INDUCTION OF THYMIC HLA-DR SIGNALING WITH ALPHA-SMOOTH MUSCLE ACTIN EXPRESSION DURING THE SECOND AND THIRD TRIMESTERS OF GESTATION

D. Tamiolakis [1], J. Venizelos [2], A. Kotini [3], P. Skafida [1], A. Cheva [4] and N. Papadopoulos [4]

1) Department of Cytology, General Hospital of Alexandroupolis, Greece
2) Department of Pathology, Ippokration Hospital of Salonica, Greece
3) Department of Medical Physics, Democritus University of Thrace, Greece
4) Department of Histology - Embryology, Democritus University of Thrace, Greece

Abstract- Less than 5% of prenatal thymocytes express HLA-DR before week 12 of gestation. However, the number of HLA-DR-positive cells increases during the late second and third trimesters of development. To determine the role of alpha-smooth muscle actin in fetal thymic HLA-DR signaling in different stages of development we examined and compared the immunohistochemical expression of alpha-smooth muscle actin in the myoid cells of the thymic medulla stroma in the 2nd, and 3rd trimesters of gestation respectively, over the equivalent expression of the protein in the 1st trimester, in relation with the appearance of HLA-DR-positive thymocytes. Our results demonstrated a quantitative difference in the second and third trimesters of development concerning the expression of alpha-smooth muscle actin in the stromal myoid cells of the thymic medulla over the equivalent expression of the protein in the first ($p<0.0001$, t-test). Similar changes in the above period were found concerning the expression of HLA-DR over the first ($p<0.0001$, t-test), suggesting a direct involvement of alpha-smooth muscle actin in the sustainence of HLA-DR reactivity. Our data provide evidence that a contractile microfilament alpha-smooth muscle actin, plays a pivotal role in HLA-DR expression, through interaction between medullary stromal cells and thymocytes.
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Key Words: Medullary thymocytes, HLA-DR signaling, alpha-smooth muscle actin, myoid cells, 2nd and 3rd trimesters of gestation.

INTRODUCTION

The subset of myoid cells (MCs) is a normal component of the thymic stroma. These cells are situated mainly in the medulla and at the corticomedullary junction. They are large, rounded cells, with a central nucleus surrounded by irregularly arranged bundles of myofilaments. In lower vertebrates, where myoid cells are often more numerous, these cells are joined to neighbouring medullary epithelial cells by desmosomes. Their functions are unknown, although it has been suggested that their contractions might aid the movement of lymphoid cells across or out of the gland.

Myoid cells share some characteristics with thymic epithelial cells (1) and it has been suggested that myoid cells may derive from myoepithelial cells within the thymus (2). Other reports have suggested that myoid cells come from pluripotent stem cells (3) or from endodermal reticular cells. It has also been postulated that they are of extrathymic origin, arising during embryogenesis from muscle precursor cells of the surrounding mesoderm (1). Experiments with chick/quail chimeras do not support the notion that myoid cells arise from transdifferentiation of thymic epithelial cells but are rather of neuroectodermal origin (4). Thymic myoid cells express several muscle-specific proteins including troponin T, desmin (5), and the acetylcholine receptor (AChR) (5,6). They have therefore the antigenic characteristics of the skeletal muscle cells within the thymus (7). Their biological role is unclear, but their involvement in human myasthenia gravis (MG) has been suggested (3). Van de Velde and Friedman reported that myoid cells were present in thymus and in thymoma of both young and adult patients with MG (8).

The major histocompatibility complex is a series of genes that participate in the regulation of the immune response. This complex encodes two classes of cell-surface glycoprotein antigens: class I, found in all

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Corresponding Author:
N. Papadopoulos, Department of Histology-Embryology, Democritus University of Thrace, Greece
Tel: +3025510-39889
Fax: +3025510-39889
E-mail: npapad@med.duth.gr

nucleated cells; and class II antigens, normally found only in a limited number of cells (B-lymphocytes, macrophages, Langerhans' cells, dendritic cells, vascular endothelial cells and some epithelial cells) (9-11). Class II antigens control cellular interactions between lymphocytes. In man at least three class II antigens (DR, DQ, and DP), each consisting of α and β glycoprotein chains, are encoded by the HLA-D region of chromosome 6 (10,12,13).

Less than 5% of prenatal thymocytes express HLA-DR before week 12 of gestation. However, the number of HLA-DR positive cells increases during the late second and third trimesters of gestation when greater than 50% of prenatal thymocytes express HLA-DR, as demonstrated by fluorescence-activated cell sorting (14). Such high-level expressions of HLA-DR in fetal thymocytes were also demonstrated by Northern-blot analysis and immunohistochemistry. After birth, the percentage of HLA-DR positive cells in thymocytes decreased gradually (14).

The aim of this study was to examine the presence, distribution and quantitation of cells expressing α-smooth muscle actin and HLA-DR, in the stroma of the thymus from embryos throughout gestation.

For this reason, we investigated the presence of myoid cells and HLA-DR positive cells in a series of 15 fetal thymic specimens, using a monoclonal antibody recognizing alpha-smooth muscle actin, a contractile microfilament expressed exclusively by smooth muscle cells, myofibroblasts and related cells, as well as another monoclonal antibody (NCL-LN3) recognizing the HLA class II (DR) positive cells.

MATERIALS AND METHODS

We studied 15 cases of human thymic fetal specimens derived from the records of the Histology - Embryology Department of Democritus University of Thrace (Alexandroupolis - Greece), in different stages of development (1st, 2nd, and 3rd trimesters). Tissue samples were processed for paraffin section immunophenotyping and stained using the monoclonal antibodies against alpha-smooth muscle actin (NCL-SMA) and HLA-DR (NCL-LN3) by Novocastra. In brief, sections were dewaxed in xylene, and passed through graded alcohols to water. Endogenous peroxidase was blocked by methanol with 0.3% H_2O_2 (30 min room temperature). Thereafter, the sections were submerged in 0.01 mol/L citrate buffer (pH 6.0) and were boiled for 10 minutes in a microwave oven to retrieve the antibody antigenecity. After cooling to room temperature, they were incubated with the relevant antibodies, followed by biotinylated antibody against mouse immunoglobulin (Vector Laboratories, Burlingame, CA: 1: 100 dilution). The sections were finally reacted with peroxidase-conjugated streptavidin (Nichirei, Tokyo, Japan), and the labeled peroxidase was visualized by the diaminobenzidine-H_2O_2 method.

The immunostained sections were examined with a × 40 objective and the distribution of NCL-SMA and NCL-LN3 within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with alpha-smooth muscle actin and HLA-DR stainings, a 10×10 square calibrated grid was inserted into the eyepiece of an Olympus BX40 binocular microscope.

Five-to-ten fields were examined for each section, and at least 1000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the relevant indices.

The indices ranged from 0-100%, with a mean of 18%. The mean index was evaluated in three ranges: low index (under 18%), moderate index (from 18 to 50%), and high index (from 51 to 100%).

$$\text{alpha-smooth muscle actin index} = \frac{\text{Number of positive cells}}{\text{Number of total (positive+negative cells)}}$$

$$\text{HLA-DR index} = \frac{\text{Number of positive cells}}{\text{Number of total (positive+negative cells)}}$$

RESULTS

The results of our study showed that the number of alpha-smooth muscle actin positive cells significantly increased during the second and third trimesters of gestation. In the above period a relevant increase in the number of NCL-LN3 positive cells was observed. Our data suggest that myoid cells are involved in the formation of an appropriate microenvironment for housing and proliferation of NCL-LN3 positive thymocytes.

The sections were examined independently by two observers, and positive cellular staining for alpha-smooth muscle actin and HLA-DR antibodies were manifested as fine yellow cytoplasmic granularity and / or surface membrane expression.

Medullary stromal myoid cells expressed alpha-smooth muscle actin in 7 of 15 fetus cases during the first trimester (46.66%) (31.75±1.46 cells/mm^2), in 8 of 15 cases during the second trimester (53.33%) (28.21±2.15 cells/mm^2) and in all 15 cases during the third trimester (100%) (47.31±3.52 cells/mm^2) (Fig. 1).

Thymocytes expressed HLA-DR in 10 of 15 fetus cases during the first trimester (66.66%) (51.36±2.32 cells/mm^2), in 13 of 15 cases during the second trimester (86.66%) (105.12±5.62 cells/mm^2) and in all 15 cases during the third trimester (100%) (64.82±3.21 cells/mm^2) (Fig. 2).

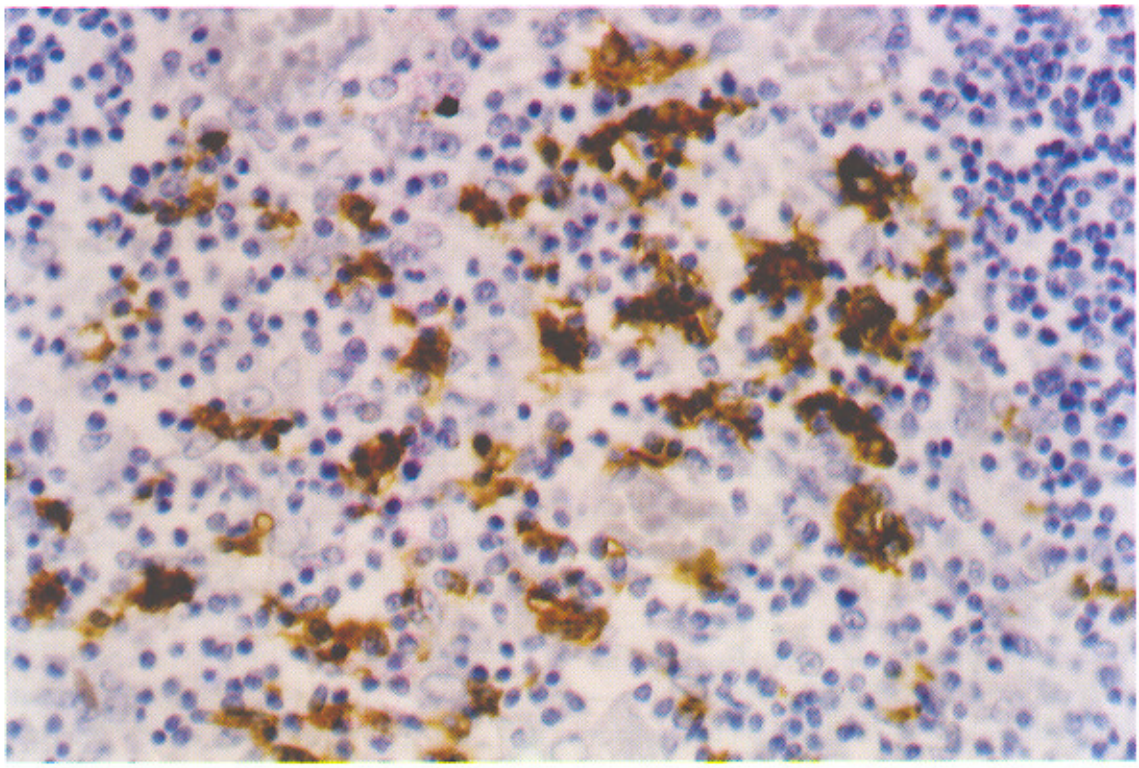

Fig. 1. Immunohistochemical control for alpha-smooth muscle actin showing a strong reactivity with the stromal myoid cells of the thymic medulla. ×250

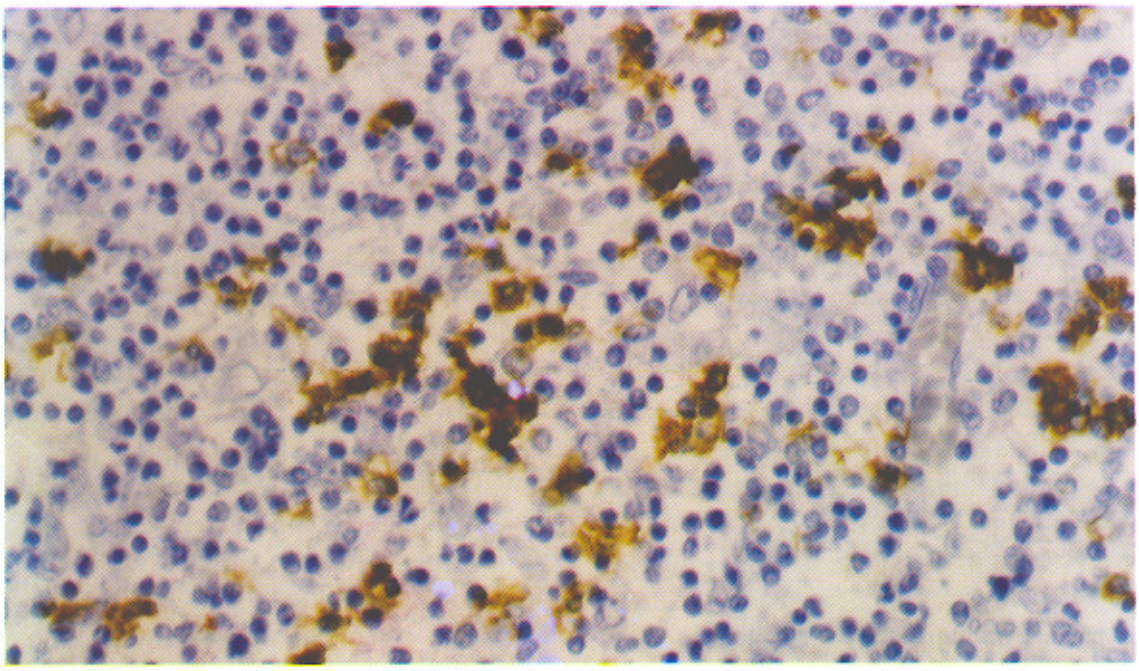

Fig. 2. Immunohistochemical control for HLA-DR showing a strong reactivity with medullary thymocytes. ×250

Our results demonstrated a statistically significant difference in the second and third trimester of gestation concerning the expression of alpha-smooth muscle actin in the connective tissue stroma of the thymic medulla over the equivalent expression of the protein in the first ($p<0.0001$, t-test). Similar changes in the above period were found concerning the expression of HLA-DR over the first ($p<0.0001$, t-test), suggesting a direct involvement of alpha-smooth muscle actin in the backing up of the capacity of thymocytes to express HLA-DR molecule.

DISCUSSION

Thymic stromal cells are thought to play a critical role in the proliferation, differentiation, and selection of precursor cells in the T cell lineage, but the precise mechanisms by which these events occur, and the particular contribution of individual thymic stromal components, are largely unknown. Most cells of the thymic stroma are of epithelial origin. Human thymic epithelial cells have been shown to produce numerous cytokines including IL-1, IL-6, granulocyte colony-stimulating factor (G-CSF), and macrophage CSF (M-CSF), that are important in various stages of thymocyte differentiation (15).

The role of myoid cells has been explored by co-culturing the myoid cells with thymocytes. Interestingly, the myoid cell line appears to protect thymic cells from spontaneous apoptosis, while the human thymic epithelial cell line has no effect. The mechanism of the protective effect needs further investigation. It could be mediated by soluble factors or by direct cell contacts. The presence of the adhesion molecule LFA-3 (CD58) on myoid immortalized thymic cells (MITC) (16) makes possible interactions between myoid cells and most thymocytes that constitutively express CD2. Indeed LFA-3 is important in the interaction with thymocytes at both immature and mature stages of development (17).

Human thymic myoid cells can be immortalized from thymic explant cultures. These cells express both the fetal and adult forms of muscle acetylcholine receptor (AChR) at the mRNA level. α-Subunit AChR protein expression is detectable in MITC line by flow cytometry. MITC line express a functional AChR, as shown by patch-clamp analysis. AChR expression on MITC line is down-modulated by myasthenia gravis (MG) sera, as on TE671 rhabdomyosarcoma cells, making MITC line an interesting tool for AChR antigenic modulation experiments. MITC line produces high levels of TNF-α and IL-8 and protects thymocytes from apoptosis, indicating that thymic myoid cells could play a role in thymocyte differentiation (16).

The following experimental arguments support the myoid nature of MITC line.

1. In the postnatal thymus, thymic myoid cells express several striated-muscle specific proteins, including myosin (18), desmin, and troponin T (5). Accordingly, Wakkach et al. (16) found that MITC line was troponin T-positive and desmin-positive, but keratin-negative. In addition, the MITC line has similar phenotype characteristics as myoid cells *ex vivo*, according to AChR, MHC class I and class II, LFA-3, and ICAM-1 antigens.

2. Wakkach et al. (16) data clearly indicate the presence of MyoD transcripts in MITC cells. MyoD is expressed only in skeletal muscle, and activates myogenesis by directly binding to the control regions of muscle-specific genes (19). Pluripotential stem cells in the thymus might express such a gene and develop into immature skeletal muscle cells in certain conditions. This is supported by the fact that cell types as different as osteocytes and chondrocytes can differentiate from thymus cells in appropriate conditions *in vitro* (20).

3. Human MITC line expressed AChR in both adult and fetal forms. In addition, anti-AChR autoantibodies induced a loss of AChR on MITC cells *in vitro*, similarly to TE671 cells; this mechanism was also observed using the anti-MIR monoclonal antibody MAb 35. These results indicate that AChR present on these cells is recognized by antibodies found in MG sera.

Some of MG patients' antibodies may show a marked preference for adult or fetal AChR, and some patients (up to 7%) are negative in diagnostic assays using only fetal AChR. That could explain why 10 to 15% of patients with clinical MG had very low titers of antibodies (0.2 to 2 nmol/L) (21). To enhance the sensitivity of the diagnostic assay for low-titer sera, Beeson et al (22) used TE671 cells transfected with the epsilon subunit of AChR as a source of AChR antigen. Thus a mix of AChR extracts from TE671-e and TE671 cells, in which adult and fetal AChR are present, was as sensitive as AChR from amputated leg muscle in MG diagnostic assay. Our data show that MITC line expresses constitutively the adult and the fetal forms of the AChR, thus MITC line might be an appropriate source of AChR for titrating anti-AChR antibodies in MG sera, as well as for studies of their functional effects.

Expression of major histocompatibility complex (MHC) molecules by components of the thymic microenvironment is required for normal T cell development (23-28) and has been implicated in the selection of the emerging T cell repertoire.

In our series: 1) The comparative study of the quantitative percentage of alpha-smooth muscle actin positive cells in thymic stromal cells at 1st , 2nd and 3rd trimesters of gestation showed a statistically significant difference in the number of the relevant cells in the thymic medulla during the second and third trimesters over the first ($p<0.0001$, t-test).

2) The comparative study of the quantitative percentage of HLA-DR positive cells in thymocytes at 1st , 2nd and 3rd trimesters of gestation showed a statistically significant difference in the number of the relevant cells of the thymic parenchyma during the second and third trimesters over the first ($p<0.0001$, t-test).

Taken together, our data indicate that myoid cells are an original thymic cell compartment. They may have a role in thymocyte property to express HLA-DR molecules.

REFERENCES

1. Puchtler H, Meloan SN, Branch BW, Gropp S. Myoepithelial cells in human thymus: staining, polarization and fluorescence microscopic studies. Histochemistry 1975; 45: 163-176.

2. Dardenne M, Savino W, Bach JF. Thymomatous epithelial cells and skeletal muscle share a common epitope defined by a monoclonal antibody. Am J Pathol 1987; 126: 194-198.

3. Wekerle H, Hohlfeld R, Ketelsen UP, Kalden JR, Kalies I. Thymic myogenesis, T-lymphocytes and the pathogenesis of myasthenia gravis. Myasthenia Gravis. Edited by Drachman DB. Ann NY Acad Sci 1981; pp 455–476.

4. Nakamura H, Ayer LE, Lievre C. Neural crest and thymic myoid cells. Curr Top Dev Biol 1986; 20: 111-115.

5. Schluep M, Willcox N, Vincent A, Dhoot GK, Newsom Davis J. Acetylcholine receptors in human thymic myoid cells in situ: an immunohistological study. Ann Neurol 1987; 22: 212-222.

6. Kao I, Drachman DB. Thymic muscle cells bear acetylcholine receptors: possible relation to myasthenia gravis. Science 1977; 195: 74-75.

7. Seifert R, Christ B. On the differentiation and origin of myoid cells in the avian thymus. Anat Embryol (Berl) 1990; 181: 287-298.

8. Van de Velde RL, Friedman NB. Thymic myoid cells and myasthenia gravis. Am J Pathol 1970; 59: 347-368.

9. Frelinger JA. Tissue distribution and cellular expression of Ia antigens. In: Ferrone S, David CS, eds. Ia Antigens I. Mice. Florida: CRC Press, Boca Raton 1983.

10. Lafuse WP, David CS. Ia antigens. Genes, molecules, and function. Transplantation 1984; 38: 443-53.

11. Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ. The detailed distribution of MHC class II antigens in normal human organs. Transplantation 1984; 38: 293-8.

12. Klein J, Figuerou F, Nagy A. Genetics of the major histocompatibility complex: the final act. Ann Rev Immunol 1983; 1: 119-42.

13. Steinmetz M, Hood L. Genes of the major histocompatibility complex in mouse and man. Science 1983; 222: 727-33.

14. Park SH, Bae YM, Kim TJ, Ha IS, Kim S, Chi JG, Lee SK. HLA-DR expression in human fetal thymocytes. Hum Immunol 1992; 33(4): 294-8.

15. Meilin A, Shoham J, Sharabi Y. Analysis of thymic cell subpopulation grown in vitro on extracellular in defined medium. IV. Cytokines secreted by human thymic epithelial cells in culture and their activities on murine thymocytes and bone marrow cells. Immunology 1992; 77: 208-213.

16. Wakkach A, Poea S, Chastre E, Gespach C, Lecerf F, De la Porte S, Tzartos S, Coulombe A, Berrih-Aknin S. Establishment of a human thymic myoid cell line. Phenotypic and functional characteristics. Am J Pathol 1999, 155(4): 1229-40.

17. Singer KM, Denning SM, Whichard LP, Haynes BF. Thymocyte LFA-1, and thymic epithelial cell ICAM-1 molecules mediate binding of active human thymocytes to thymic epithelial cells. J Immunol 1990; 144: 2931-2939.

18. Drenckhahn D, von Gaudecker B, Muller Hermelink HK, Unsicker K, Groschel Stewart U. Myosin and actin containing cells in the human postnatal thymus: ultrastructural and immuno-histochemical findings in normal thymus and in myasthenia gravis. Virchows Arch B Cell Pathol 1979, 32: 33-45.

19. Lassar AB, Buskin JN, Lockshon D, Davis RL, Apone S, Hauschka SD, Weintraub H. MyoD is a sequence-specific DNA binding protein requiring a region of myc homology to bind to the muscle creatine kinase enhancer. Cell 1989; 58: 823-831.

20. Friedenstein AJ, Lalykina KS. Thymus cells are inducible to osteogenesis. Eur J Immunol 1972; 2: 602-603.

21. Vincent A, Newsom Davis J. Acetylcholine receptor antibody characteristics in myasthenia gravis. III. Patients with low anti-AchR antibody levels. Clin Exp Immunol 1985; 60: 631-636.

22. Beeson D, Jacobson L, Newsom Davis J, Vincent A. A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis. Neurology 1996; 47: 1552-1555.

23. Kruisbeek AM, Mond JJ, Fowlkes BJ, Carmen JA, Bridges S, Longo DL. Absence of the Lyt-2-, L3T4+ lineage of T cells in mice treated neonatally with anti-I-A correlates with absence of intrathymic Ia-bearing antigen-presenting cell function. J Exp Med 1985; 161: 1029-1047.

24. Lind EF, Prockop SE, Porritt HE, Petrie HT. Mapping precursor movement through the postnatal thymus reveals specific microenvironments supporting defined stages of early lymphoid development. J Exp Med 2001; 194(2): 127-34.

25. Prockop SE, Palencia S, Ryan CM, Gordon K, Gray D, Petrie HT. Stromal cells provide the matrix for migration of early lymphoid progenitors through the thymic cortex. J Immunol 2002; 169(8): 4354-61.

26. Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. Nat Rev Immunol 2001; 1(1): 31-40.

27. Suniara RK, Jenkinson EJ, Owen JJ. An essential role for thymic mesenchyme in early T cell development. J Exp Med 2000; 191(6): 1051-6.

28. Anderson G, Jenkinson EJ, Moore NC, Owen JJ. MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. Nature 1993; 362(6415): 70-3.