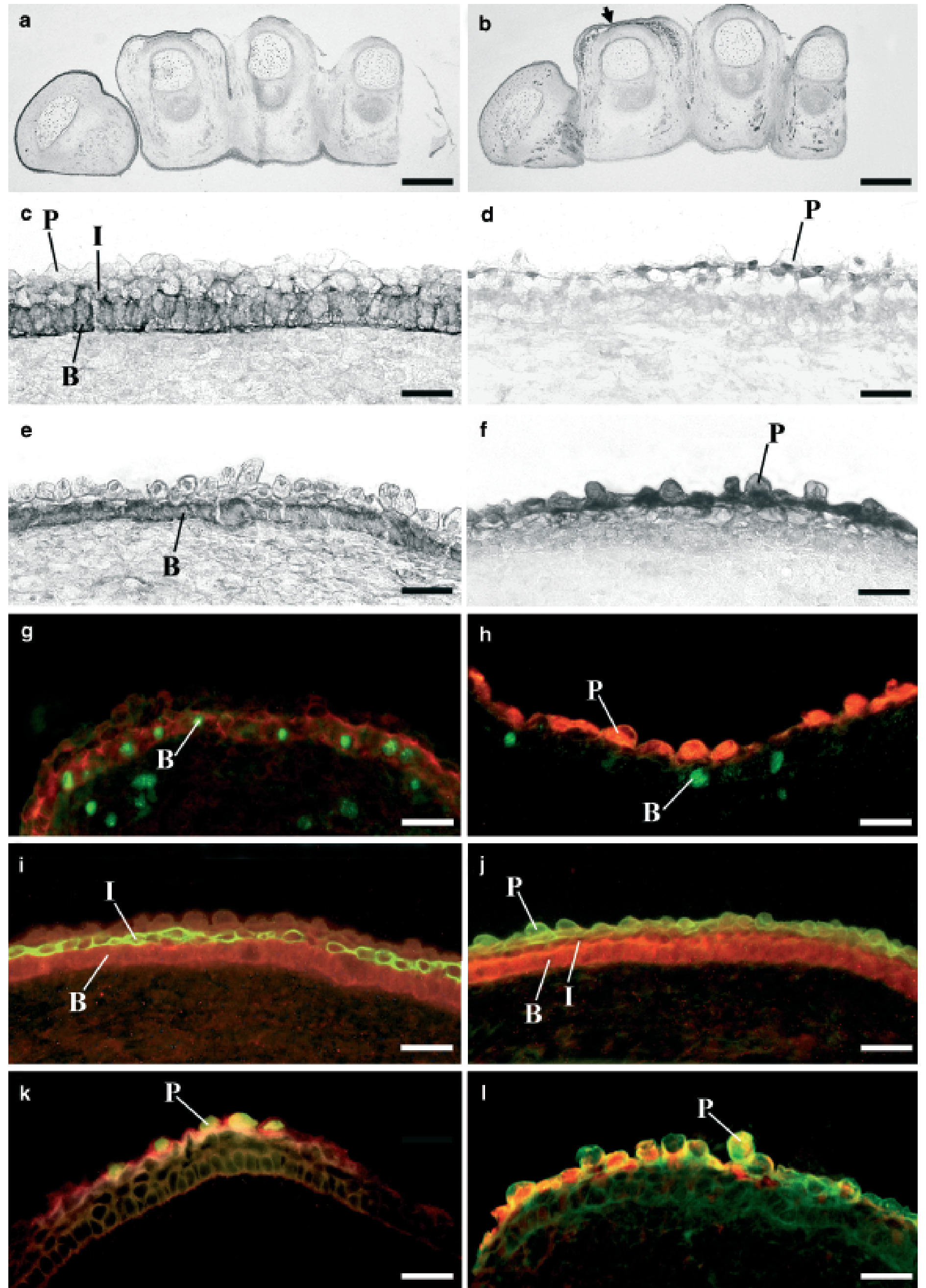

Figure 1. Expression of $P2X_5$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptors in human fetal epidermis and double labeling with markers for cellular proliferation, keratinocyte differentiation, and markers for apoptosis. (*a*) Cross-section of hand of 9-wk-old fetus showing $P2X_5$ receptor immunostaining. *Bar,* 375 µm. (*b*) Cross-section of hand of 9-wk-old fetus show-ing $P2X_7$ receptor immunostaining, with a predominance on the dorsal surface (*arrow*). *Bar,* 375 µm. (*c*) High-power view of (*a*): $P2X_5$ receptor staining was mainly found in the basal layer (*B*) of the epidermis and to a lesser extent in the intermediate layer (*I*). There was little staining in the periderm (*P*). *Bar,* 25 µm. (*d*) High-power view of (*b*): $P2X_7$ receptor immunoreactivity was associated with cells within the periderm (*P*). *Bar,* 25 µm. (*e*) $P2Y_1$ receptors were found in the basal layer (*B*) of the epidermis. *Bar,* 25 µm. (*f*) $P2Y_2$ receptors were found in the periderm (*P*). *Bar,* 25 µm. (*g*) Double labeling of fetal epidermis with $P2Y_1$ receptors (*red*) and Ki-67 (*green*) to show that $P2Y_1$ receptors are found in proliferating cells in the basal layer (*B*). *Bar,* 25 µm. (*h*) Double labeling of $P2Y_2$ receptors (*red*) and PCNA (*green*). PCNA identified a proliferating subpopulation of only basal keratinocytes (*B*), but $P2Y_2$ receptors were only found in periderm cells (*P*). *Bar,* 25 µm. (*i*) Double labeling of $P2X_5$ receptors (*red*) with cytokeratin K10 (*green*). Cytokeratin K10 was found in cells in the intermediate cell layer (*I*) and not in basal keratinocytes (*B*). The stratum basale (*B*) stained only for $P2X_5$ receptors, indicating that no differentiation was taking place in these cells. *Bar,* 25 µm. (*j*) Double labeling of $P2X_5$ receptors (*red*) with involucrin (*green*). Involucrin was expressed only in the periderm cells (*P*) and did not colocalize with $P2X_5$ receptor staining in the basal (*B*) and intermediate (*I*) layers. *Bar,* 25 µm. (*k*) Double labeling (*yellow*) of $P2X_7$ receptors (*red*) with TUNEL (*green*). Only intermittent, rounded periderm cells (*P*) had nuclei that stained strongly positive for TUNEL although $P2X_7$ receptors were expressed in most of the cells in the periderm layer. *Bar,* 25 µm. (*l*) Double labeling (*yellow*) of $P2X_7$ receptors (*red*) with anti-caspase-3 (*green*) showed colocalization within the periderm (*P*). *Bar,* 25 µm.

Double labeling of $P2X_5$ receptors with markers for keratinocyte differentiation Double labeling of $P2X_5$ receptors with cytokeratin K10 (**Fig 1*i***) showed that $P2X_5$ receptors were expressed in differentiating keratinocytes within the fetal epidermis. Cytokeratin K10, an early marker of keratinocyte differentiation, was found in cells in the intermediate cell layer and not in basal keratinocytes. The basal layer stained only for $P2X_5$ receptors, and not for markers of differentiation, indicating that no differentiation was taking place in these cells. Involucrin, a late marker of keratinocyte differentiation in adult skin, was expressed only in the periderm cells and did not colocalize with $P2X_5$ receptor staining (**Fig 1*j***).

Double labeling of $P2X_7$ receptors with markers for apoptosis in fetal epidermis show colocalization Individual rounded periderm cells had nuclei that stained strongly positive for TUNEL (**Fig 1*k***). This corresponded to a transitional stage when the periderm is beginning the formation of a series of complex surface blebs. Double staining of the $P2X_7$ receptor and TUNEL showed that only intermittent cells were positive for TUNEL, although $P2X_7$ receptors were expressed in most of the cells in the periderm layer. The negative control for TUNEL showed no reaction. Double labeling of $P2X_7$ receptors with anti-caspase-3 showed colocalization within the periderm (**Fig 1*l***).

Controls Both the omission of the primary antibody and the preabsorption with corresponding peptides were performed as controls. The immunoreaction was abolished after preabsorption of the $P2X_5$, $P2X_7$, $P2Y_1$, or $P2Y_2$ receptor antibodies with the corresponding peptides, confirming the specificity of the immunoreaction.

DISCUSSION

This study has shown the first direct evidence for the expression of $P2X_5$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptors in 8- to 11-wk-old

human fetal epidermis, using immunohistochemistry. Four periods of human epidermal development have been identified (Dale *et al*, 1985). The first is the embryonic period (before 9 wk estimated gestational age) and the others are within the fetal period: stratification (9–14 wk), follicular keratinization (14–24 wk), and interfollicular keratinization (beginning approximately 24 wk). We have studied epidermal tissue at the end of the embryonic period and during the period of stratification. It would be of interest to have studied fetal tissue from different stages of gestation but fresh-frozen tissue was not available for other time points.

Purinergic receptors bind ATP, which is found in all cells. Intracellular ATP can be released passively from dying, necrotic cells undergoing cytolysis, or it can be actively released from living cells by vesicular release or via ATP-binding cassette proteins (Bodin and Burnstock, 2001). In our study, we have seen that purinoceptors are distributed on cell membranes and in the cytoplasm. Internalization of purinoceptors has been observed in a variety of circumstances including synthesis of receptors in the cytoplasm and transport to the cell membrane (Loesch and Burnstock, 2001) and following prolonged exposure to ATP (Li *et al*, 2000).

$P2Y_1$ receptors showed a strong immunopositive signal in the basal layer of the fetal epidermis, which is known to be where keratinocyte proliferation occurs (Bickenbach and Holbrook, 1987). Double labeling of $P2Y_1$ receptors and keratinocyte proliferation markers Ki-67 and PCNA was performed. This confirmed the presence of $P2Y_1$ receptors in proliferating cells, indicating that this receptor is involved in keratinocyte proliferation. The distribution of $P2Y_1$ receptors was the same as in adult epidermis (Greig *et al*, 2003).

$P2X_5$ receptors were expressed in the basal layer of human fetal epidermis and were also expressed in the intermediate cell layer, where cytokeratin K10, an early marker of keratinocyte differentiation, was found. It is known that keratinocytes that initially occupy the intermediate layer of fetal epidermis from 9 to 20 wk gestation undergo only an incomplete type of differentiation (Holbrook, 1991) that is not characteristic of the mature epidermis. Although not morphologically different, the intermediate cells at this stage have initiated biochemical differentiation, characterized by the synthesis of epidermal differentiation-specific markers, keratins 1 and 10 (Dale *et al*, 1985) as seen in our study. The expression of these keratins is thought to herald the commitment of the keratinocyte to a program of epidermal keratinocyte differentiation (Haake and Cooklis, 1997). Double labeling of $P2X_5$ receptors with cytokeratin K10 showed that $P2X_5$ receptors were expressed in differentiating keratinocytes within the fetal epidermis, so it seems likely that $P2X_5$ receptors are involved in fetal epidermal keratinocyte differentiation. The basal layer stained only for $P2X_5$ receptors and not cytokeratin K10, indicating that basal cells were not differentiated, so $P2X_5$ receptors may also have an additional role in basal keratinocytes. There is evidence from other tissues regarding the role of $P2X_5$ receptors. In fetal rat skeletal muscle, $P2X_5$ receptors are sequentially expressed during development (Ryten *et al*, 2001). $P2X_5$ receptors have been implicated in the regulation of osteoblastic differentiation and proliferation (Hoebertz *et al*, 2000) and in triggering the differentiation of skeletal muscle satellite cells (Ryten *et al*, 2002).

Involucrin, a late marker of keratinocyte differentiation in adult skin and a cornified cell envelope precursor protein, was expressed only in periderm cells and did not colocalize with $P2X_5$ receptor staining. The lack of dual labeling might simply be due to the incomplete nature of epidermal differentiation at this stage in development, because involucrin is a late marker of differentiation. Involucrin has previously been found to be expressed only on periderm cells in fetal epidermis at the same stage of development (Akiyama *et al*, 1999) and is thought to be representative of a process of keratinization within the periderm, separate from the keratinization of the underlying "proper" epidermal layers that occurs later in development. In adult epidermis, we found that involucrin colocalized with $P2X_5$ receptors only in the upper layers of the stratum spinosum (Greig *et al*, 2003).

Cells in the periderm were intensely stained for TUNEL and caspase-3. Double staining of the $P2X_7$ receptor and TUNEL showed that only intermittent cells were positive for TUNEL, although $P2X_7$ receptors were expressed in most of the cells in the periderm layer. Double labeling of $P2X_7$ receptors with anti-caspase-3 showed colocalization within the periderm. The $P2X_7$ receptor is unlike other P2X receptors because it is a bifunctional molecule that can be triggered to act as a channel permeable to small cations or on prolonged stimulation can form a cytolytic pore permeable to large hydrophilic molecules of up to 900 Da (Surprenant *et al*, 1996). The opening of this pore results in the increase in intracellular cytosolic free calcium ions and the induction of cell death and there is increasing evidence that this process is dependent on the caspase signaling cascade (Ferrari *et al*, 1999). Caspase-3 is expressed in adult terminally differentiating keratinocytes (Weil *et al*, 1999). During apoptosis, the nucleus condenses and DNA is fragmented by endonucleases, which can be detected by TUNEL. TUNEL-positive keratinocytes have been found in the upper regions of the granular layer of the adult epidermis, before cornification (Polakowska *et al*, 1994; Tamada *et al*, 1994; Gandarillas *et al*, 1999). Nuclei of individual periderm cells have been previously reported to stain positively for TUNEL from about 14 wk estimated gestational age (Polakowska *et al*, 1994). In this study, the periderm was shown to exhibit characteristics consistent with apoptosis before its loss from the epidermis, which occurs at midgestation (20–24 wk). The periderm undergoes a series of morphologic changes that have been described ultrastructurally including: blebbing of the cell surface and pinching off of cytoplasmic fragments, accumulation of cytoplasmic filaments, formation of a submembranous cell envelope, and a pyknotic nucleus (Holbrook and Odland, 1975). These morphologic changes in the periderm are similar to those described for other cell types undergoing apoptosis (Kerr *et al*, 1972) and the time of their onset correlates with the appearance of markers of apoptosis (Polakowska *et al*, 1994). Following the bleb stage, periderm cells also exhibit a number of regressive changes involving partial organelle degeneration and accumulation of filaments in the cytoplasm. It is thought that apoptosis is the mechanism by which periderm cells are deleted from the epidermis after serving their function (Polakowska *et al*, 1994). In this study, it is likely that periderm cells at 8 to 11 wk estimated gestational age, which double label for $P2X_7$ receptors and markers of apoptosis, are showing a preliminary stage in the journey toward apoptosis, because they do not have the characteristic histologic appearance of apoptosis. The expression of $P2X_7$ receptors may be important in the regulation of apoptosis intrinsic to correct development.

Interestingly, $P2Y_2$ receptors were also found only in periderm cells. In adult skin, $P2Y_2$ receptors were found in the basal layer of the epidermis, where proliferation markers Ki-67 and PCNA were also found (Greig *et al*, 2003). The $P2Y_2$ receptor agonist, uridine 5′-triphosphate (UTP), caused a significant increase in human keratinocyte cell number *in vitro* (Greig *et al*, 2003). Previous work has localized $P2Y_2$ receptor mRNA in adult human epidermal basal cells via *in situ* hybridization (Dixon *et al*, 1999). UTP, the potent $P2Y_2$ receptor agonist, has also been shown to cause proliferation in keratinocytes (Dixon *et al*, 1999; Greig *et al*, 2003). Nevertheless, in fetal epidermis, $P2Y_2$-receptor-positive periderm cells were not positive for proliferation markers PCNA and Ki-67. Therefore, $P2Y_2$ receptors may have an alternative role in periderm cells. For example, $P2Y_2$ receptors on periderm cells could be involved in chloride and fluid secretion into the amniotic fluid. $P2Y_2$ receptors are involved in ion and fluid secretion in lung and conjunctival epithelial cells (Yerxa, 2001). Studies have suggested that nonkeratinized fetal epidermis may have an osmoregulatory function and also that the fetal epidermis contributes to the formation of amniotic fluid in the first trimester and

becomes the primary source in the second trimester (Holbrook, 1991). Periderm cells have microvilli and surface blebs that increase the surface area of the plasma membrane exposed to the amniotic fluid, there are large numbers of membrane-bound vesicles adjacent to the plasma membrane, and tight junctions join adjacent periderm cells. This suggests that the periderm cell layer may have potential for fluid and ion transport (Holbrook, 1991).

In summary, P2 purinergic receptors are likely to be involved in fetal keratinocyte proliferation via $P2Y_1$ receptors found on basal cells and fetal keratinocyte differentiation via activation of $P2X_5$ receptors. $P2X_7$ receptors are likely to be involved in apoptosis of periderm cells, but the role of $P2Y_2$ receptors in periderm cells is currently unknown; it is suggested that they may play a role in chloride and fluid secretion into the amniotic fluid.

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