Regulation of Differentiation of Murine Intestinal Epitheliocytes

T. M. Yavisheva, E. G. Khlynina, and O. Yu. Semenyak

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The most important epithelial cells of murine small intestine are the "initial" maternal and daughter cells functioning as a single unit and presenting two main types of cell populations. One type is characterized by the balance between the maternal and daughter cells, while in the other type this balance is impared. The predominance of maternal cells provides maturation of the daughter cells.

Key Words: *maternal and daughter cells; differentiation*

The problem of regulation of cell proliferation and differentiation is now extensively studied, because imbalance in these processes can result in the development of different pathological states including tumor growth. At the population level proliferation and differentiation are regulated through different mechanisms, including neurohormonal control and chalon-mediated maintenance of the total cell mass. However, complex processes of cell division and maturation have not been completely elucidated. The important part of these studies was carried out on cells of basal epithelial layer and the cornea [6,7].

In murine cornea and intestinal epithelium, as well as in human epidermis the most important functions are performed by so-called "initial" cells giving rise to maternal (MC) and daughter (DC) cells in the course of proliferation. These similar in size cells differ by their surface orientation. In cell population they exist in two types (I and II). Each of these types is presented by a functional unit of MC and DC. The cells of type I maturate under the differentiative influence of the II type. Thus, the relationship between cells of types I and II is the key factor of differentiation.

The aim of the present study was to elucidate the mechanisms of interaction between MC and DC of types I and II.

N. N. Blokhin Russian Oncology Research Center, Russian Academy of Medical Science, Moscow

MATERIALS AND METHODS

Intestinal epitheluim of 20 adult female BALB/cj mice was examined. The animals were killed with ether at 3.00, 9.00, 15.00 and 21.00 (5 mice per point). Epithelium imprints were treated with silver nitrate according to Ranvier. Morphometric analysis was performed on a Kontron image analysis system. Epitheliocyte area, cell ellipticity (CE), and the angle between the long cell axis and the abscissa were estimated in different periods of epithelium proliferation. Definite orientation of the long cell axes allowed to analyze fields formed by parallel cell accumulations [1,2]. The histograms of cell distribution by the above mentioned parameters were obtained. For each parameter 2000 cells were examined. Earlier we revealed a correlation between mitotic activity in different periods of a day and cell distribution by CE [8]. This allowed us to estimate the dynamics of epithelium proliferative activity by these histograms. The data were analyzed statistically using Wilcoxon—Mann—Whitney *U*-test.

RESULTS

The comparison of the area of basal cell layer with CE and the angle between long cell axis and the abscissa revealed a definite orientation of epithelial cells in relation to each other. The main subject of our investigation was MC and DC. MC and DC of type I

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had similar areas (61.14 μ^2) and CE (0.9258). The same was observed in type II cells, though the area (52.41 μ^2) and CE (0.6124) differed from those of type I cells. Types I and II MC differed from the corresponding DC by surface orientation. MC and DC of types I and II had perpendicular orientation: MC are arranged vertically, while DC horizontally. The number of type I cells was 2-fold lower than type II cells and cells of both types could transmutate into each other [6]. Type II cells had bipolar shape (CE=0.6124) in contrast to round-shaped type I cells (CE=0.9258). Similarly oriented bipolar cells or dipoles are known to form fields [1,9].

During resting period characterized by low proliferative activity, bipolar MC and type II DC are arranged perpendicularly to each other and their content is equal (by 3.6%; Fig. 1). At this period type I cells are presented by MC only (about 1.8%). Such a balance between MC and type II DC would eliminate the field effect of the cells, because the fields created by two equal but perpendicularly oriented cell populations annihilate [4,5]. In live epithelium it does not occur because type II cells do not act separately but form a single system with type I cells regulating differentiation processes. Type I cells presented by weak dipoles consist exclusively of MC showing the same orientation as type II MC. The fields created by similarly oriented cell dipoles do not compensate but add to each other [2]. Consequently, the balance between type II MC and DC is shifted to enhancement of the field effect of MC. Thus, the conditions for the influence of MC on DC appear, because maximum effect of one dipole on anothers is provided by their perpendicular orientation and predominance of one type of dipoles [3]. This induces transformation of DC into other cells: the number of type II DC decrease from 3.6 to 0.5% ($p<0.05$), while the content of other cell types increases. The number of type II DC decreases and during the period of intense proliferation type I is presented by MC only (Fig. 1). As a result of these processes types I and II are presented by MC only. As mentioned above, type I cells can transmutate into type II and vice versa. The content of type II MC increases from 3.6 to 6% ($p<0.05$), while the number of type I MC decreases to 0.2%. Thus, a dynamic pool of maternal cells is formed. During this period MC concentrate in type II only and start changing into "initial" cells. The number of MC decreases by 4%, while the number of "initial" cells increases correspondingly. Thus, autorenewal of "initial" cells occurs. Type I MC are transformed into type II which stimulates the recovery of the I type cell population from "initial" cells. In type I the number of MC and DC becomes equal (by 2%; Fig. 1) due to intense proliferation of "initial" cells. During this period the balance between MC and DC is maintained only in type I, while type II is presented by MC only. Since these MC are strongly dipolar, they create a field trans-

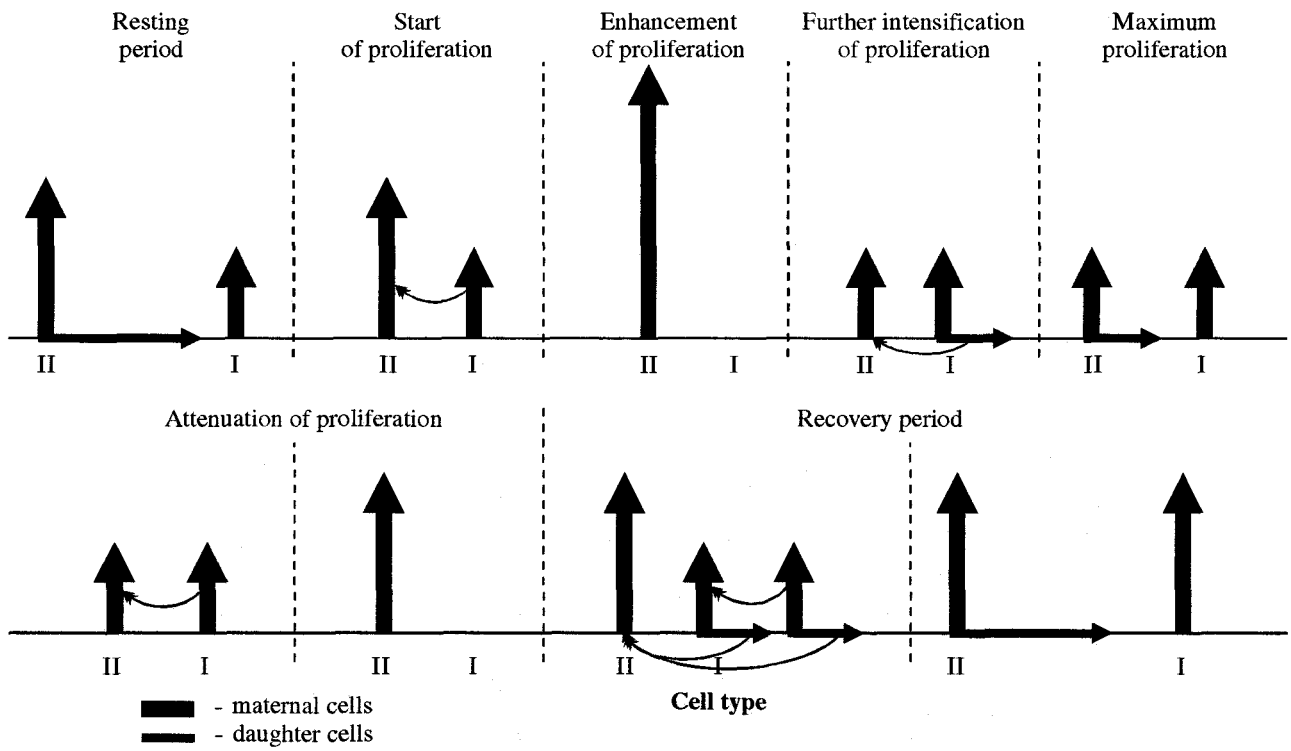

Fig. 1. Scheme of interrelations between types I and II maternal and daughter cells in the course of proliferation.

forming type I DC into type II. The number of type II DC sharply increases and reaches the MC level. Consequently, during maximum proliferation, the balance between MC and DC is established in type II, while type I is presented by MC (Fig. 1).

Thus, types I and II cells interact during various periods of proliferation: the balance between MC and DC of one type can be established due to disbalance between these cells of another type.

The coordinated functioning of types I and II cells is maintained throughout the whole proliferative process in the epithelium (Fig. 1). During the recovery period the number of type II cells returnes to baseline value. We showed that during this period the number of type I DC increases significantly and reaches the level of type II MC (Fig. 1). Then type I DC are transformed into corresponding type II cells, thus restoring the balance between type II MC and DC. Thus, during the recovery period "initial" cells undergo not one but several successive divisions giving rise to new DC. Thus, DC perform a double function in cell population: first, they play a role of a buffer compensating the content of type II MC, which can block cell differentiation acting as strong dipoles, and second, they serve as a source of cells, which maturate under the differentiative effect of MC.

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