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Expression of MHC Antigens in Fetal Rat Thymus

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Abstracts Major histocompatibility complex (MHC) Class I antigen is known distinct on cell surface of the mature cells including lymphocytes but not detected on early stage of thymocytes.

Monoclonal antibody, OX-18 or HAM-2 is the specific antibody to rat MHC Class I antigen and OX-6 is that to MHC Class II. These antibodies are detected immunohistochemically or flow cytofluorometrically by using HRP or FITC conjugated anti-mouse immunoglobulin.

Main findings are as follows : MHC Class I antigen first appeared on thymic epithelial cells at day 15 of gestation and with the progress of fetal stage MHC Class I antigen positive cells appeared to increase in number and its intensity of the staining. MHC Class I antigen on thymocytes at about day 15 of gestation was negative, however at day 19 of gestation was about 36%, which is same as in normal young adult thymus. The degree of total antigen amount expressed in fetal thymus was compared with that in adult thymus, using radioactive binding inhibition assay. The amount of antigen is quite low at days 18 and 20 of gestation compared to that of adult. From these findings it is suspected that MHC Class I antigen first appeared on epithelial cells and a few days later on thymocytes. Thus, the interaction between MHC Class I positive epithelial cells and thymocytes may be important for the differentiation and maturation of thymocytes.

Key Words : MHC antigen, Fetal thymus, Monoclonal antibodies, Thymic epithelial cells

Introduction

Architecture of the thymus is mainly built up by endodermally derived epithelial reticular cells, neural crest derived myoid cells[1] and connective tissue and blood vessels invaded into it. Macrophages, interdigitating cells[2] and Langerhans cells[3] are thought to be derived from precursor cells in bone marrow. Among these comoponents, epithelial reticular cell and macrophage are important on the immunological aspect and thymocytes education.[4~6] In mammals, bone marrow or fetal liver derived prethymocytes are thought to migrate to thymus especially subcapsular region and differentiate into thymocytes[6] but the detailed mechanism for this is still under proof. In this process many of them died.[7] Avian hemopoietic precursor cells are recruited to the thymus by chemotactic peptides secreted by thymic epithelial cells.[8,9] Also thymic hormones, which are secreted by thymic epithelial cells, are important for thymocytes to differentiate.[10,11]

Ontogenical expression of MHC antigens and the role of MHC antigens on epithelial cells in the thymus of the mice are reported by Jenkinson et al.[12] but those of rats are scanty excepts the reports of Grein et al.[13] and Duijvestijn et al..[14] Thus, we examined the appearance of MHC antigens in the fetal rat thymus immunohistochemically by mono-

clonal antibody. This will be important to resolve the mechanism of thymocytes maturation. Monoclonal antibody (OX-18 or HAM-2) is the specific antibody to rat MHC Class I antigen, and OX-6 is that to MHC Class II.[15,16] We used HRP or FITC conjugated anti-mouse immunoglobulin (Ig) to detect these antibodies immunohistochemically or flow cytofluorometrically.

Materials and Methods

Animals : DA strain (RT-1^a) rats were maintained at the Institute of Laboratory Animals of Yamaguchi Univiersity School of Medicine. Young adult,neonatal and fetal DA rats were used. Fertilization and completion of the pregnancy were checked on vaginal plugs ; this day was determined as day 0 of fetal life. In this time schedule, day 21 was the day of birth, determined as day 0 of postnatal life. We used fetal rats at days from 14 to 19. Under ether or chloroform anesthesia, fetal rats were delivered artificially. Fetal rats were anesthetized on ice and thymus was taken out gently from mediastinum under the binocular microscopy (Wild M 650).

Monoclonal antibodies : Monoclonal mouse anti-rat MHC Class I (RT1-A) antisera (OX-18 or HAM-2) were culture supernatants or ascites. Further details of the specificity of this antisera were given elsewhere.[15,16] Monoclonal antibody OX-6 ascites (sera-lab.) was also used to recognize MHC Class II (RT1-B) antigen.

Immunohistochemistry for light microscopy : Freshly isolated thymic tissues were rinsed in phosphate-buffered saline (PBS), mounted in an OCD compound and frozen at −20℃. Frozen sections were cut at about 10 μm by microtome (Bright Model). Tissues were mounted on slides, air dried and fixed by dipping in acetone. These sections were overlayered with 100 μl of a non-diluted or a 1/10 diluted of monoclonal antibodies OX-18, HAM-2 or OX-6 respectively for 60 min at room temperature. Being kept 10 min at 4℃, the sections were washed 3 times in cold PBS and incubated for a further 60 min at 4℃ with 100 μl of a 1/10 dilution of horseradish peroxidase conjugated goat $F(ab')_2$ anti-mouse IgG (Cappel lab. Inc., U.S.A.) which contained 2% heat -inactivated normal rat serum. After washing with PBS, sections were incubated at room temperature for about 15 min with 0.05% 3-3' -diaminobenzidine (DAB) HCl (Sigma Chemical Company). Counter staining was done with hematoxyline or methylgreen.

Radioactive binding inhibition assay : The inhibition assays were performed on thymic tissue specimens obtained from fetal and young adult DA rats. Thymus weights were measured precisely, equal amounts of PBS was added and homogenized. After homogenization, tissues were washed once with PBS. Diluted monoclonal antibody (OX-18) was absorbed with the homogenates at various dilutions overnight at 4℃. These samples were centrifuged at 1800 rpm for 10 min. Duplicated 50 μl aliquots were then assayed by cellular radioimmunoassay (cRIA) ; namely, glutalaldehyde-fixed thymocytes were used for target cells after adjusting the cell number to $1\text{-}3\times10^7$ cells/ml. Fifty μl of absorbed monoclonal antibodies were incubated with 50 μl of target cells for 60 min at 4℃. The cell bound monoclonal antibodies were detected with ^{125}I -rabbit $F(ab')_2$ anti-mouse IgG (Cappel lab. Inc., U.S.A.) using Auto Well gamma system (Aloka, ARC-202).

Immunohistochemistry for fluorescence microscope : The 5 μm frozen sections of thymic tissues were incubated with 100 μl of each monoclonal antibody for 1 hour at room temperature, washed three times in cold PBS and then incubated at 4℃ for 1 hour with 100 μl of FITC-conjugated sheep $F(ab')_2$ anti-mouse IgG (Cappel lab. Inc., U.S.A.), which contained 2% heat -inactivated normal rat serum. The binding patterns of the monoclonal antibodies to cell surface of thymic cells were detected by fluorescence microscope (Nikon XF-EF, Japan).

Flow cytofluorography : The binding profiles of the monoclonal antibodies to cell surface of fetal and young adult thymic cells were assayed with indirect immunofluorescence methods using a fluorescence activated cell sorter or EPICS (Coulter Corp., U.S.A.) ; Gently pieced and freed thymocytes (5×10^5) were centrifuged and condensed into 1-2 ml saline. These cells were incubated for 60 min at 4℃ with OX-18 or HAM-2 monoclonal antibodies. Thymocytes, conjugated with these antibodies, were washed with PBS and resuspended into FITC conjugated rabbit $F(ab')_2$ anti-mouse IgG at 4℃ for 60 min. After washing 3 times these cells were analyzed.

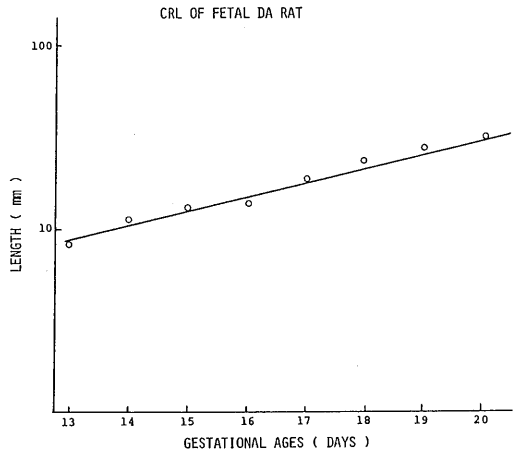


Fig. 1 Crown-rump length (CRL) of fetal DA rat at days 13 to 20 of gestation. Relationship of CRL and gestational day is almost linear, when plotted in a semilogarithmic scale.

Results

a) Crown-rump length (CRL)

CRL of the fetal rats at days 13 to 20 of gestation were measured. Relationship of length and fetal days was almost linear (Fig. 1).

b) Onset and changes of MHC Class I antigen in rat thymus : Ontogenically, primitive thymus was observed at day 14 of gestation of DA rat near the primitive heart (data not shown). This organ was occupied by the cells which had pale nuclear chromatin and clear nuclear envelope as those of reticular cells (Fig.2-A). At this stage both MHC Class I and II antigens were not observed (Fig.2-B) (Table 1). At day 15 of gestation, this organ was invaded by connective tissue and vessels. Lymphoid cells with dense nucleus were observed and some of them were on the way of mitosis (Fig.2-A). MHC Class I antigen was first detected immunohistochemically at this stage (Table 1). The lobulus was completed at days 16-17 of gestation when thymocytes were clearly observed, but cortex and medulla were not demarcated (data not shown). At about this stage MHC Class I positive cells were found (Fig.2-C, 2-E). Thymocyte rich cortex was separated from medulla at days 18-19 of gestation. MHC Class I antigen positive cells were slightly large epithelioid cells and occupied in the center of compacted cell mass (data not shown). With the progress of fetal stage, the number of antigen positive cells increased (Table 1). The percentage of MHC Class I antigen positive cells of neonatal rats were nearly as same as that of young adult rats (Fig. 2-F). This antigen was strongly detected immunohistochemically among almost all of the medullary cells of the young adult rats at 3-4 months of ages (Fig. 2-F). MHC Class II antigen was detected immunohistochemically after day 17 of gestation (Table 1).

c) Expression of MHC Class I antigen on fetal thymocytes : To examine whether thymocytes of these developmental stages express MHC Class I antigen or not,

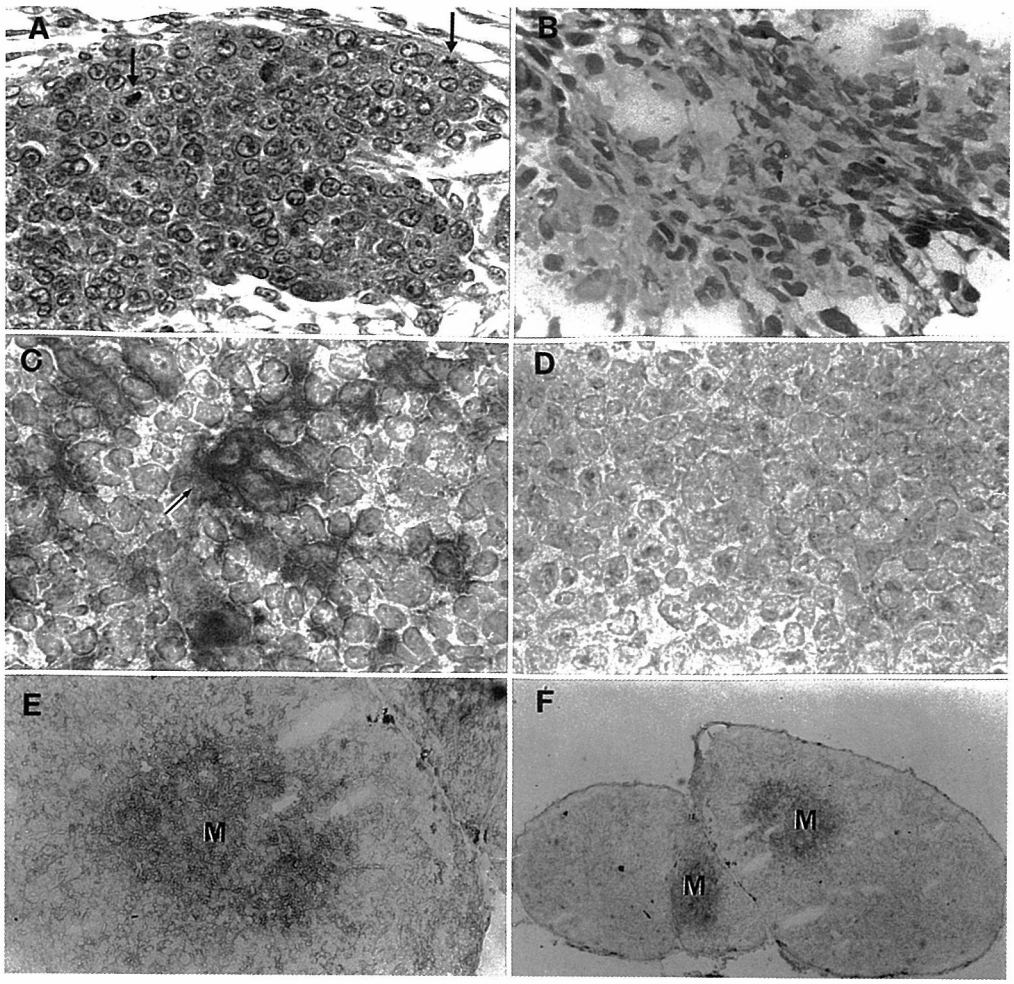
Table 1. Immunohistochemical examination of the membrane antigen in the rat thymus

Antigens	mAbs*	Gestational ages (days)					
		14	15	16	17	18	20
MHC Class I	OX-18	−	±**	+	+	+	++
MHC Class II	OX-6	−	−	−	+	+	+

mAbs* : monoclonal antibodies to membrane antigens

** : The degree of membrane antigen expression was shown as follows ;

++ : stolongly positive, + : positive, ± : weakly positive, − : negative



Figs. 2

A : Thymus of fetal DA rat at day 15 of gestation. Thymus consists of epithelial reticulum cells and a few round cells. Note some cells on way of mitosis (arrows). H.E. x 300

B : The frozen section of the same day specimen of A. stained by indirect immunoperoxidase method with Ox-18. Note no OX-18 positive epithelial cells are recognized. x 300

C : The frozen section of fetal rat thymus at day 18 of gestation stained by the same way as B. Some positive cells are recognized and typically OX-18 positive epithelioid cell is shown (an arrow). x 800

D : The frozen section of the same day specimen of C. This section is not overlayered with OX -18 monoclonal antibody. No positive stainings are observed. x 800

E : Lower magnification of C. OX-18 positive cells are mainly observed in medulla (M). x 30

F : Young adult DA thymus stained by the same way as B, C and E. Strongly positive cells are compacted in medulla (M). x 15

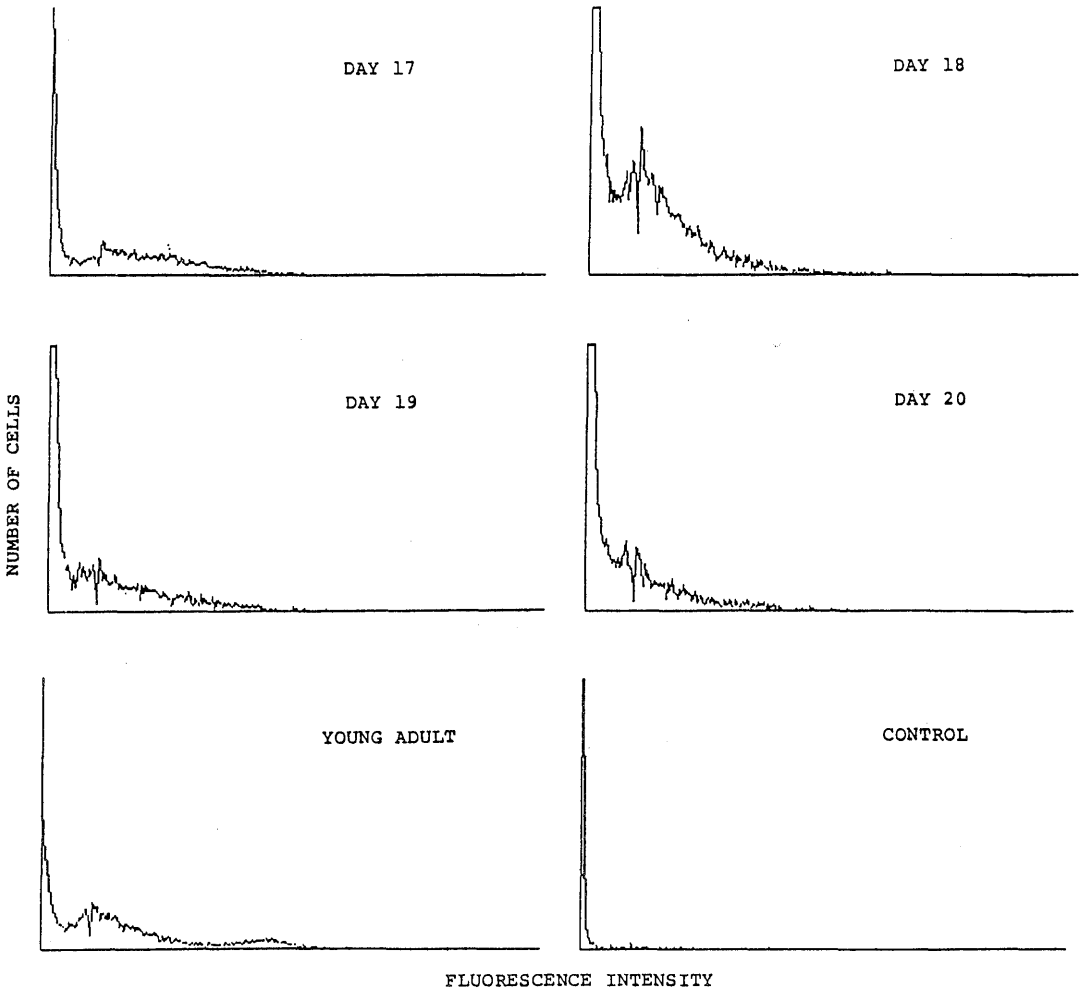


Fig. 3 EPICS profiles of fetal rat thymocytes.Fluorescence histogram from EPICS analyses of isolated DA rat thymocytes at days 17 to 20 of gestations labelled with OX-18 or saline control.

thymocytes were analyzed by EPICS after labelled with monoclonal antibody OX-18 or HAM-2 and FITC conjugated $F(ab')_2$ rabbit anti-mouse IgG. The labelling profile of fetal and adult thymocytes with anti-MHC Class I antibody was shown in Fig. 3. Percentage of MHC Class I antigen positive thymocytes at days 17 to 20 of gestation was about 20-35%, which was as same as in normal young adult thymus.

d) Amount of MHC Class I antigen in fetal thymus : To examine the amount of MHC Class I antigen expressed in the fetal thymus, inhibition assays were performed on thymic tissue specimens obtained from fetus at days 18 and 20 of gestation and young adult rats. Monoclonal antibody OX-18 was inhibited significantly by adult whole thymus but not much by fetal thymus (Fig. 4).

Discussion

The activation of T helper cells for both

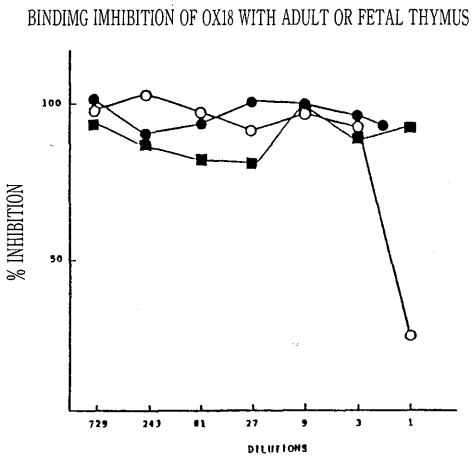


Fig. 4 Binding inhibition assay of rat thymus. Thymic tissue specimens obtained from 18 and 20 days old fetus and young adult rats. OX-18 was inhibited by adult thymus but not by fetal thymus.
○——○ Adult, ●——● 18 day fetus,
■——■ 20 day fetus

antibody and cytolytic T lymphocyte (CTL) responses is restricted by products of the I-region of MHC, while the activation of CTL themselves is restricted by products of the K/D regions of the MHC of the mouse.[17~19] However, it is less clear how and when during their developmental pathway in the thymus, T cells acquire their MHC -restricted self-recognition specificity. Distribution of MHC antigens in the thymus of mouse was well examined immunohistochemically and detected on dendritic cells of adult mouse.[4] Ewijk et al.[20] characterized the MHC Class I positive dendritic cell types as epithelial reticular type because they had tonofilaments and desmosomes. Ontogenically in mouse, MHC Class I antigen first appeared on thymic epithelial cells at about day 15 of gestation when this antigen was not expressed on thymocytes.[12] Studies on ontogenical expression of MHC antigen in rat thymus are scanty except some reports.[13,14] Duijvestijn et al.[21] studied the ontogeny of the rat thymus and reported that MHC Class II antigen was first identified at day 16 of gestation, which is equivalent to day 15 of this report, but they did not study MHC Class I antigen. So, we examined the onset and amount of MHC Class I antigen in fetal rat thymus (Table 1, Fig. 2). The onset of MHC Class I antigen in fetal rat was almost similar to the results of mice. Namely, MHC Class I antigen first appeared on epithelioid cells at day 15-16 of gestation when this antigen was not expressed on thymocytes. It was suspected that the expression of this antigen on thymocytes might be induced by cell to cell interaction as the same way of MHC Class II antigen.[22] Percentage of MHC Class I positive thymocytes at the late stage of fetus was as same as that of adult life (Fig. 3), whereas it was demonstrated by inhibition assay that the amount of this antigen at the late stage of fetal life is lesser than that of adult life (Fig. 4). The reasons of this discrepancy may be due to the difference of sensitivity between radioactive binding inhibition assay and cell sorter. In radioactive binding inhibition assay total cell population including epithelioid cells were measured, while in cell sorter only thymocytes in suspension were measured.

We examined MHC Class II antigen on the thymus of rats by OX-6 monoclonal antibody and showed that thymic epithelial cells expressed this antigen at day 17 of gestation, a few days later than the appearance of MHC Class I antigen (Table 1). These results are similar to those of Duijvestijn et al.[21] These findings may suggest during developmental pathway in the thymus the interaction of epithelial cells and thymocytes may be important for thymocytes to differentiate and acquire self recognition specificity.

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