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The distribution of factor XIIIa-positive cells in the human fetus and placenta *

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Summary. Immunohistochemical staining for factor XIIIa, a transglutaminase, revealed a variety of positively stained cells in human fetal tissues. Factor XIIIa-positive cells were most numerous in the dermis and connective tissues. Numerous large, stellate cells in placental villi, decidua, and chorionic membranes also expressed factor XIIIa at 7–9 weeks gestational age, before the onset of fetal hematopoiesis. There was heterogeneity in the staining for factor XIIIa in the early and late fetal tissues, in both rounded and in dendritic cells. In preparations of consecutive sections and in double-labelling experiments, some cells expressed both factor XIIIa and certain monocyte markers and were identified in close association with blood vessels and lymphoid organs in the late fetus and in the placental villi at the end of gestation. Other rounded and dendritic cells expressed factor XIIIa but not monocyte markers, and were found in adult and fetal connective tissues at all gestational ages. These results suggest that there are two factor XIIIa-positive cell populations. One population is present at all developmental stages, does not express monocyte markers, and probably differentiates in situ from primitive mesenchyme. The other population appears mainly after the onset of fetal hematopoiesis, co-expresses some monocyte markers, is HLA-DR positive and may be capable of antigen presentation.

Key words: Factor XIIIa – Macrophage – Dermal dendrocyte – Placenta – Skin

Introduction

Factor XIII is a plasma proenzyme which contributes to the blood coagulation pathway by catalysis of the formation of cross-linking amide bonds between glutamine and lysine residues on fibrin (Fear et al. 1984; Reid et al. 1986; Nemes et al. 1988; Nickoloff and Griffiths 1989a, b; Gray et al. 1990; Greenberg et al. 1991) and several other proteins, for example, fibronectin (Barry and Mosher 1990). It circulates in the plasma as a tetramer composed of two "a" subunits (factor XIIIa) and two "b" subunits (factor XIIIb). The transglutaminase activity is located on the "a" subunits and the "b" subunits may stabilize or regulate the activation of the enzyme (Barry and Mosher 1990; Greenberg et al. 1991). Factor XIIIa is found within a variety of dendritic cells in connective tissues as well as in platelets and megakaryocytes (Cerio et al. 1990). Some classes of monocytes and macrophages may also contain factor XIIIa (Henriksson et al. 1985; Cerio et al. 1988). It is controversial whether or not true tissue fibroblasts express factor XIIIa (Fear et al. 1984; Henriksson et al. 1985; Reid et al. 1986; Glukhova et al. 1988; Nemeth et al. 1988; Nemeth and Penneys 1989; Cerio et al. 1990).

The characteristics of factor XIIIa-positive cells have been studied most extensively in human skin, in which there is a population of dermal dendritic cells, called "dermal dendrocytes", that bind antibodies against factor XIIIa (Headington 1986; Cerio et al. 1989; Headington and Cerio 1990). These cells are concentrated in the papillary dermis, near the superficial vascular plexus, and are distinct from Langerhans' cells since they are CD1 negative, but express HLA-DR, as well as several other markers typical of monocytes and macrophages, such as OKM5 and LeuM3 (Henriksson et al. 1985; Kradin et al. 1987; Cerio et al. 1989; Headington and Cerio 1990). The co-expression of factor XIIIa and monocyte markers has suggested that the dermal dendrocyte may be derived from bone marrow stem cells and may possess the capability to present antigen (Cerio et al. 1989; Headington and Cerio 1990). Dermal dendrocytes proliferate in a number of inflammatory skin diseases, including early wound healing, psoriasis, and atopic dermatitis (Cerio et al. 1989, 1990; Nickoloff and Griffiths 1989b; Morganroth et al. 1991). Several tu-

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mours of "fibrohistiocytic" origin stain for factor XIIIa, including dermatofibroma (Adany and Muszbek 1987), benign fibrous histiocytoma (Adany and Muszbek 1987), fibrous papule of the nose (Nemeth et al. 1988), and malignant fibrous histiocytoma (Nemes et al. 1988), suggesting that these tumors arise by transformation of dermal dendrocytes or dendritic cells in other connective tissues. In other tumors, dermal dendrocytes may become entrapped by the proliferation of tumor cells, such as by proliferating endothelial cells in the case of Kaposi's sarcoma (Nickoloff and Griffiths 1989b; Cerio et al. 1990; Gray et al. 1991).

In this study, we tested the hypothesis that factor XIIIa dendritic cells are a heterogeneous population of cells and are not all derived from monocytes from the bone marrow.

Immunohistochemistry on fetal tissues before and after the onset of fetal hematopoiesis and double-labelling experiments confirmed this hypothesis.

Materials and methods

Seventeen human fetuses of 8–20 weeks gestational age were studied after they had been submitted to the Surgical Pathology Division, Department of Pathology, New York Hospital, following either the legal termination of pregnancy or after miscarriage. The gestational ages were determined by the crown/rump length when possible, and otherwise on the basis of long bone lengths (Arey 1954). Samples of skin, central nervous system, intestine, liver, spleen, and placenta were fixed in formalin within 24 h, and embedded in paraffin.

Table 1. Antibodies used for immunohistochemistry

Antibody	Source	Dilution
CD4	Becton Dickinson	1:200
CD68	Dakopatts	1:50
Factor XIIIa	Cal Biochem	1:400
HLA-DR	Becton Dickinson	1:100
HLe-1	Becton Dickinson	1:100
Leu-M3	Becton Dickinson	1:100
Leu-M5	Becton Dickinson	1:50
LN3	ICN Immunobiologicals	1:100
Mac 387	Dakopatts	1:100

Immunohistochemistry was performed usually on paraffin-embedded tissue but also on frozen sections in double-labelling experiments. The formalin-fixed, paraffin-embedded tissues were sectioned a 5 μm, deparaffinized in xylene, and rehydrated in phosphate buffered saline (PBS). The sections were incubated with primary antibody overnight at 4° C, including the following antibodies (Table 1): factor XIIIa, anti-HLA-DR, Mac 387, anti-CD68 (KP1), LN3, and HLe. Consecutive paraffin sections were stained for factor XIIIa and HLA-DR (using LN3) and with Mac 387. The sections were washed in PBS, and the bound antibodies were localized by the avidin-biotin-peroxidase method using diaminobenzidine as the chromogenic substrate.

The double-labelling procedure used either rehydrated paraffin sections as described above or frozen sections that were cut at 6 μm, fixed in cold acetone for 5–10 s, air-dried, incubated in 0.3% hydrogen peroxide for 10 min to reduce endogenous peroxidase activity, and then incubated in horse serum for 20 min to reduce nonspecific protein binding. The sections were incubated in primary antibody for 1 h in the case of frozen sections and overnight at 4° C for deparaffinized sections. The first antibody used stained factor XIIIa, which was localized by the avidin-biotin-alkaline phosphatase reaction using a red chromogen (Vector Laboratories, Burlingame, Calif., USA). The red reaction product had better resolution than the blue chromogen. The slides were washed in PBS, blocked again with 5% goat serum, and the second stage primary antibodies were applied (including LN3, anti-CD68, Leu M1, and Mac 387 for paraffin sections and anti-CD4, anti-human leukocyte, anti-HLA-DR, Mac 387, and Leu M3 for frozen sections) (Table 1). These antibodies were then localized by the avidin-biotin-peroxidase method using diaminobenzidine as the chromogen. This produced a contrast between the red and the brown reaction products.

Results

In the stroma of placental villi, at 8 weeks' gestation, before the onset of fetal hematopoiesis, there were large,

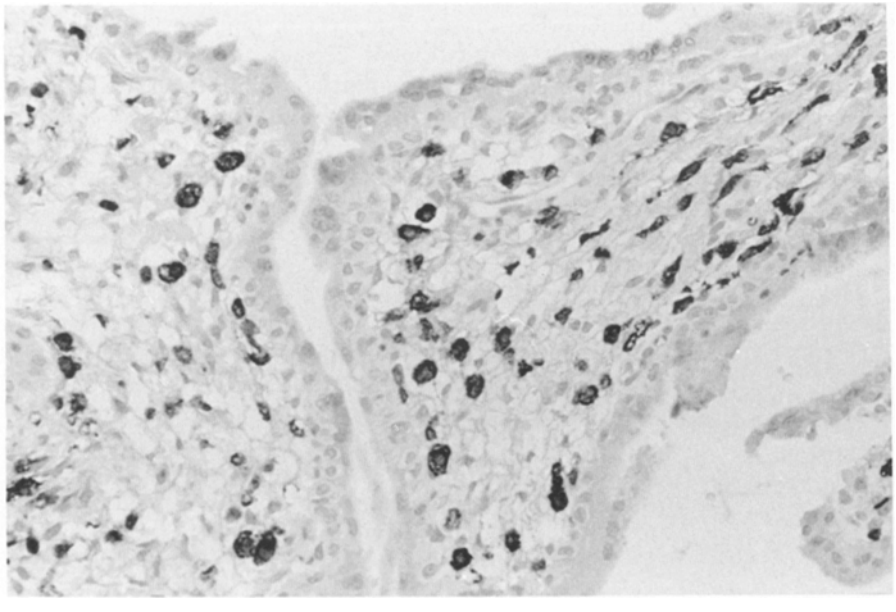

Fig. 1. Placental villi, 8 weeks gestational age. Numerous Hofbauer cells in the center of the villi are stained darkly, indicating that they contain factor XIIIa. Immunoperoxidase, × 340

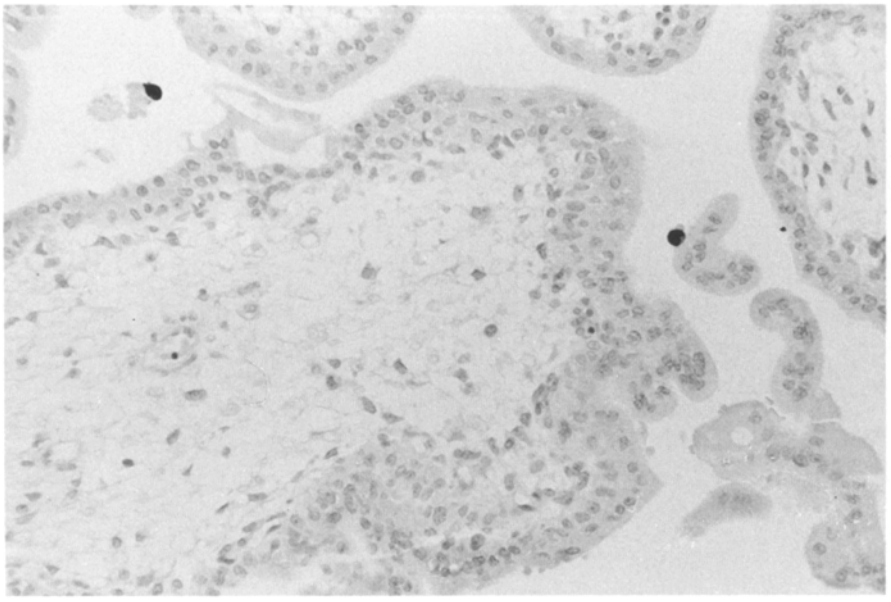

Fig. 2. Placental villi, 8 weeks gestational age. This preparation has been stained only for macrophages with Mac 387. The Hofbauer cells do not stain but a few maternal leukocytes on the surface of the villi are positive. Immunoperoxidase, ×350

Table 2. Distribution of factor XIIIa-positive cells

Anatomical Site	Early fetus (8 weeks)	Late fetus (after 12 weeks)
Placental villi	+++ large; +/− small	+++ large; + small
Dermis	++ large; + small	++ large; ++ small
Fascia	++ large; 0 small	++ large; + small
Intestinal stroma	+/− large	+ large
Heart stroma	+ large	ND
Lung stroma	+ large; +/− small	+ large
Kidney capsule	+ large	+ large
Spleen	++ large/+ small	+++ large/− small
Lymph node		
cortex	ND	+ large/+ small
capsule/sinuses	ND	+ large/− small
Thymus		
Cortex	− large/+ small	++ large
Medulla	− large/+ small	− large/− small
Meninges	+ large	+ large
Brain	− large/− small	− large/− small
Umbilical cord	++ large	++ large

ND, Not done; −, negative; +/−, a few faintly stained cells; +, a few strongly stained cells; ++, moderate numbers of strongly stained cells; +++, the maximum number of strongly stained cells

factor XIIIa-positive cells that were abundant and resembled the large cells named Hofbauer cells (Arey 1954; Enders and King 1970) (Fig. 1). Smaller stellate cells in the stroma varied in their staining; most were negative and a few were weakly positive. Fine strands of stromal material or very small processes of dendritic cells also were weakly positive. At this early gestational age the Hofbauer cells and the smaller cells did not co-express the macrophage or monocyte markers: HLA-DR, Mac 387, CD68, CD4, or Hle (Fig. 2). Factor XIIIa-positive cells were found in loose connective tissues throughout the early embryos (Table 2), as well as in the blood pools. The presumptive fascia and capsular regions of organs had large stellate factor XIIIa positive cells. Small bipolar cells which stained lightly for factor XIIIa were aggregated to form a cambium layer in focal regions of the dermis, particularly on the limb buds and on the face and upper back, sites which have a thick dermis in the adult.

Fetuses older than 12 weeks gestational age, as well as adult tissues, contained factor XIIIa-positive cells in a wide distribution throughout multiple organ systems. They were most numerous in the dermis and in the connective tissues that were collagen-rich (Table 2). The morphology of the positive cells varied. In the placenta, the large, darkly stained stellate cells that resembled Hofbauer cells were abundant in villi, decidua, and chorionic membranes in term placentas. In term placental

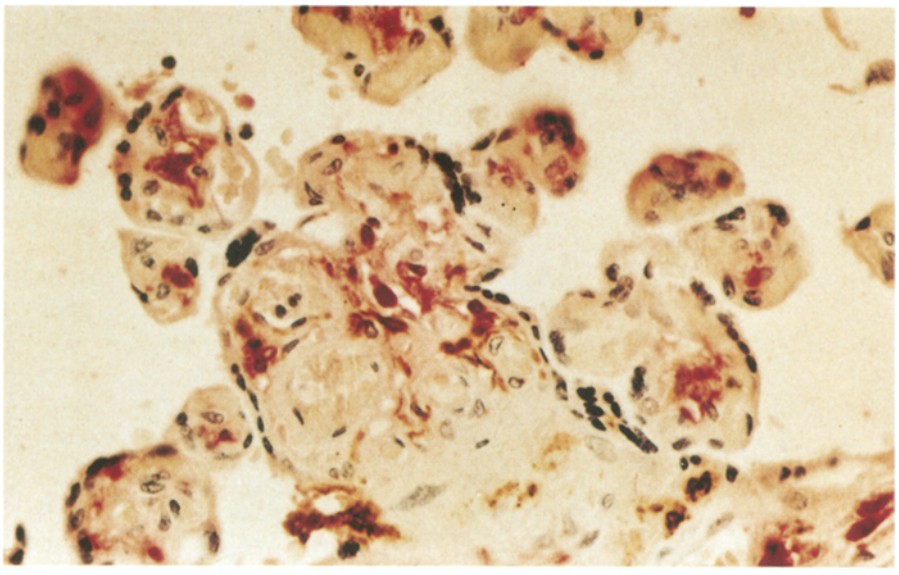

Fig. 3. Term placental villi that have been double-stained for factor XIIIa (*red*) and Mac 387 (*brown*). Most of the large Hofbauer cells stain only for factor XIIIa. A few smaller interstitial cells in the center of the villi stain positively with Mac 387. Immunoperoxidase, × approx. 340

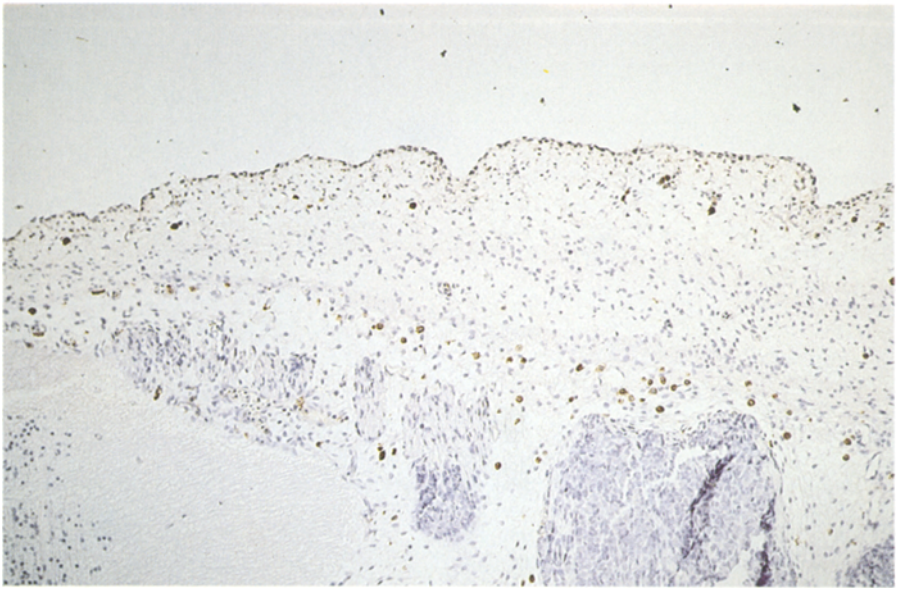

Fig. 4. Skin of a fetus at 8 weeks gestational age. The epidermis is very thin. The dermis contains few cells, but large stellate cells are positive for factor XIIIa in the deep dermis or fascial plane. Immunoperoxidase, ×130

villi, some of the stromal cells did stain with Mac 387 (Fig. 3), even in the absence of evidence of chorioamnionitis.

In the skin of embryos and fetuses, the dermis contained cells that were positive for factor XIIIa. One such cell type was small, bipolar or dendritic in shape, and, in focal areas, formed a cambium layer just beneath the epidermis which was rather thin in the 8-week gestation fetus and more developed in the fetus after 12 weeks (Figs. 4, 5). This layer was most developed at the tips of the limbs and at the sites of flexures, and on the skin of the face and the upper back. In contrast, larger more polygonal and stellate cells were scattered throughout the developing papillary and reticular dermis, and morphologically resembled the large stellate cells in the placental villi (Figs. 4, 5). These larger cells stained more intensely for factor XIIIa than the smaller cells. In many areas the larger cells tended to lie in a plane similar to that of the future fascia, below the dermis, and around the developing muscles.

In the fetal central nervous system, large cells that were factor XIIIa-positive were distributed in the meninges and dura, predominantly in the perivascular areas. These cells did not co-express macrophage/monocyte markers. Similar large cells that were strongly factor XIIIa-positive were found in the mesenteries and loose connective tissues surrounding much of the parenchyma (Table 2) of the gastrointestinal tract, heart, lung, pancreas, kidneys, and adrenal glands. In these organs the factor XIIIa positive cells tended to concentrate in the region of the future capsule of the organ, for example the pleura, the pericardium, or the renal capsule. Near the heart valves, there were occasional factor XIIIa-positive large cells. Similar large positive cells were found

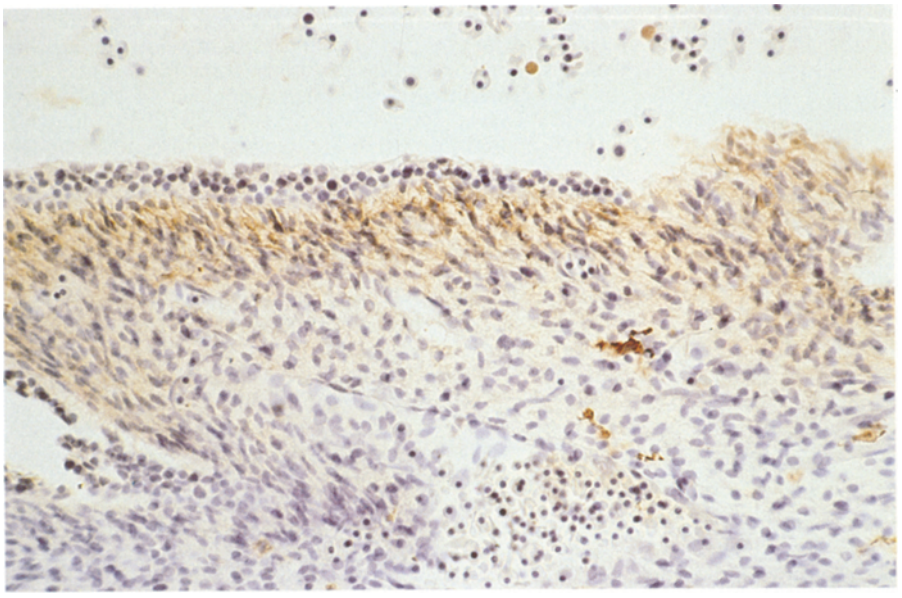

Fig. 5. Skin of a 10- to 20-week-old fetus, stained only for factor XIIIa. Small, positive, bipolar cells form a layer near the epidermis. Large polygonal and stellate cells that are strongly positive for factor XIIIa tend to be in the deep dermis to fascia. Immunoperoxidase, ×330

in the retroperitoneal lymph nodes and were in the peripheral regions of the spleen in the late fetuses. In the thymus, there were bipolar cells that stained intensely for factor XIIIa in the cortical areas and in the loose connective tissue around the thymus. In fetuses less than 12 weeks of age, neither Mac 387-positive nor LN3-positive cells were identified in these areas. Megakaryocytes were positive for factor XIIIa in the hematopoietic zones of fetuses after 16 weeks of gestation. In the lymph nodes and hematopoietic sites of the late fetus, there were admixtures of factor XIIIa-positive cells and Mac 387-positive cells. The lamina propria of late fetuses also contained admixtures of large cells that were positive for factor XIIIa and large cells that were Mac 387 positive. Double staining was not observed.

Discussion

A remarkable variety of cell types stain for factor XIIIa in human fetal tissues from a wide range of organs. These cells are usually in the connective tissues, particularly in areas that are to be the locations of capsules of organs, fascia, joint capsules, meninges, near heart valves, and in the dermis. They are also found in lymph nodes, spleen, and thymus, in addition to the megakaryocytes that are in areas of hematopoiesis.

This study has shown that at least two phenotypic variants of factor XIIIa-positive cells exist, one type that co-expresses monocyte/macrophage markers and another type that does not. The morphology of the cells in routine sections, stained with hematoxylin and eosin, does not always allow a prediction as to whether or not the cells will co-express factor XIIIa and the monocyte markers. The cells that do co-express these markers are evident in a variety of locations in the human fetus after the development of the fetal hematopoietic system. They are prominent in the extraembryonic membranes, in the reticuloendothelial system, and in the connective tissues of the respiratory system, gastrointestinal system, and in sites of hematopoiesis. In the skin, they are in the dermis near the blood vessels. Since some of these cells are HLA-DR positive, they may be capable of antigen presentation and of phagocytosis (Henriksson et al. 1985; Headington 1986; Cerio et al. 1989; Headington and Cerio 1990).

The second cell phenotype that is factor XIIIa positive but does not co-express monocyte/macrophage markers is most prevalent in the connective tissues such as the superficial dermis, the interstitium of deep soft tissues, and in the loose connective tissues and capsules of the visceral organs. In the placental villi, the large factor XIIIa-positive cells correspond to the cells that have been named Hofbauer cells (Arey 1954; Enders and King 1970; Zaccheo et al. 1989; Lewis et al. 1990), that are present prior to the development of the fetal hematopoietic system. These large cells may represent non-hematopoietic cells derived from primitive mesenchyme from the somatic and splanchnic mesoderm. From our studies in normal tissues, it is not clear to what extent the expression of monocyte/macrophage markers of HLA-DR might be inducible in these cells. In the term placenta, the number of cells co-expressing factor XIIIa and Mac 387 seemed approximately equal in placentas with and without evidence of chorioamnionitis. Other studies have indicated that Hofbauer cells can express monocyte or macrophage markers during the second and third trimester but this was not found in the first trimester (Zaccheo et al. 1989; Berkowitz et al. 1990). Hofbauer cells from aborted 8-week gestations can be HIV-1 positive on ELISA and Western-blot assays (Lewis et al. 1990). Hofbauer cells also have been demonstrated to be capable of immune-mediated and non-immune phagocytosis, as well as the elimination of exogenous antigen-antibody complexes (Benirschke and Kaufmann 1990). Further studies are necessary to determine to what extent pathological changes can induce the co-expression phenotype in Hofbauer cells or other

factor XIIIa-positive cells that normally do not co-express monocyte/macrophage markers in the fetus and adult.

Recent studies indicate that factor XIIIa and the macrophage marker Mac 387 label separate, possibly non-overlapping cell populations in normal adult skin and in a variety of pathological conditions (Cerio et al. 1990). These results complement our findings in the fetus that factor XIIIa-positive cells can arise from early mesenchyme without a relationship to blood monocytes or bone marrow precursors. In other investigation, factor XIIIa has been found to bind to the cell surface of fibroblasts and to remain catalytically active in the cross-linking of fibronectin in the extracellular matrix (Barry and Mosher 1990). Further studies are necessary to determine to what extent the cells labelled for factor XIIIa in the fetus synthesize factor XIIIa and to what extent they bind exogenous factor XIIIa, and how the factor XIIIa transglutaminase activity functions in the formation of the dense collagenous tissues of the fetus and adult.

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