Fluorescent Granular Cells of the Thymus Can Be Identified as Dendritic Macrophages

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Fluorescent granular cells of the thymus lobule containing neurotransmitter monoamines express Ia antigen and S-100 protein, which attests to their macrophage origin; positive staining with aldehyde fuchsin points to secretion of peptide hormones by these cells. These facts and the absence of phagocytic activity allow to identify these cells as dendritic macrophages.

Key Words: *thymus*; *catecholamines*; *serotonin*; *histamine*; *aldehyde fuchsin*; *catecholamines*; *fluorescent granular cells*; *Ia antigen*; *S-100 protein*; *dendritic macrophage*

Previous histochemical studies of the thymus demonstrated the presence fluorescent granular cells (FGC), which cannot be detected by routine staining techniques [5].

Premedullary, subcapsular, and deep cortical FGC are distinguished. Each autofluorescent cell contains fluorescent granules (white, yellow, or green) of various sizes. Paraformaldehyde-induced chemiluminescence significantly enhances fluorescence of granular cell. Hence, FGC are characterized by true fluorescence and contain catecholamines, serotonin [5], and histamine [3].

Further experiments revealed different functional responses of premedullary and subcapsular FGC to modulators of the immune and autonomic nervous systems: secretion of serotonin and histamine (but not catecholamines) by premedullary FGC, and the presence of biogenic amine uptake mechanisms in subcapsular FGC. Moreover, FGC exhibited no direct phagocytic activity, reaction for acid phosphatase was negative. Sequential staining of the same FGC with several staining techniques showed that apart from biogenic amine-specific fluorescence it possesses all properties typical of APUD cells (argentophilia, metachromasia, aldehyde fuchsin-positive reaction). It was hypothesized that lymphoid FGC possess the properties of both macrophage and APUD cells. However, experimental data are insufficient to proof macrophage, or neuroendocrine origin of thymic FGC.

Our aim was to find out whether thymic FGC belong to the APUD system and to acquire more evidence on their macrophage origin.

MATERIALS AND METHODS

Experiments were performed on 250 albino rats weighing 150-200 g. The control group consisted of intact rats (n=90); group I comprised ovariectomized rats (n=50); group II rats included ovariectomized rats treated with estradiol (n=50); and group III comprised rats at different terms of pregnancy (n=60).

The thymus was extracted under deep ether anesthesia and 15-μ cryostat sections were prepared. Monoamine neurotransmitters were detected by Falck fluorescence-histochemical method (modified by E. M. Krokhina). Lipid complexes were stained with Sudan Black B with 60-min hydrolysis in 0.1 N HCl. Secretory process in cells was evaluated by staining with aldehyde fuchsin, argentaffinity by Masson—Fontana reaction, metachromasia by toluidine blue staining by the method of Unna, and expression of Ia antigen and S-100 protein with FITC-labeled antibodies using a filter transmitting only specific fluorescence.

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RESULTS

Thymus responses to different immune events was studied on a natural model of pregnancy. Trimester I is characterized by T-cell immune conflict [2], trimester II by immunotolerence, and trimester III by progressive T- and B-cell immune conflict. Fluorescence-histochemical method and staining with sudan black B demonstrated that subcupsular FGC were primarily detected in trimester I, premedullary FGC predominated in trimester II, while trimester III was characterized by the appearance of numerous FGC in the subcapsular zone and in deep cortical layers and disappearance of premedullary FGC.

Expression of Ia antigen in trimester I was observed on subcupsular FGC and during trimester II on premedullary FGC forming a continuous layer around the medulla. Abundant FGC in the deep cortical layer were seen in trimester III [12].

In intact animals, aldehyde fuchsin treatment yielded week positive staining. In ovariectomized rats, solitary aldehyde fuchsin-positive cells appeared in the premedullary zone. In estradiol-treated ovariectomized rats the aldehyde fuchsin-positive cells were detected in both the subcapsular and premedullary zones.

Our experiments showed that the premedullary zone of the thymus lobule contains cells exhibiting no phagocytic activity, but expressing macrophage markers, *i.e.* major histocompatibility complex proteins and class II (Ia antigens). Moreover, these cells contained S-100 protein, characteristic of dendritic macrophages.

Our hypothesis that FGC combines the activities of macrophages and secreting APUD-cells would be inconsistent because premedullary FGC of the thymus manifested no phagocytic activity. However, taking into account recent data on dendritic macrophages, one can conclude with a high degree of confidence that premedullary FGC belong to the population of dendritic macrophages.

It was demonstrated that dendritic cells are macrophages of the mesenchymal origin migrating from the red bone marrow to the epidermis, where they form dendrites, lose phagocytic activity, and acquire properties of antigen-presenting cells [6,9,10,11]. A population of dendritic macrophages was identified in lymphoid organs; these cells can not present the whole antigen, but only its fragments [4].

Since maximum detection of premedullary FGC in the specific reaction for lipid complexes and expression of Ia antigen coincided with the period of immune tolerance during pregnancy, it can be hypothesized that antigen-representing activity of premedullary FGC is associated with the appearance of fetal B-dependent antigens occurring in trimester II [2].

The fact that aldehyde fuchsin-positive staining of subcapsular FGC appeared only in ovariectomized rats treated with estradiol suggests that these FGC form an independent subpopulation, despite their morphological similarity to premedullary FGC. The third subpopulation consisted of FGC abundantly seen in the deep cortical layer in trimester III.

The existence of three FGC subpopulations (with different antigen properties and different sensitivity to pharmacological agents) in the thymus is confirmed by immunocytochemical studies [10,13,14].

A positive aldehyde-fuchsin-positive staining in thymic subcapsular and deep cortical FGC in ovariectomized rats treated with estradiol and in pregnant rat during trimester III can be considered as a shift in thymocyte differentiation in response to new immune or hormonal status. Realization of such cellular response accompanied with phenotypic transitions was confirmed experimentally [15].

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