HISTOLOGY OF THE STROMA IN DEVELOPING RAT SUBCUTANEOUS ADIPOSE TISSUE

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Summary

Microscopic changes in the stroma of developing adipose tissues were studied. An organized arrangement of collagen fibers was associated with spindle-shaped cells with basophilic cytoplasm. An unorganized arrangement of collagen fibers was associated with cells of irregular shape, little cytoplasmic basophilia and a large pale-staining nucleus. Adipocyte development was apparent only in unorganized collagen fibers. The unorganized stroma was located near muscle-tendon junctions. Other aspects of muscle were surrounded by organized stroma. Physical interactions between expanding muscle and connective tissue cells may prevent or delay collagen maturation, resulting in a matrix suitable for adipocyte development. (Key Words: Adipocyte, Muscle Stroma, Muscle-Tendon Junction.)

Introduction

Preadipocytes in developing rat (Napolitano, 1963) and mouse (Slavin, 1979) adipose tissue are ultrastructurally identical to active fibroblasts. Fibroblasts are distributed throughout the body in connective tissue proper, and yet, specific areas of the body are prone to adipose tissue accumulation. This paradox indicates that unknown local factors may control the fibroblast-to-adipocyte conversion.

Cellular shape is a primary factor regulating the mitogenic response of a given cell type to mitogens (Gospodarowicz et al., 1978). The extracellular material upon which the cells rest can dictate cellular shape (Gospodarowicz et al., 1978) and induce cells to shift their pattern of differentiation (Reddi, 1976). For example, demineralized bone matrix implanted in subcutaneous sites can induce the entire endochondral sequence (Reddi, 1976). Fibroblasts migrate to the matrix surface, and after migration, proliferate and give rise to cartilagenous tissue. Formation of bone marrow is complete by 24 d after implantation. Therefore, the substrate upon which cells rest can shift the pattern of genetic expression of the already differentiated fibroblasts.

Similarly, the fibroblast-to-adipocyte conversion may be controlled by nature of the substrate. In adipose depots, where fibroblasts apparently undergo the conversion to adipocytes (Napolitano, 1963; Slavin, 1979), the nature of the collagenous stroma has not been reported. We have analyzed this stroma in the rat inguinal fat pad during a period of rapid and abundant adipocyte differentiation (Greenwood and Hirsh, 1974). The purpose of the present study was to determine whether morphological alterations in the stroma are linked spatially or temporally to the conversion of fibroblasts to adipocytes.

Materials and Methods

Preliminary observations indicated that clusters of small, multilocular adipocytes were often located adjacent to the connective tissue stroma surrounding inguinal adipose tissue in rats 3 d old and older. Histological techniques were employed to study the characteristics and integrity of the stroma in combination with techniques that highlight cytoplasmic and nuclear changes of stromal cells.

Tissues for microscopy were obtained from Sprague-Dawley rats (n = 70) ranging in age from newborn, unsuckled to 21 d. Rats were rendered unconscious and then decapitated. Whole and hemibody 1-cm sections were obtained from the younger animals (<14 d), whereas the inguinal adipose pads were removed from older rats. Tissues were either fixed in Bouin’s fixative or 10% neutral buffered formalin and then routinely processed into...
paraffin blocks after 2 to 3 d fixation. Paraffin sections (10 μm) were stained with the periodic acid Schiff (PAS) reagents (Humason, 1972) and Harris hematoxylin, toluidine blue (Humason, 1972), Lillie's allochrome reagents and the picric ponceau reagents (Humason, 1972).

Results

Stromal Morphology of Rats Younger Than 3 Days. The stroma surrounding areas of adipocyte and capillary formation was a loosely arranged collection of collagenous fibers containing a few cells, no blood vessels (figure 1A,C) and a nonmetachromatic (toluidine blue) intercellular substance. The cells contained a deeply stained cytoplasm (toluidine blue) and nuclei. Stromal adjacent to muscles was of two varieties depending on the particular location...
along the muscle. Areas close to muscle-tendon junctions contained collagenous fibers with no particular arrangement (figure 1 B). The cells present in this unorganized stroma did not resemble typical fibroblasts (figure 2 A,B,D). Unorganized stromal cells were characterized by an irregular outline and large, pale-staining nuclei surrounded by a pale-staining, often vacuolated, cytoplasm. Examination of serial sections indicated that as the distance from the muscle-tendon junction increased, the stromal morphology gradually changed and eventually became very organized (figure 2 C). The intercellular substance of organized stromas was not metachromatic (toluidine blue) but was slightly metachromatic in unorganized stromas. Adipocytes and blood vessels were not present within the unorganized stroma. Areas around muscles deeper in the body contained mesenchymal-like cells, reticular fibers (red colored, Lillie's stain) and nonmetachromatic (toluidine blue) intercellular substance (figure 3).

Stromal Morphology of Rats Older Than 3 Days. As in the younger rats, there were two distinct types of stromal morphology around inguinal adipose tissue from rats 3 d and older. However, adipocytes and blood vessels were present in stromal areas close to muscle-tendon junctions (figure 4 A,B,C,D). These adipocytes and vessels were surrounded by unorganized stromal cells and fibers (figure 4 B) and a metachromatic (toluidine blue) intercellular substance. Adipocytes were never present within organized stromas.

Small, multilocular adipocytes were darkly stained and consistently present in clusters of

Figure 2. The cellular nature of the stroma close to the muscle-tendon junction (A, t; B and D) and at points away from the muscle-tendon junction (A, a; C). Stromal cells near the muscle-tendon junction (B and D) are pale-staining and have irregular and variable morphology. In contrast, at points away from the muscle-tendon junction, the stromal cells (C) are darkly stained and are more morphologically homogenous. The section in A demonstrates that the sectioning plane of the muscle had no effect on stromal cell morphology. Both muscles in A are cut in similar tangential planes. As demonstrated in B and C, stromal cell morphology is different. The section in D is from an area similar to the area indicated in figure 1 A by an asterisk. Obviously this is an area between two muscles that is close to the muscle-tendon junction. Indicated are muscle (m) and stromal cells (arrows). Paraffin sections (10 µm) stained with toluidine blue. (A × 63; B, C, D × 150.)
Figure 3. The collagenous stroma around deep muscles in the shoulder region of a newborn rat. Undifferentiated mesenchymal cells (arrow) and reticular fibers (red color, Lillie's stain) indicate an undifferentiated stroma. Paraffin section (10 μm) stained with Lillie's allochrome (×460).

two or more cells (figure 5 A). Nuclei of these cells contained one to three large and prominent nucleoli surrounded by a pale-staining nuclear material. In the unorganized stroma, there were clusters of cells with nuclei containing prominent nucleoli but few or no cytoplasmic lipid droplets (figure 5 B). Clumping of stromal cells was prerequisite to the acquisition of nuclear morphology typical of small adipocytes. Mast cells were rarely located in layered stromas. Transitional areas between layered and unlayered stromas often contained large numbers of mast cells.

Discussion

This study establishes that connective tissue cell morphology is associated with a particular arrangement of collagen fibers. Typical fibroblasts were present among layered connective tissue fibers. An unorganized arrangement of fibers contained cells morphologically distinct from fibroblasts. Adipocyte development was seen only among the unorganized connective tissue fibers. Stromal and cellular relationships have not been reported previously (Napolitano, 1963; Slavin, 1979). An interaction of stroma and cells may not have been obvious in previous experiments for several reasons. Staining techniques specific for demonstrating the nature of the stroma were not used, and, therefore, possible interactions went unnoticed. The small and limited sample size used in electron microscopic studies (Napolitano, 1963; Slavin, 1979) would not allow the recognition of the anatomical associations that were noted in the present study. The presence of the unorganized stroma near muscle-tendon junctions offers an explanation for the coexistence of two types of stromata. The longitudinal growth of the muscle at the muscle-tendon junction, in conjunction with the generalized radial growth, may physically interfere with connective tissue. It is assumed that connective tissue cells are synthesizing connective tissue proteins and are, therefore, stationary (fixed in position) cells. Tissue movement (muscle, bone, etc.) resulting from growth must result in physical interactions with the stationary connective tissue cells. A unidirectional movement may merely compress the connective tissue cells and fibers. In contrast, a two-directional movement, such as around the muscle-tendon junction, would be physically disruptive because of the tearing action of moving in two directions. Physical interactions might delay or prevent collagen maturation, resulting in a matrix suitable for adipocyte development.

In culture, human and rat preadipocytes
Adipocyte development in muscle stromata (A, B, C) close to the muscle-tendon junction in tissue from a 4-d-old rat. Groups of small adipocytes (a) are located in an area between two muscles that is close to the muscle-tendon junction. Section in C is serial to the section shown in A and B. Note the similarity of the area in A and C to that shown in figure 2 D. In young rats (<3 d) these areas (figure 2 D and figures 4 A and C) never contained adipocytes. The size of adipocytes present in the subcutaneous stroma is shown in D. Note the larger and mostly unilocular cells (ua) in D as opposed to generally smaller and more multilocular adipocytes in B and C. Paraffin sections (10 μm) stained with toluidine blue (B) and with PAS and Harris hematoxylin (A, C, D). (A, C and D X 192; B X 320.)

(Poznanski et al., 1973; Roncari and Van, 1978) became mature adipocytes, while dermal fibroblasts cultured similarly did not. Presumably, the dermal fibroblasts are not exposed to physical and chemical environment, as would be the adipose tissue fibroblasts, and, therefore, have not received the necessary stimuli.

The nuclear morphological changes in clusters of differentiating adipocytes have not been reported previously. The nuclei of adipocytes in newborn rats do not contain the prominent nucleoli that are present in adipocyte nuclei of 18 h and older rats (G. J. Hausman, unpublished observations). Other researchers have studied the fetus (Desnoyers and Vodovar, 1977) or have pooled data on newborn and early postnatal animals (Napolitano, 1963; Slavin, 1979). A rapid rate of adipocyte differentiation is evident in early postnatal animals (Greenwood and Hirsch, 1974). Therefore, the morphological changes in nuclei of developing adipocytes (present study) may represent histological evidence that the postnatal environment hastens adipocyte differentiation much more than does the fetal environment.

The arrangement of preadipocytes in clusters (present study) was not reported in a previous study of developing rat adipose tissue (Napolitano, 1963). In the present study, the cluster arrangement was prerequisite to the changes in nuclear morphology that were indicative of adipocyte development. Therefore, the cell-to-cell contact in the clusters may be a necessary stage of adipocyte differentiation. Napolitano's
(1963) micrographs showed differentiating adipocytes closely applied to endothelial cells. Napolitano (1963) considered the assumption of an oval or rounded shape as an initial stage of adipocyte differentiation. If the adipocyte differentiation process is considered to be without stages or steps, then a cell cluster arrangement may be considered a consequence of development instead of an integral phase. In vitro data (Roncari and Van, 1978) indicate that cells with enzymes characteristics of mature adipocytes but containing no lipid are present in adipose tissue. The presence of several types of preadipocytes (lipid-free, no enzymes; lipid-free, enzymes characteristic of adipocytes) implies several phases of adipocyte development. With these new perspectives and reports of apparent cell-to-cell contact in developing sheep (Wensvoort, 1967), pig (Hermans, 1973) and mice (Slavin, 1979)
adipocytes, the significance of cell cluster arrangement cannot be ignored.

Literature Cited