Levels of the Thymic Factor Thymalin in Human and Murine Skin Epithelia During Ontogeny

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Skin specimens from humans, CBA mice, and nude mice are studied during ontogeny using antithymalin and antithymosin α_1 antisera in order to reveal extrathymic thymalin-positive cells and to collect more information on the involution of these cells with age. Thymalin is identified in young actively proliferating skin cells. In humans and mice, the number of thymalin-containing cells decreases with age. It is concluded that thymalin-positive cells not only of the thymic epithelial stroma but also of the epidermis involute with age.

Key Words: *skin; thymic factor thymalin; ontogeny*

The importance of thymic epithelium increases during ontogeny. At the early stages of embryogenesis, thymic parenchyma is represented by a network of reticuloepithelial cells producing hormonal factor which attracts undifferentiated lymphoid elements [6]. Involution of the thymus with age starts from the stromal epithelium. Progressive degeneration of the epithelial component coincides with decreased ability of the thymus to attract the precursors of lymphoid cells and reduction in the number of hormone-producing cells [9].

The properties and functions of thymic epithelium are morphogenetically similar to those of the epidermis. It has been shown that epidermal keratinocytes and cells of thymic secretory epithelium derive from the ectoderm and express identical antigens (TE-4, TE-8, TE-15, TE-16) [8]. In athymic (nude) mice, the epidermis may serve as a site of extrathymic differentiation of T cells [7]. Thymopoietin (identified by amino acid sequence) is synthesized in the skin during embryogenesis, this ability being lost by the epidermis after birth [10].

We have found that in human embryo the keratin phenotype of most cells in the thymic epithelial reticulum resembles that of undifferentiated basal cells of the epidermis [2]. Poorly differentiated cells of embryonal epidermis and thymic epithelium react with antiserum to the polypeptide thymic factor thymalin [5]. These findings point to the genetic similarity of the thymus and the skin.

Previously, we showed that all cells in human embryonal reticuloepithelium react with antithymalin antiserum at the early stages of embryogenesis (7-8 weeks). On the 23rd week of gestation, thymalin-positive cells were identified only in the subcapsular region and medulla. In a 2-year-old child, these cells are concentrated predominantly in the medulla [4].

The present study is an attempt to collect additional information regarding extrathymic location of thymalin-positive cells and their involution with age in humans and mice. For this purpose, skin specimens from humans, CBA mice, and athymic (nude) mice were studied by immunomorphological methods using antithymalin and antithymosin α_1 antisera.

MATERIALS AND METHODS

Skin specimens from 8-23-week-old human fetuses and two adults (40- and 70-year-old) who died from

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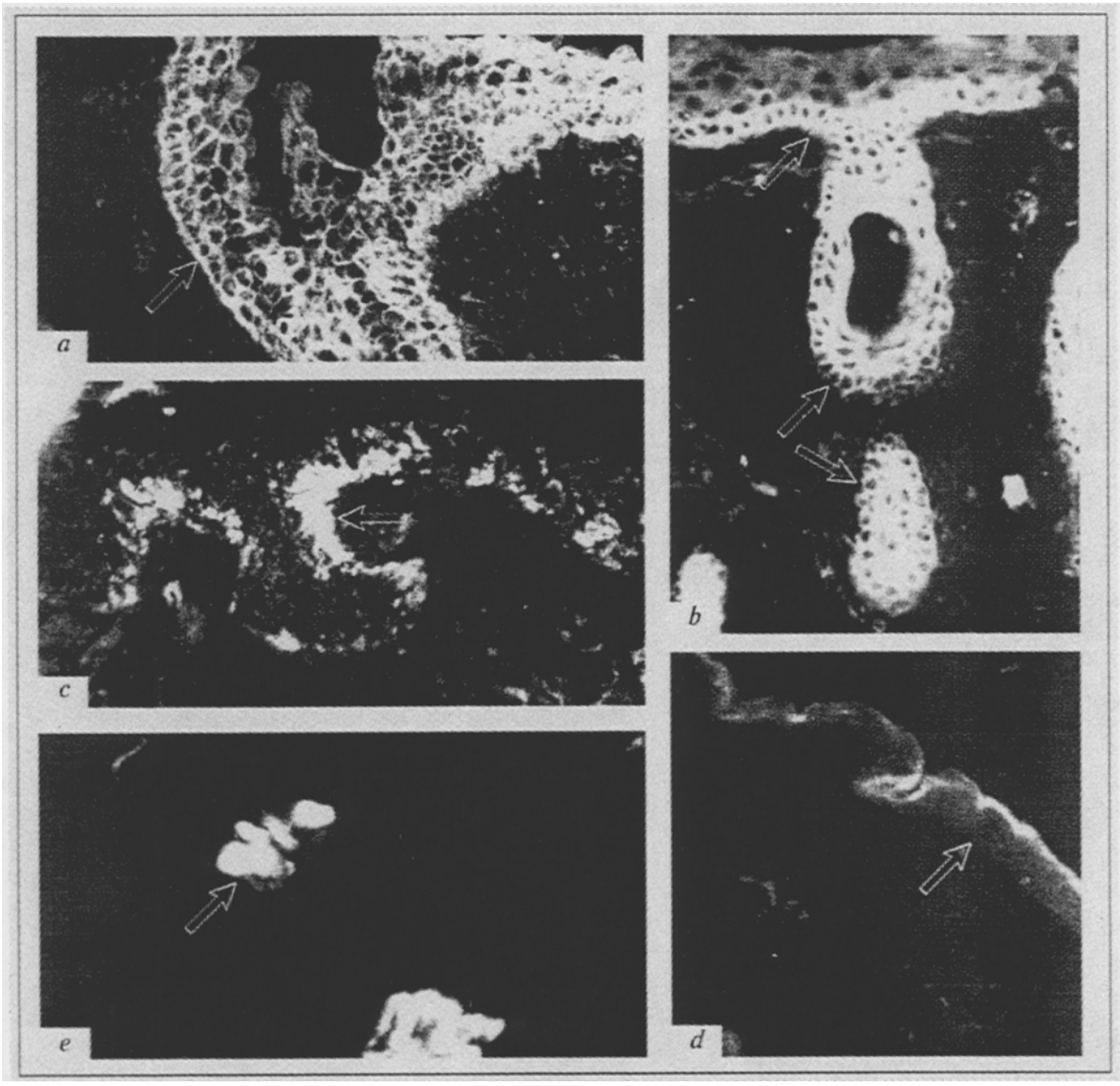

Fig. 1. Human and murine epidermis labeled with antithymalin antiserum. Immunofluorescent staining of skin specimens with rabbit anti-thymalin antiserum and FITC-labeled donkey antiserum to rabbit immunoglobulin, ×800. Arrows indicate thymalin-positive cells. *a*) epidermis from the hand of a 9-week-old human fetus: cells of all keratinocyte layers are thymalin-positive; *b*) epidermis from the head of a 23-week-old human fetus: the basal layer keratinocytes are thymalin-positive, intense fluorescence is confined to the hair follicle cells; *c*) epidermis of a 70-year-old man: only small groups of thymalin-positive cells occur in the basal layer of keratinocytes; *d*) epidermis from an old mouse: no immunofluorescence is seen; *e*) epidermis from a nude mouse: there is no immunofluorescence in keratinocyte layers, and only the rudiments of hair follicles are fluorescent.

ischemic heart disease, CBA mice (embryos, young mice aged 5-6 months, and old mice aged 24 months), and athymic adult mice were studied.

Serial cryostat sections were incubated with rabbit antisera to the polypeptide thymic factor thymalin and thymosin α_1 [3].

Thymalin is a set of polypeptides with Mr= 1-5 kD (as determined by gel chromatography on Sephadex G-25 and Sephadex G-50) which participate in T-cell differentiation by activating the expression of specific receptors on these cells [1].

Cryostat sections 4-6 μ thick were cut from specimens frozen in liquid nitrogen, air-dried, fixed in cold acetone, washed in cold phosphate-buffered saline, and incubated first with rabbit antithymalin or antithymosin antiserum and then with FITC-conjugated donkey antiserum to rabbit IgG. The preparations were examined under a LYuMAM-R3 fluorescence microscope. Control tests were carried out with intact serum, buffered saline, and anti-Thy1, anti-IgG, and antitriiodothyronine antisera. Intestinal and hepatic epithelia, which derive from the germ layer other than ectoderm, were studied in parallel.

RESULTS

Skin specimens from the hand, foot, back, and abdomen of 9-10-week-old human fetuses differed

from each other in the number of keratinocyte layers. These layers were formed by undifferentiated cells reacting with antithymalin antiserum (Fig. 1, *a*). The keratinized epithelium layer was absent.

At later stages, stratification of the epithelium and formation of sweat glands and hair follicles were observed at various sites. The number of keratinocyte layers increased in the foot and hand epidermis. A layer of keratinized epithelium and numerous sweat glands appeared on the 25th week of prenatal life. Thymalin-positive cells were located in the basal keratinocyte layer. The immunofluorescence of these cells was more intense than that of the epithelium of the secretory unit and excretory duct of sweat glands. The epidermis on the back and abdomen was thinner, being composed of fewer keratinocyte layers. Thymalin-positive cells were located in the basal epidermal layer and in the secretory units and excretory ducts of sebaceous and sweat glands. In the epidermis of the head, only the basal layer cells reacted with antithymalin antiserum. Strongly fluorescent were the cells forming hair follicles (Fig. 1, *b*). In a 40-year-old individual, the entire basal layer of the epidermis was composed of thymalin-positive cells, while in the epidermis of a 70-year-old individual only small groups of such cells were seen (Fig. 1, *c*).

These findings agree with the distribution of thymalin-positive cells in thymic medulla and subcapsular zone, which is a compartment of undifferentiated lymphoid and epithelial cells, and with the expression of keratins typical of undifferentiated keratinocytes by epithelial cells of medullar and subcapsular zones [2].

Thymalin- and thymosin α_1-positive cells were present in all epidermal layers of CBA mouse embryos and neonates. The epidermis of old CBA mice contained neither thymalin- nor thymosin α_1-positive cells, as evidence by the absence of immunofluorescence (Fig. 1, *d*).

The skin of nude mice which is devoid of hair and contains rudiments of hair follicles with large numbers of undifferentiated elements can be regarded as a model of atypical epidermis. After treatment with antithymalin antiserum, intense immunofluorescence was confined to the rudiments of hair follicles (Fig. 1, *e*). This finding is consistent with the hypothesis that extrathymic T-cell differentiation in nude mice occurs in the skin epithelium [7].

There was no fluorescence on control sections treated with intact serum or other hyperimmune antisera in the presence of normal saline. No fluorescence was observed in intestinal or hepatic sections.

Thus, both in humans and mice, the thymic hormonal factor thymalin is present in young actively proliferating cells not only in the thymus, but also in the epidermis, sweat glands and hair follicles. The time course of thymalin distribution in human skin does not differ from that in murine skin, the number of thymalin-containing cells decreasing with age. When keratinized epithelium is formed, thymalin-positive cells are concentrated in the basal epidermal layer. These cells gradually disappear from this layer with age. In sweat glands and hair follicles, specific fluorescence is confined to young cells. Thymalin-positive cells not only of the thymic epithelial stroma but also of the epidermis involute with age.

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