

# Immunohistochemistry of Human Fetus

—Ig G, A and M Cells in the Thymus and Spleen—\*)

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## ABSTRACT

In order to study the development and differentiation of the immunological activity of human fetus, Ig G, A and M cells are demonstrated in the thymus and the spleen by means of Avidin Biotin Peroxidase Complex method.

The results show (1) The Ig G cells appear in the thymus about 4 months of gestation and Ig A and M cells about 6 months of gestation in the thymus and the spleen in our human fetal specimens. (2) The Ig G cells are seen more often than Ig A and M cells both in the thymus and in the spleen. (3) Ig G, A and M cells are observed mainly in the medulla of the thymus and in the red pulp of the spleen mostly around the capillary vessels.

## INTRODUCTION

For a long time, we have been working on autopsy of normal and abnormal human fetuses and newborns to make clear the mechanism of abnormal development. In this histological study, the Ig G, A and M cells in the thymus and spleen of normal human fetuses are examined by the immunoperoxidase method for the purpose of studying the development and differentiation of immunological activities of human fetuses, and to use them as control for the purpose of making a comparison with the group of abnormal development.

## MATERIALS AND METHODS

About 5,000 fetuses were autopsied by the Department of Pathology, Nagasaki University, School of Medicine (Director, Professor Takayoshi IKEDA, Professor Emeritus Ichiro HAYASHI) from 1947 to 1962, and about the same number of fetuses has been done by the Department of Geneticopathology, Research Institute for Nuclear Medicine and Biology, Hiro-

shima University (Director, Professor Yukio SATOW, Professor Emeritus Naomasa OKAMOTO) from 1962 to the present<sup>4)</sup>. Among the artificially aborted fetuses, we considered those with no abnormal findings by autopsy to be normal control cases. In this study 9 cases of the normal fetuses in the gestation periods of 4, 6, 7, 8, and 10 months were examined by the light and electron microscopes and the immunoperoxidase staining method (Avidin Biotin Peroxidase Complex Method, Biomed Corp. Foster City, CA., U. S. A., Maruzen Sekiyu Biochemical Co. Tokyo)

## RESULT AND DISCUSSIONS

### Histological findings in the thymus of human fetus

At the age of 4 months of gestation, the line demarcation between cortex and medulla is not clear yet. The connective tissue septa among lobules are relatively wide (Fig. 1).

About 6-7 months of gestation (Fig. 2), the line demarcation between cortex and medulla

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is clear. The larger part of the lightly stained medulla is reticulum and the lymphocytes are few. In the medulla, the Hassall's corpuscles are beginning to appear.

Around 8-10 months of gestation, the dark-stained cortex is a network of reticulum packed densely with free lymphocytes (Fig. 3).

Haematopoiesis is seen in the connective tissue septa among lobules.

Fig. 4 shows the electron microscopic finding of the border line between cortex and medulla. On the cortex side many lymphocytes with round and dense nuclei are seen, while around the capillary vessels in the medulla, the large cells with irregular shaped nuclei are observed.

#### Histological findings in the spleen of human fetus

About 4-6 months of gestation, two types of cells are seen in the spleen. One is a stellate cell with fibrils that attach themselves to one another and form a supporting reticulum network of the organ. The other is a free cell within the network. This free cell looks like a lymphocyte (Fig. 5).

About 7-10 months of gestation (Fig. 6 and 7), the initial figure of the white pulp appears around small branching arterial vessel. The red pulp is composed of reticulum cells with fibers supporting the free cells of blood and forming the so-called splenic cords. The white pulp scattered in the red pulp is packed with lymphocytes and grows to be typical lymphoid tissue. This white pulp is around the small branching arterial vessels.

Fig. 8 and Fig. 9 are the electron microscopic pictures of the white pulp and the red pulp of the organ at the 10 months of gestation respectively. In the red pulp, cells with irregular shaped nuclei and cytoplasm are observed among lymphoid cells, while in the white pulp many lymphoid cells are seen around the arterial vessels.

#### About the ABC (Avidin Biotin Peroxidase Complex) Method

The immunoperoxidase staining series are based on the avidin-biotin affinity cytochemistry technology<sup>5-7,9)</sup>. High affinity monospecific antibodies are labeled with a modified biotin structure which places the biotin residues away from the antibody mass, resulting in a readily accessible and reactive biotin label. The avidin-peroxidase complex is a stable and highly effi-

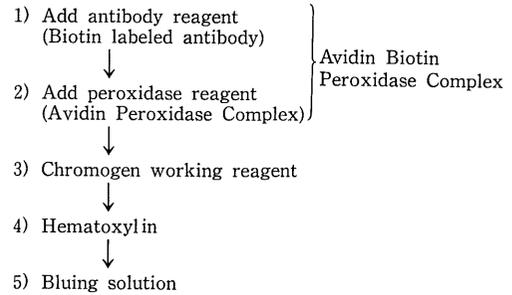


Fig. 22. Schema of immunoperoxidase staining procedure

cient polymeric structure possessing very low non-specific binding characteristics. This peroxidase reagent recognizes the extended biotin residues associated with the specific antibody which is bound to the antigenic site under investigation. The peroxidase which becomes associated with those sites on the tissues is visualized by using a chromogenic substrate which forms insoluble deposits on the areas containing the enzyme. Finally, the unclear stain hematoxylin resolves the cells not stained by the immunohistochemical procedure.

In this study, such human immunoglobulin antigens as Ig G ( $\gamma$  heavy chain), Ig A ( $\alpha$  heavy chain) and Ig M ( $\mu$  heavy chain) are demonstrated.

The simultaneous processing of pertinent controls is an absolute requirement in order to achieve meaningful interpretations of the immunoperoxidase staining.

Negative controls: (A) Delete antibody reagent and add phosphate buffered saline instead. (B) Delete peroxidase reagent and add phosphate buffered saline instead.

Any stain in control (A) is due either to nonspecific binding of the peroxidase reagent or to endogenous tissue peroxidase-like activity. Control (B) reveals the degree of endogenous peroxidase-like activity in the tissue.

Fig. 10 and 12 (control A) shows a few cells with red stained cytoplasm and Fig. 11 and 13 (control B) shows no Ig positive cells.

#### Immunohistochemical findings in the thymus and spleen of the human fetus

##### a) Ig G cells in the thymus

About 4 months of gestation, the Ig G positive cells are scarcely seen.

About 6 months the number of the Ig G cells is increasing slightly around the capillary vessels in the medulla (Fig. 14).

About 7-10 months almost the same phenomenon is seen in the medulla (Fig. 15). The nuclei of these cells are round and the red stained cytoplasm are round and oval.

#### b) Ig G cells in the spleen

About 4 months of gestation, no Ig G positive cells are seen in our specimens. About 6-7 months of gestation, small amount of the Ig G positive cells are seen mainly in the red pulp. About 8-10 months of gestation, the number of the Ig G cells is increasing slightly (Fig. 16 and 17). In the white pulp, dark-stained lymphocytes are seen, but Ig G positive cells are scarcely seen.

#### c) Ig A cells in the thymus

The Ig A positive cells are hardly seen in the period of about 4 months, and after 6-7 months of gestation, the cells are observed near

the capillary vessels in the medulla. Most of the Ig A positive cells look like plasma cells (Fig. 18).

#### d) Ig A cells in the spleen

The Ig A positive cells are scarcely seen about 4 months as in the thymus. The cells appear after 6 months, and the number of the cells increases gradually in the red pulp (Fig. 19).

#### e) Ig M cells in the thymus

The Ig M positive cells appear mainly in the medulla around 6 months of gestation. The number of cells is increasing more rapidly than that of the Ig A cells about 7-10 months (Fig. 20).

#### f) Ig M cells in the spleen

These Ig M positive cells are also observed mainly in the red pulp around 6 months (Fig. 21).

Table 1. Abstract of the amount and appearance of Ig cells

fetal months	Thymus			Spleen		
	Ig G cell	Ig A cell	Ig M cell	Ig G cell	Ig A cell	Ig M cell
10	++	+	+	+	+	+
8	++	+	+	+	+	+
7	+	+	+	+	+	+
6	+	+	+	+	+	+
4	+					

The outline of the results are shown in Table 1.

In general, the Ig G and A cells are believed to be produced from plasma cells and the Ig M cells are from lymphoid cells, and its genetic regulation is thought not to be controlled by the simple Mendelism but by polygenic factors. In our study the number of Ig G cells is greater than that of Ig A and M cells both in the thymus and in the spleen.

The same is observed to the amount of Ig in the human fetus serum. Our observation shows that most of the Ig G, A and M cells are seen near the capillary vessels in the medulla of the thymus and in the red pulp of the spleen. This suggests that the vicinity of the capillary vessels should play an important role to immunological activity.

The serum examination shows that the maternal Ig G transfers to fetus after 37 weeks (about 8.6 months) of gestation. Furth<sup>1)</sup>, using

the tissue culture or immunofluorescent method, demonstrates that the Ig G cells are produced from the lymphoid or plasma cells in the spleen after 20 weeks (about 4.6 months) of gestation.

Tanaka<sup>8)</sup> reports that the average value of serum Ig G is about 5.4% of the adult value at 17-20 weeks (about 4 months), 21% at 21-27 weeks (about 5 months), 42% at 29-32 weeks (about 6.7 months), 55% at 33-36 weeks (about 7.7 months) and 86% at 37-40 weeks (about 8.6-9.3 months) of gestation.

About the Ig M, 0-2% of the adult value are measured at 17-20 weeks (about 4 months), 0-7.1% at 21-24 weeks, 2.6-12% at 29-32 weeks, 3.5-4.7% at 33-36 weeks and 6-16% at 37-40 weeks of gestation.

These fetal values of serum Ig M are smaller than those of Ig G. For this reason it is recognized that the maternal Ig M does not transfer to the fetus. Gitlin<sup>2)</sup> demonstrates that the ABO saline isoagglutinin, which does not move

from mother to fetus, belongs to the Ig M. Gitlin et al.<sup>2,9)</sup> observe that the maternal Ig M labeled with <sup>131</sup>I does not transfer to the fetus. On the other hand it is suggested that the fetal Ig M should come out around 17-20 weeks (about 3.9 months) of gestation, because at this stage the Ig M appear in the fetal serum. Furth<sup>1)</sup> also observes by the immunofluorescent method that the fetal spleen produces Ig M 19 weeks of gestation, and the thymus cells possess the Ig M at 11 weeks of gestation<sup>9)</sup>. We observe Ig M cells both in the thymus and in the spleen about 6 months of gestation.

About of the Ig A, small amount of Ig A is measured in the fetal serum around 17-20 weeks (about 4 months) of gestation, and 1% of adult serum value at 32 weeks (about 6.7 months) of gestation.

About 16% of the adult serum Ig A value is calculated at 7-12 months old newborns, and it increases to 79% at 6-8 years old. It is known that Ig A does not move from mother to fetus. Although Furth et al.<sup>1)</sup> by the immunofluorescent method, point out that no cells producing Ig A are observed around 13-31 weeks (4-6 months) of gestation, Tanaka<sup>9)</sup> mentions that the presence of Ig A cells both in the thymus at 11 weeks (2.5 months) and in the spleen at 20 weeks (4.6 months) of gestation. These observations and ours correspond each other.

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## EXPLANATIONS OF FIGURES

- Fig. 1.** Human fetal thymus, 4 months of gestation. Autopsy number: HG 4169 (♀), Body Weight (BW) 38.3 g, Cranio-rump distance (CR) 7.5 cm, thymus 0.03 g. The borderline between cortex and medulla is not clear. Hematoxylin and Eosin (HE) staining.
- Fig. 2.** Thymus, 6 months of gestation. HG 4402 (♂), BW 350 g, CR 17 cm, thymus 0.7 g. The line demarcation between cortex and medulla is clear. HE staining.
- Fig. 3.** Thymus, 10 months of gestation. HG 3250 (♀), BW 2950 g, CR 32.6 cm, thymus 10.1 g. The darkstained cortex and lightly stained medulla are observed. Hassall's corpuscles are seen. HE staining.
- Fig. 4.** Electron microscopic picture of the thymus, 10 months of gestation shows the borderline between the cortex (C) and medulla (M).
- Fig. 5.** Human fetal spleen, 4 months of gestation. HG 4169, spleen 0.06 g. Two types of cells are seen in the spleen. One is a stellate cell which forms a supporting reticulum network. The other is a free lymphocyte. HE staining.
- Fig. 6.** Spleen, 6 months of gestation. HG 4402, spleen 0.7 g. The lymphocyte are mainly observed around the arterial vessels (A). This shows initial figure of developing white pulp. HE staining.
- Fig. 7.** Spleen, 7 months of gestation. HG 3574 (♂), BW 250 g, CR 15.4 cm, spleen 0.2 g. The red pulp is composed of reticulum cells forming the so-called splenic cords.
- Fig. 8.** Electron microscopic picture of red pulp of spleen, 10 months of gestation. Some irregular shaped reticulum and blood cells are observed.
- Fig. 9.** Electron microscopic finding of white pulp of spleen, 10 months of gestation. Many lymphoid cells are seen.
- Fig. 10.** Negative control staining of delete antibody reagent of thymus, 10 months of gestation. HG 3250. Few cells with red stained cytoplasm are seen.
- Fig. 11.** Negative control staining of delete peroxidase reagent of thymus, 10 months of gestation (HG 3250) shows no Ig positive cells.
- Fig. 12.** High magnification of Fig. 10 shows around blood vessels of the thymus. Few cells with red stained Ig positive cells are observed.
- Fig. 13.** High magnification of Fig. 11 shows next section of the preparat of Fig. 12. No Ig positive cells are observed.
- Fig. 14-21** show immunohistochemical findings.
- Fig. 14.** Ig G staining of the thymus, 6 months of gestation. HG 4402. Some Ig G positive cells are observed around the capillary in the medulla (↑).
- Fig. 15.** Ig G staining of the thymus, 8 months of gestation. HG 3080 (♂), BW 1810 g, CR 27.5 cm, thymus 6.0 g. Ig G cells with red stained cytoplasm are observed (↑).
- Fig. 16.** Ig G staining of the spleen, 10 months of gestation. HG 3250 (♀) BW 2950g, CR 32.6 cm spleen 8.2 g. Ig G positive cells look like plasma cells (↑).
- Fig. 17.** Ig G staining of the spleen, 7 months of gestation. HG 3574. Some Ig G positive cells are seen in the red pulp (↑).
- Fig. 18.** Ig A staining of the thymus, 8 months of gestation. HG 3080. Ig A positive cells are seen in the medulla (↑).
- Fig. 19.** Ig A staining of the spleen, 8 months of gestation. HG 3080. Relatively small number of the cells are seen in the red pulp of the organ.
- Fig. 20.** Ig M staining of the thymus, 8 months of gestation. HG 3080. Most of the cells are observed in the medulla (↑).
- Fig. 21.** Ig M staining of the spleen, 6 months of gestation. HG 4402. The cells are also mainly seen in red pulp (↑).

