MORPHOLOGY AND PATHOMORPHOLOGY

Radioautographic Study of Palatine Tonsillar Cells

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Proliferative and functional (protein producing) activities of palatine tonsillar cells were studied by light radioautography (semithin sections). ^{3}H-Thymidine incorporation demonstrated the dynamics of renewal of palatine tonsillar cell populations in various pathological states. ^{3}H-Uridine labeling objectively evaluated functional activity of different cells and more accurately showed distribution of destructive processes in the organ.

Key Words: *radioautography; palatine tonsils; proliferation; protein producing activity*

The incidence of chronic tonsillitis in Russia is 22.1-40.1% [4,5,6]. There are no precise criteria indicating when the tonsils transform from organs performing useful functions into a focus of infection.

The development of a diagnostic test or a series of tests (algorithm) for evaluation of functional and proliferative activity of palatine tonsillar cells will help to detect their functional reserve in various diseases, clear out whether this activity changes with age, and whether this organ is subject to "justified age-associated involution" [1,2]. These data are essential for the choice of rational treatment strategy, specifically, for making a solution on tonsillectomy, especially in childhood.

Radioautographic study with low-molecular-weight precursors of nucleic acids (^{3}H-uridine and ^{3}H-thymidine) was carried out in order to evaluate functional (protein production) and proliferative activities of the palatine tonsillar cells. No study of this kind were previously performed.

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MATERIALS AND METHODS

Specimens of palatine tonsillar tissue for the analysis were obtained after tonsillectomy from 2 children aged 4 and 7 years with the diagnosis of hypertrophic palatine tonsils and 4 adults aged 25-48 years with the diagnosis of chronic tonsillitis.

Tissue specimens were incubated in medium 199 with ^{3}H-uridine (100 μCi/ml; specific activity 26.0 Ci/mM) or ^{3}H-thymidine (20 μCi/ml; specific activity 21.6 Ci/mM) at 37-38°C for 1.5 h. After incubation and washout from free precursor the material was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, embedded in epon-araldite resin, and semithin sections and radioautographs were made as described previously [3]. The sections were examined under a Leitz light microscope.

RESULTS

Radioautographic study of biopsy specimens of palatine tonsils with labeled DNA precursor ^{3}H-thymidine showed that this method can be used for evaluation of proliferative activity of various cells of the organ. Today radioautography is the best method for evaluating proliferative activity of mor-

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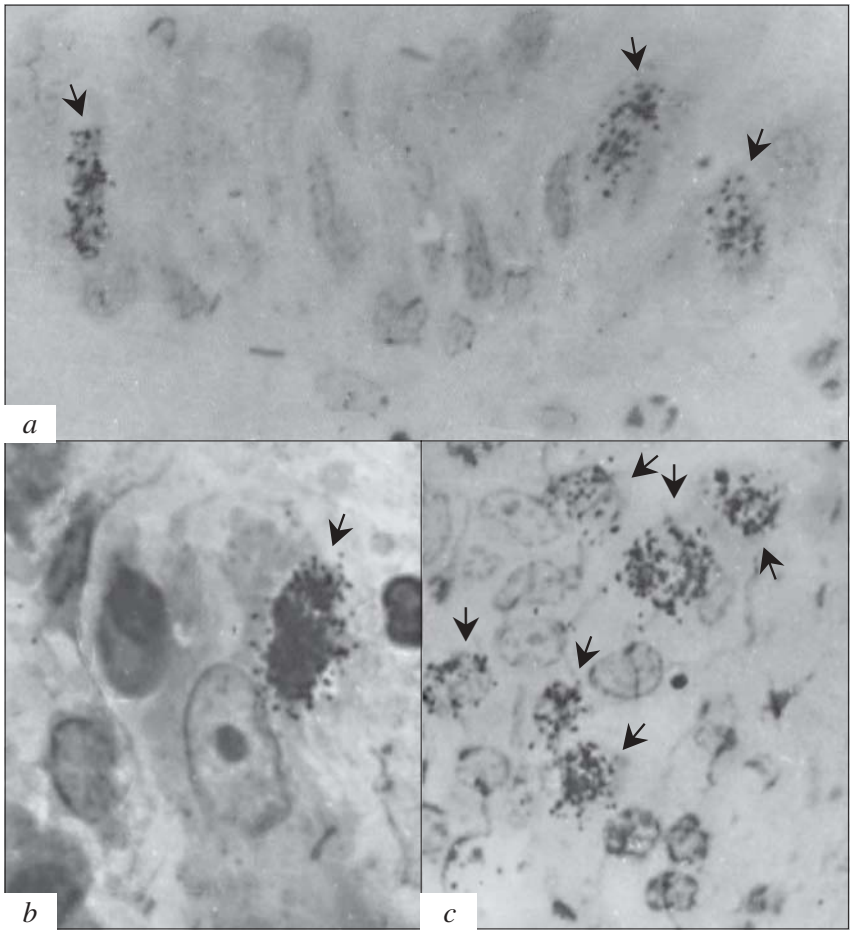

Fig. 1. ^{3}H-Thymidine incorporation (DNA synthesis, dark silver grains) in palatine tonsillar cells (arrows). Semithin sections. *a*) epithelial cells; *b*) fibroblast; *c*) lymphocytes. Toluidine Blue staining, ×1000.

phologically identifiable cells. ^{3}H-thymidine incorporation into a cell is a reliable evidence of DNA synthesis in this cell during incubation. The synthesis of DNA indicates cell preparation to division or increase in ploidy. Palatine tonsillar epithelial cells incorporated ^{3}H-thymidine (Fig. 1, *a*); connective tissue cells and fibroblasts (Fig. 1, *b*) and capillary wall cells (endotheliocytes and pericytes) incorporated it more often; lymphocytes incorporated the label rarely and only in young patients (aged below 18 years) (Fig. 1, *c*).

Analysis of radioautographs made after incubation of biopsy sample with ^{3}H-uridine evaluated the rate of RNA synthesis in the studied cells. Since various RNA types maintain protein production, the density of ^{3}H-uridine label indicates the intensity of differentiation process provided by new proteins; when the process is completed, it leads to the formation of other proteins. RNA synthesis is also essential for the maintenance of protein synthesis in differentiated cells. In contrast to DNA synthesis, limited to just one (synthetic) period of the cell cycle, RNA is produced during the entire cell cycle except a short period of mitosis when all chromatin is condensed. Moreover, RNA is produced in cells not participating in the cell cycle (in the G_0 period). These characteristics of RNA synthesis allow using ^{3}H-uridine label as an indicator of cell viability, as we can assume that RNA is produced in all live cells. This latter fact is very important for the study of palatine tonsils in which some cells and whole follicles die. In these cases ^{3}H-uridine label more accurately than morphological analysis differentiate live and dead cells (Fig. 2, *a*). On the other hand, the study of palatine tonsillar tissues showed that the use of ^{3}H-uridine label as a criterion of cell viability is justified for the majority, but not for all cells. Mast cells, numerous in the palatine tonsillar tissue, were poorly labeled or not labeled at all (Fig. 2, *b*), probably because they represent the final stage of cell differentiation and produces protein only for provision of intracellular processes. Plasma cell is also a cell at the final stage of differentiation, but it releases proteins into extracellular space, and ^{3}H-uridine is intensive incorporated in these cells (Fig. 2, *c*).

We think that the radioautographic method can be used as an objective indicator (by ^{3}H-thymidine incorporation) of the dynamics of the main cell

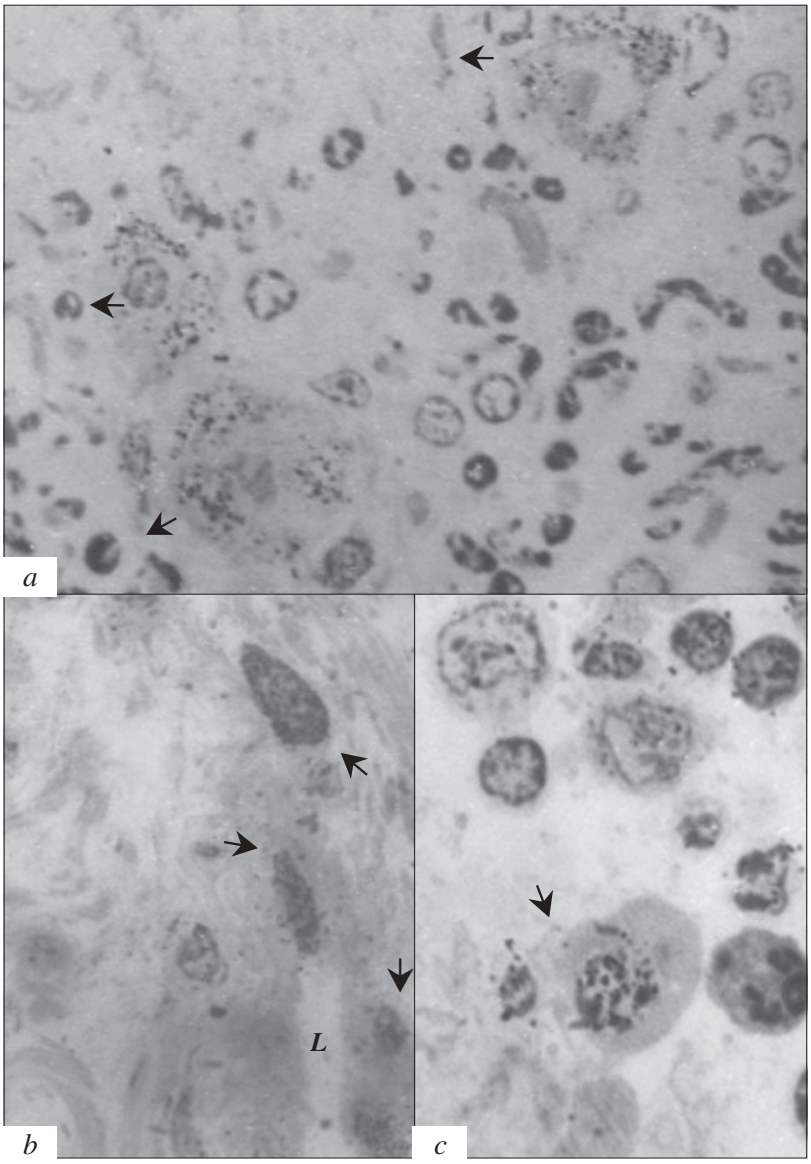

Fig. 2. ^{3}H-Uridine incorporation (RNA synthesis, black silver grains). Semithin sections. *a*) labeled capillary endotheliocytes (arrows) among degenerating (dead) lymph follicle cells; *b*) no label in mast cell (short arrow) against the background of intense label in endotheliocytes (long arrow) of a neighboring vessel; *c*) RNA synthesis in plasmacyte nucleus (arrow). *L*: vascular lumen. Toluidine Blue staining, ×1000.

populations in palatine tonsils in various diseases. ^{3}H-Uridine label helps to objectively evaluate functional activity of cells and more accurately assess the distribution of destructive processes.

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