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Medullary *HLA-DR* immunopositive cells of human fetal thymus are involved in negative T-lymphocytes selection

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Accumulating evidence shows that the T-cell precursors move from the bone marrow to the thymus where they are selected for self-tolerance by exposure to MHC antigens on stromal cells. Those developing thymocytes that bind too strongly to self MHC molecules will be induced to undergo apoptosis (negative selection) because these cells would have the potential to cause autoimmune diseases. We applied monoclonal antibodies, immunocytochemistry, electron microscopy and flow cytometry to investigate the HLA-DR immunoreactivity and apoptosis of thymus medullary cells in human fetuses. The results presented provide new proofs about the role of fetal HLA-DR immunopositive cells in thymus medulla as eventual sites of negative prenatal T-cell selection processes.

Key words: fetal thymus, negative T-lymphocytes selection.

Introduction

It is well established that the mature T cells expressing αβT cell receptors (TCRs) are generated in the thymus via a complex process of positive and negative selection. Those developing thymocytes that bind too strongly to self major histocompatibility complex (MHC) molecules will be induced to undergo apoptosis (negative selection) because these cells would have the potential to cause autoimmune diseases [1, 2]. Evidence is presented that negative selection occurs at a relatively late stage of thymocyte differentiation and affects a population of CD4+CD8-/αβTCR+ and CD4-CD8+/αβTCR+ cells found in the medulla. Several important questions about positive and negative selection in the thymus still remain to be answered though [3, 4]. Some kinds of thymic cells such as epithelial cells, dendritic cells, macrophages, B lymphocytes, activated T lymphocytes and thymic "nurse" cells (TNC) express MHC class II antigens, including human leukocyte-associated antigen-DR (HLA-DR). However, relatively little is known of the details of HLA expression and

T-lymphocyte selection in human thymus [1, 5, 6]. In view of the above, the present study was focused on the detection and analysis of HLA-DR immunoreactivity and apoptosis of medullary cells in fetal human thymuses as possible sites of prenatal negative T-cell selection, using monoclonal antibodies, immunocytochemistry, electron microscopy and flow cytometry.

Material and Methods

Our study covered human fetuses (6th-7th month of gestation; n=12) during interruption of normal pregnancy and thymuses of old (aged 60-70 years; n=6) individuals, obtained from thoracic surgery cases. They had no pathological disorders. Annexin V (FL), sc-4252, (Santa Cruz Biotechnology) and three kinds of monoclonal antibodies (Ab), namely Anti-Pan cytokeratin Ab (C 1801, Sigma Chemical Co.), Anti-CD 14 Ab (UCH-M1, sc-1182, Santa Cruz Biotechnology) and Anti-HLA-DR Ab /HK 14, IgG2a, Sigma Chemical Co.) were tested. Indirect immunoperoxidase (IIP), immunofluorescence and transmission electron microscopy (TEM) were performed according to the protocols that we described previously [5, 6]. To define the nature of the thymic cell types we stained serial tissue sections with Anti-cytokeratin Ab, Anti-CD14 Ab and Annexin V which reacted with epithelial cells, monocyte/macrophages and apoptotic cells, respectively (according to the manufacturer's instructions). Control experiments were carried out in parallel. Labomikroscop Axioskop 20 (Fb Carl Zeiss Opton) and electron microscope Hitachi H500 were used. Two-colored flow cytometry was performed using FACSalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA). Histogram and dot plots of HLA-DR positive cells were presented [5].

Results

Normal fetal thymus showed a lobulated structure and prominent Hassall's corpuscles in the medulla (Fig. 1). HLA-DR immunopositive epithelial cells, macrophages and lymphocytes, as well as CD14 immunopositive macrophages were scattered throughout the thymus medulla. Strong HLA-DR staining of Hassall's corpuscles and adjacent thymic cells was seen (Fig. 2). Some lymphocytes, macrophages and epithelial cells showed co-localization of Annexin V and HLA-DR immunopositivity. Double staining demonstrated that the green Annexin V signal co-localized with the red HLA-DR signals, leading to yellow-staining cells and clusters of cells (data not shown). The ultrastructural characteristics of lymphocyte apoptosis, i.e., cell shrinkage, rearrangement of the chromatin structures (chromatin margination, aggregation and condensation), nuclear fragmentation, cytoplasm condensation and/or vacuolization, cellular disintegration and formation of apoptotic bodies which are phagocytosed and digested by adjacent macrophages were observed. The apoptotic bodies were degraded within the lysosomes. Clusters of apoptotic cells were organized by thymus macrophages (Fig. 3). We quantified the presentation of MHC molecules by flow cytometry. The two-color flow cytometric analysis revealed the presence of HLA-DR positive and HLA-DR/CD3 double positive lymphocytes (Fig. 4).

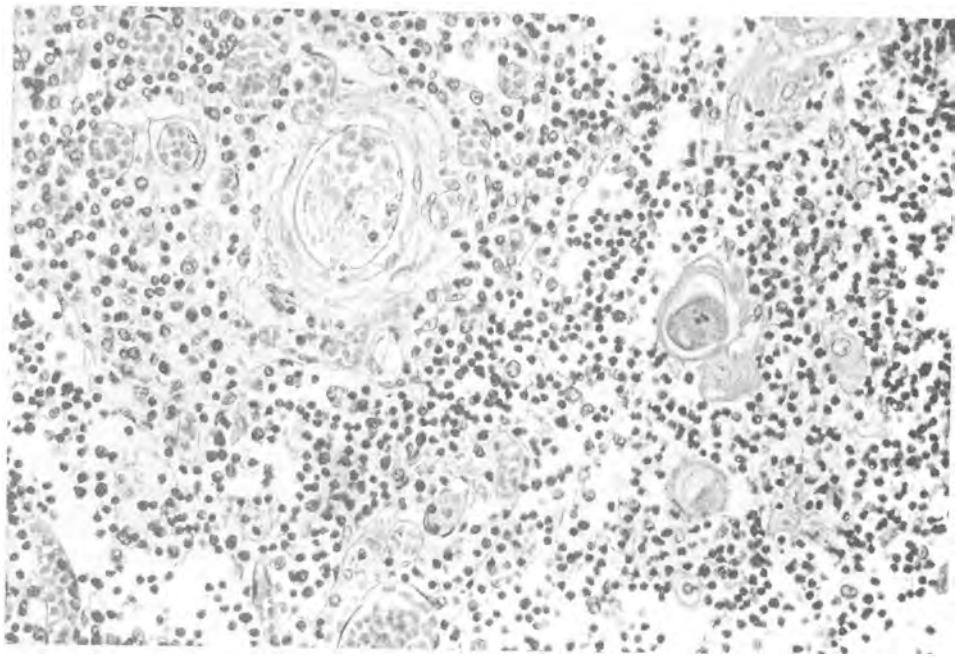


Fig. 1. Medulla of fetal thymus with Hassall's corpuscles, containing keratinized epithelial layers, intact epithelial cells, macrophages and thymocytes; HE staining; Magnification × 100

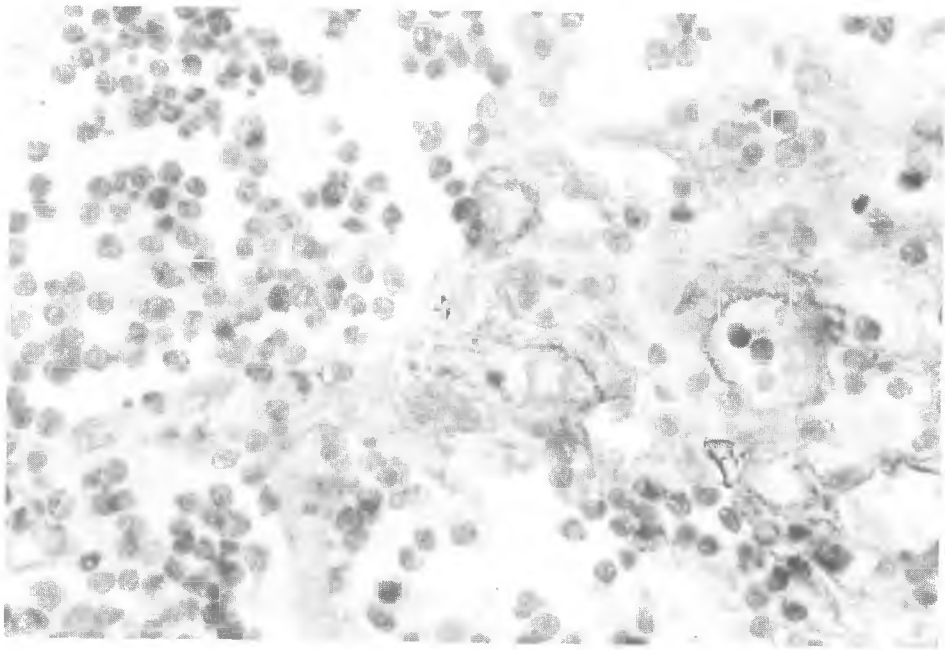


Fig. 2. HLA-DR immunopositive medullary epithelial cells, macrophages and Hassll's corpuscles; IIP staining; Magnification × 200

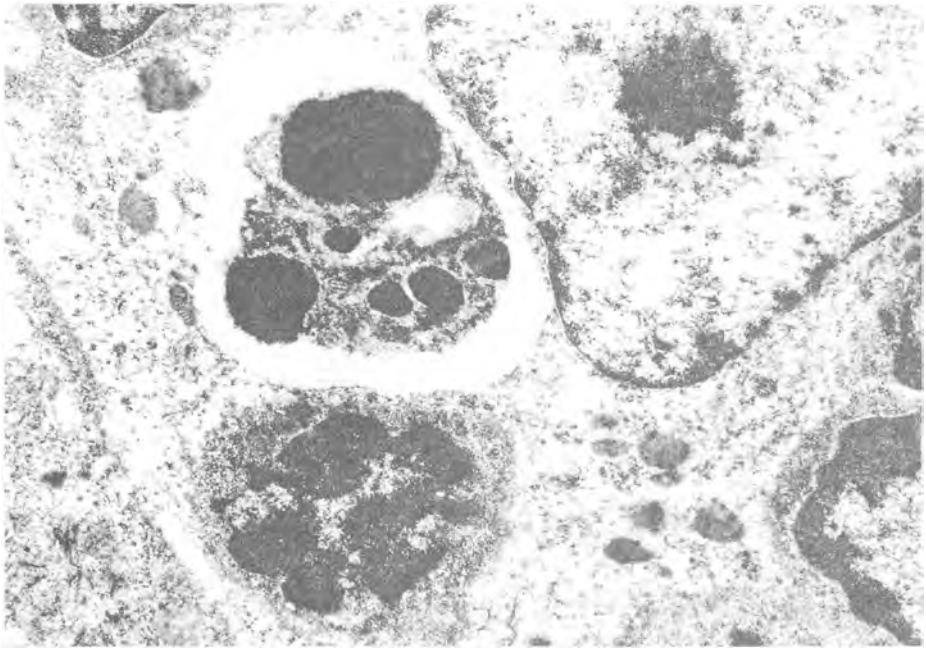
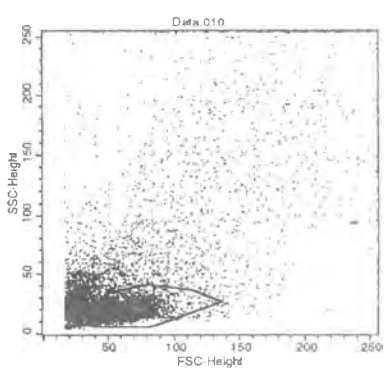
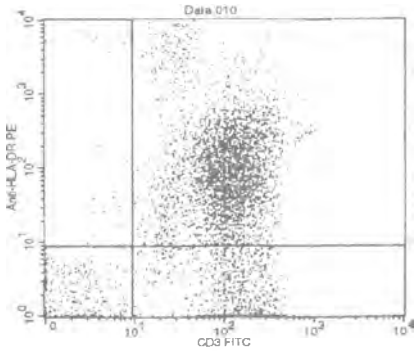
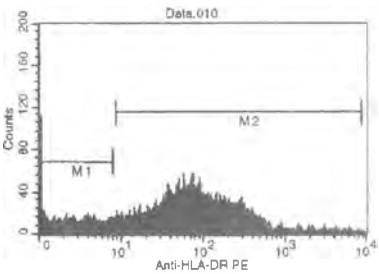


Fig. 3. Medullary macrophage containing a phagolysosome with nuclear fragments of an apoptotic thymocyte; TEM; Magnification × 10 000







Quad	Events	% Gated	% Total
UL	23	0.48	0.26
UR	3539	73.33	40.07
LL	262	5.43	2.97
LR	1002	20.76	11.34

Marker	Left, Right	Events	% Gated	% Total	CV	Median	Peak Ch
All	1, 9647	4826	100.00	54.64	333.85	48.70	1
M1	1, 8	1224	25.36	13.86	77.62	1.43	1
M2	8, 8354	3584	74.26	40.58	277.72	80.58	74

Fig 4. Representative flow cytometric analysis of lymphocytes from fetal human thymus: Status of HLA-DR and CD3 expression; Histogram and dot plot analysis

Discussion

Accumulating evidence shows that the T-cell precursors move from the bone marrow to the thymus where they are selected for self-tolerance by exposure to MHC antigens (class I and/or class II) on stromal cells. Self-tolerance induction is largely a reflection of negative selection (deletion) of autoreactive T cells in the thymus by apoptosis. [1, 4]. Whether thymocytes undergo negative selection in the cortex or medulla or in both sites has long been controversial [4, 7]. It is still controversial if a specialized class of thymic antigen-presenting cells (APCs) are responsible for the negative selection [3, 4]. We found an elimination of thymocytes in human fetal thymus by apoptosis and subsequent phagocytosis. Under physiological conditions, thymocytes selection probably occurs largely in the thymus medulla with the participation of HLA-DR immunopositive macrophages, epithelial cells and Hassll's corpuscles as sites of negative T-lymphocytes selection.

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