The Seoul Journal of Medicine
Vol. 32, No. 4: 177-189, December 1991

Immunohistochemical Study on Changes of the T- and B-Cell Population in Uterus Draining Lymph Nodes of Mouse during Pregnancy[+]

Wang Jae Lee, Young Sil Hwang*, Douk Ho Hwang, Kwang Ho Lee and Ka Young Chang

Department of Anatomy, Seoul National University College of Medicine, Seoul 110-460, Korea and
Department of Internal Medicine, Gyeongsang National University College of Medicine, Jinju 660-280, Korea*

=Abstract=**The following experiment was performed to investigate changes of immune status in the uterus draining lymph nodes of pregnant ICR mice. The experimental animals were divided by duration of pregnancy as follows; Group I, virgin control; Group II, pregnancy 2nd day; Group III, pregnancy 5th day; Group IV, pregnancy 8th day; Group V, pregnancy 14th day; Group VI, pregnancy 19th day. The objective of this study was to focus on the changes in both B and T-cell systems. To note changes in the B-cell system, the nunber and distribution pattern of activated B cells were observed, the number of IgM and IgG positive plasma cells was counted,, and the distribution pattern of surface IgM and IgG positive cells was observed. For changes in the T cell system, the distribution pattern of L3T4 positive and Lyt-2 positive cells was observed. Histochemical staining for activated B cells by means of membrane alkaline phosphatase activity (mAP) and immunohistochemical staining, such as avidin-biotin-peroxidase complex (ABC) and alkaline phosphatase-anti-alkaline phosphatase (APAAP) techniques for T cell and other B-cell systems were carried out. L3T4 positive cells in the deep cortex and IgM- and IgG-positive plasma cells in the medulla were significantly increased near the term. Lyt-2 positive cells were increased in the medullary cord or corticomedullary junction on the 5th day of pregnancy. The results obtained from the above experiment suggest that the conceptus evokes the immune responses locally as well as systemically and that immune responses against the conceptus involve changes in both the B cell and T cell systems, and especially the the T-cell system in early pregnancy and the B-cell system in late pregnancy may be operating.**

Key Words: *Histochemical and immunohistochemical staining, Pregnant mice, Uterus draining lymph nodes, Activated B cells, IgM- and IgG positive cells, L3T4- and Lyt-2 positive cell*

INTRODUCTION

Even in cases of normal pregnancy, antibo

[+] This study was supported by a research grant from Seoul National University Hospital (1991).

dies against fetal blood antigens (red blood corpuscles) are formed when there is an incompatibility between the fetal and maternal red blood corpuscles antigens, and most Rh antigen incompatibilities lead to fetus death from the second pregnancy. However, no fatal immune responses are evoked in most pregnancies even if the conceptuses react to the maternal immune system as semiallogenic antigens. Several immunological studies show that all immune responses for and against the conceptus involve both the T- and B-cell systems (Goodlin *et al.* 1964; Maroni *et al.* 1973; Baines *et al.* 1976).

First, considering the effects of pregnancy on the T-cell system, in the 1970s many investigators reported a depression of cell-mediated immunity (Thong *et al.* 1973), an increase in weight of the iliac lymph nodes along with and a decrease in weight of the thymus (McLean *et al.* 1974), and a decrease of T lymphocytes in the peripheral blood (Strelkauska *et al.* 1975) during pregnancy. Sridama and his colleagues (1982) insist that the cause of immunosuppression in pregnancy is a decrease of helper T lymphocytes in the peripheral blood. Kim and his co-workers (1987) also report the same result as Sridama.

Meanwhile, Clark *et al.* (1977, 1983, 1984a&b, 1985, 1986) and Slapsy *et al.* (1983, 1984, 1985) show that the graft-vs-host response in pregnant mice is better suppressed than that in non-pregnant mice and that it is due to the presence of the suppressor cells in the uterine decidua and the suppression of the maternal cytotoxic T lymphocytes by them.

Reviewing the trends of research on B-cell system changes caused by pregnancy, Voisin (1983) insists that there are enhancing antibodies, immune complexes (so-called blocking factors), and anti-idiotypic antibodies that act as the protecting mechanisms of the fetus from maternal rejection. Hwang and his colleagues (1986) report that many IgG2 bearing plasma cells are present in the uterus-draining lymph nodes of allogeneically mated mice and that IgG2b would be the blocking antibodies. After all, both T- and B-cell systems are involved in the immune responses for and against conceptus.

Our research team (Lee *et al.* 1990) reported the changes in the T- & B-cell systems in the spleens of the pregnant mouse. We noted in that experiment that the conceptus evoked the systemic immune responses and that the supressor/cytotoxic T cells in early pregnancy and the helper/inducer T cells in late pregnancy might be operating. In this experiment, we studied the changes in the T- and B-cell systems in the uterus draining lymph nodes of pregnant mice as part of the supplementary work recommended and advised in the above experiment.

MATERIALS AND METHODS

1. Animals

Eight-week-old female ICR mice weighing approximately 30 gm (Seoul National University Laboratory Animals) were mated with male mice of the same strain. On the morning of the next day, which was determined as pregnancy 0 day, the vaginal plug was confirmed. The experimental animals were divided into 6 groups by duration of pregnancy (Table 1).

Table 1. Experimental groups

Groups		No. of animals
Group I	Virgin	12
Group II	Preg. 2nd day	11
Group III	Preg. 5th day	13
Group IV	Preg. 8th day	12
Group V	Preg. 14th day	12
Group VI	Preg. 19th day	10

2. Tissue processing

The experimental animals were anesthetized with ethyl ether and the uterine horns were revealed by laparotomy. 11% patent blue was injected into their wall and some minutes later only the blue stained ones among the scattered lumbar lymph nodes were resected. They were infiltrated by 30% sucrose-phosphate buffered saline (0.05 M, pH 7.2) in a 4℃ refrigerator for 12 to 16 hours. The tissue was embedded in embedding media for cryocut and were cryocut at 6 μm thickness. Each section was fixed in cold acetone (−20℃) before use.

3. Histochemical staining for activated B lymphocytes

For the detection of alkaline phosphatase activity in the membrane of the activated B lymphocytes, the lymph node sections were reacted with a Tris buffered saline solution containing 2 mg of naphthol AS-MX-phosphate sodium salt (3-hydroxy-2-naphthoic acid 2,4-dimethyl-anilide phosphate, Sigma Chemical Co.) and 10 mg of fast red TR salt (4-chloro-2- methylbenzene-diazonium chloride, hemizinc chloride salt, Sigma Chemical Co.) or fast blue BB salt (diazothized-4-amino-2,5'-diethoxyhenzanilide, zinc chloride salt, Sigma Chemical Co.) for 10 to 20 minutes.

4. Immunohistochemical staining

1) IgM positive cells

For the localization of surface and cytoplasm IgM positive cells (plasma cells), biotinylated goat anti-mouse IgM (μ chain speci- fic) antibody (Vector Co.) was diluted to 1 : 200 with a phosphate buffered saline (PBS) solution and reacted on the sections for 30 minutes. Endogenous peroxidase was removed by treatment with 0.5% periodic acid for 3 minutes. Avidin-biotin-peroxidase complex (ABC) was reacted for 30 minutes. For visualization of the positive cells, the tissue sections were finally reacted with PBS solution becoming 0.025% of 3,3'-diaminobenzidine (DAB), 0.001% of H_2O_2, and 0.04 % of nickel chloride until prominent visualization of the positive cells was achieved.

2) IgG positive cells

Goat anti-mouse IgG antibody (Sigma Chemical Co.) was diluted to 1:100 with PBS solution and reacted on the sections for 30 minutes. For the secondary step, monoclonal alkaline phosphatase-anti-alkaline phosphatase (APAAP) complex in 1 : 50 dilution with PBS solution was reacted for 30 minutes at room temperature. For visualization of positive cells, the sections were reacted with TBS (pH 8.2) solution containing 2 mg naphthol AS-MX-phosphate sodium salt, 10 mg of fast red TR salt or fast blue BB salt and 2.4 mg of Levamisole (Sigma Chemical Co.).

3) T cell subtypes

Three primary antibodies (Rat anti-Lyt 1 for pan T lympho-cytes, Rat anti-L3T4 for helper T lymphocytes and Rat anti-Lyt-2 for a part of suppressor or cytotoxic T lymphocytes) (Beckton-Dickenson Immunocytometry Systems) were commonly diluted to 1 : 100 with PBS (pH 7.2) and reacted for 30 minutes at room temperature. In the second step, Goat biotinylated anti-Rat-IgG antibody (Sigma Chemical Co.) was reacted for 30 minutes, and then ABC (Vector Lab.) was finally reacted for the same time. Visualization of each of the positive cells was made by reaction with a peroxidase substrate solution in PBS becoming 0.025% of DAB, 0.04% nickel chloride, and 0.001% of H_2O_2. All slide sections on which the histochemical and immunohistochemical staining procedure were completed were counterstained with hematoxylin or methyl green and wet-mounted with glycerogel (DAKO Corp.) for preservation and microscopic observation.

5. Microscopic observation

The items of microscopic observation were as follows: (1) place of appearance, and number of activated B cells (2) the number of IgM- and IgG-positive plasma cells (3) the distribution pattern of surface IgM- and IgG-positive cells (4) the distribution pattern of T-cell subtypes (Lyt-1, L3T4- & Lyt-2 positive cells).

RESULTS

1. Activated B cells

Activated B cells, expressing membrane alkaline phosphatase activity, will be designated as mAP positive cells.

Few mAP positive cells were observed in the outer cortex of the virgin control group (5 ± 1/unit area) and the pregnancy 2nd day group (7 ± 2 /unit area). In the pregnancy 5th day group, many positive cells were observed in the outer cortex (26 ± 3/unit area) and a few positive cells were observed in the deep cortex. In the pregnancy 8th day group, many more positive cells were observed in the outer cortex (128 ± 14/unit area).

In the pregnancy 14th and 19th day groups, the number of mAP positive cells in the outer cortex decreased compared with that of the pregnancy

Table 2. The number of mAP positive cells per unit area* of deep cortex of uterus draining lymph nodes

Groups		No. of mAP + cells	p value#
Group I	Virgin	5 ± 1@	—
Group II	Preg. 2nd day	7 ± 2	$p > 0.05$
Group III	Preg. 5th day	26 ± 3	$P < 0.05$
Group IV	Preg. 8th day	128 ± 14	$P < 0.01$
Group V	Preg. 14th day	99 ± 10	$P < 0.01$
Group VI	Preg. 19th day	91 ± 8	$P < 0.01$

* 0.2 cm × 0.2 cm ocular lattice in high power field (×200)
# P values are compared with that of Group I
@ Mean ± standard deviation

Table 3. The number of IgM positive plasma cells per unit area* of medulla of uterus draining lymph nodes

Groups		No. of IgM plasma cells	p value#
Group I	Virgin	2 ± 1@	—
Group II	Preg. 2nd day	3 ± 1	$p > 0.05$
Group III	Preg. 5th day	8 ± 2	$P < 0.05$
Group IV	Preg. 8th day	10 ± 3	$P < 0.01$
Group V	Preg. 14th day	22 ± 5	$P < 0.01$
Group VI	Preg. 19th day	11 ± 2	$P < 0.01$

* 0.2 cm × 0.2 cm ocular lattice in high power field (×200)
# P values are compared with that of Group I
@ Mean ± standard deviation

8th group but was greater than that of the virgin control group (Table 2).

2. IgM positive cells

1) Surface IgM (sIgM) positive cells

In the virgin control group, sIgM positive cells were localized well in the outer cortex and they were forming lymphatic follicles (Fig. 1).

In the pregnancy 5th and 8th day groups, the staining intensity of the positive cells became weaker than that of the other groups. In the pregnancy 14th day group, the spread out positive cells were localized in the outer cortex, and the staining intensity became stronger that that of the pregnancy 8th day group.

In the pregnancy 19th day group, the staining intensity was strongest.

2) IgM positive plasma cells

The number of IgM positive plasma cells in the medulla was compared. There was no difference in number between the virgin control group (2 ± 0.6/unit area) and the pregnancy 2nd day group (3 ± 0.5/unit area) (Fig. 1). In the pregnancy 5th day (8 ± 2/unit area) and 8th day (10 ± 3/unit area) groups, there was a statistically significant increase ($p < 0.05$) (Fig. 2). In the pregnancy 14th day group, the most numerous plasma cells were observed (22 ± 5/unit area) (Fig. 3). In the pregnancy 19th day group, the number of plasma cells decreased compared with that of the pregnancy 14th day group but significantly increased compared with that of the virgin control group (Table 3).

3. IgG positive plasma cells

The number of IgG positive plasma cells was counted in the medulla. The number of plasma cells in the virgin control and the pregnancy 2nd day groups was 7 ± 2 and 9 ± 2 per unit area, res-

Table 4. The number of mAP positive cells per unit area* of deep cortex of uterus draining lymph nodes

Groups	No. of IgG plasma cells	p value#
Group I Virgin	7 ± 2@	—
Group II Preg. 2nd day	9 ± 2	p > 0.05
Group III Preg. 5th day	25 ± 3	P < 0.05
Group IV Preg. 8th day	58 ± 7	P < 0.01
Group V Preg. 14th day	141 ± 13	P < 0.01
Group VI Preg. 19th day	139 ± 14	P < 0.01

* 0.2 cm × 0.2 cm ocular lattice in high power field (×200)
# P values are compared with that of Group I
@ Mean ± standard deviation

Table 5. Distribution and approximate number of B cells (surface IgM and IgG positive cells) in uterus draining lymph nodes

Groups (Pregnancy date)	Regions	Items	
		Surface IgM	Surface IgG
Group I (Virgin)	Cortex	++*	++
	C-M junction	—	—
	Medulla	—	—
Group II (2nd D.)	Cortex	++	+++
	C-M junction	—	±
	Medulla	—	—
Group III (5th D.)	Cortex	++	+++
	C-M junction	+	+
	Medulla	—	—
Group IV (8th D.)	Cortex	++	+++
	C-M junction	+	+
	Medulla	—	—
Group V (14th D.)	Cortex	+++	++++
	C-M junction	+	++
	Medulla	—	—
Group VI (19th D.)	Cortex	+++	++++
	C-M junction	+	+
	Medulla	—	—

* Approximate number of positive cells per high power field (×400)
— ; none
± ; 1~10
+ ; 10~50
++ ; 50~100
+++ ; 100~300
++++ : 300~500

Table 6. Distribution and approximate number of T cells (Lyt-1, L3T4 and Lyt-2 positive cells) in uterus draining lymph nodes

Groups (Pregnancy date)	Regions	Items		
		Lyt-1	L3T4	Lyt-2
Group I (Virgin)	Cortex	++++*	+++	+
	Medulla	+	±	±
Group II (2nd D.)	Cortex	++++	++	+
	Medulla	++	±	+
Group III (5th D.)	Cortex	++++	++	+
	Medulla	+++	±	++
Group IV (8th D.)	Cortex	++	++	+
	Medulla	±	±	±
Group V (14th D.)	Cortex	++++	+++	+
	Medulla	±	±	±
Group VI (19th D.)	Cortex	++++	+++	+
	Medulla	±	±	±

* Approximate number of positive cells per high power fields (×400)

− ; none
± ; 1~10
\+ ; 10~50
++ ; 50~100
+++ ; 100~300
++++ ; 300~500

pectively. In the pregnancy 5th day group, 25 ± 3 plasma cells were counted, and there was a significant increase ($p < 0.05$) compared with that of the virgin control group. In the pregnancy 8th day group, 58 ± 7 plasma cells were counted, and there was a very significant increase ($p < 0.01$). The pregnancy 14th and 19th day groups showed 141 ± 13 and 139 ± 14 plasma cells, respectively and reached a peak (Table 4, 5).

4. T cells

1) Lyt-1 positive cells

Lyt-1 positive cells were localized mainly in the deep cortex, and some positive cells were scattered in the medulla of the virgin control (Fig. 4a). In the pregnancy 2nd and 5th day groups the density of the positive cells in the medulla increased compared with that of the virgin control group (Fig. 5a).

In the pregnancy 8th day group, the density of the positive cells in the deep cortex decreased compared with that of the virgin control group, the pregnancy 2nd and 5th day groups and in the medulla it decreased compared with that of the pregnancy 2nd and 5th day groups (Fig. 6a).

In the pregnancy 14th and 19th day groups, the density of the positive cells in the deep cortex was the same or increased compared with that of the virgin control, pregnancy 2nd and 5th day groups (Fig. 7a & 8a).

2) L3T4 positive cells

Generally, the distribution patterns of L3T4 positive cells were similar to those of Lyt-1 positive cells. Most positive cells were localized only in the deep cortex. The density of the positive cells was lower than that of Lyt-1 positive cells (Fig. 4b).

There was a remarkable decrease in the number of positive cells in the pregnancy 5th and 8th

day groups (Fig. 5b & 6b). In the 14th and 19th day groups, the densities of the positive cells were the same or slightly increased compared with those of the virgin control and the pregnancy 2nd day groups (Fig. 7b & 8b).

3) Lyt-2 positive cells

Generally, the distribution patterns of Lyt-2 positive cells were similar to those of L3T4 positive cells. Most positive cells were localized only in the deep cortex. The density of the positive cells was much lower than that of L3T4 positive cells.

In the pregnancy 5th day group, the density of the positive cells in the medulla increased compared with that of the virgin control and the pregnancy 2nd day groups. In the 8th day group, however, it decreased in the deep cortex as well as in the medulla compared with that of the virgin control and the pregnancy 2nd day groups. In the pregnancy 14th and 19th day groups, there were no changes in the density of the positive cells from that of the virgin control and the pregnancy 2nd day groups (Table 6).

DISCUSSION

As the experimental groups were determined, we have considered that implantation took place 3 to 4 days after fertilization. Early pregnancy groups, therefore, were divided into two groups: those just prior to implantation (the pregnancy 2nd day group) and those just after implantation (the pregnancy 5th day group).

The pregnancy 8th day group was determined, because the 8th day of pregnancy was the period of organogenesis, and it was the end of the first trimester.

Detection of activated B cells by checking the membrane alkaline phosphatase activity was reported by several investigators at the end of 1970. Garcia-Rozas (1982) and Mosbach-Ozmen (1986) reported that only activated B cells could show membrane alkaline phosphatase activity through in vitro experiments in which B cells were stimulated by several kind of mitogens. The same result was reported in in vivo experiments using murine spleens by Lee and his colleagues (1991a&b). The above experiments testify that the detection method for activated B cells by membrane alkaline phosphatase activity is a very simple and good one.

The number of activated B cells significantly increased for the first time in the pregnancy 5th day group, while the most numerous activated B cells were observed in the pregnancy 8th day group, indicating that the largest amount of fetal antigens might be exposed to the maternal immune system around the 8th day of pregnancy. After the 8th day of pregnancy there were still significant increases in the number of mAP cells. This suggests that pregnancy- associated antigens may have been challenged during the whole period of pregnancy. The number of activated B cells in each stage of pregnancy was similar to that reported by Lee and his co-workers (1990), who performed a similar experiment in the spleens of pregnant mice.

IgM positive plasma cells showed their peak number on the 14th day of pregnancy. As a whole the number of IgM positive plasma cells was much less than that of IgG positive plasma cells.

The number of IgG positive plasma cells gradually increased, and there was a peak at the 19th day of pregnancy. As these results were considered in combination with those of mAP cells and IgM positive plasma cells from the 8th day of pregnancy, IgM positive plasma cells might have been cross-switched into IgG positive plasma cells. Consequently, from the 14th day of pregnancy, the number of IgM positive plasma cells decreased and that of the IgG positive plasma cells increased and reached a peak at the term of pregnancy. Hwang and his colleagues (1986) reported that IgG positive plasma cells increased even in the case of syngeneic mating. Several investigators (Rocklin *et al.* 1973; Devey and Voak 1974; Pence *et al.* 1975; Riggio *et al.* 1978; Martnez *et al.* 1980) reported that IgG antibody plays a role as a blocking antibody in preventing the fetus from being rejected.

The density of Lyt-1 positive cells, representing pan-T lymphocytes, increased in the medulla and L3T4 positive cells, representing helper or inducer T lymphocytes decreased in the medulla at the 5th day of pregnancy. And at the 8th day of pregnancy Lyt-1 positive cells much decreased in the deep cortex, while L3T4 positive cells did not

decrease in the deep cortex. This means that Lyt-2 positive cells may be moved from the uterus draining lymph nodes somewhere around the 8th day of pregnancy. Actually Lyt-2 positive cells increased in the medulla at the 5th day of pregnancy and they decreased in the deep cortex from the 8th day of pregnancy.

Near the term of pregnancy, L3T4 positive cells were gathered into the deep cortex. Lee and his colleagues (1990) report the same result in the spleens of pregnant mice as that described here.

The density of Lyt-2 positive cells, representing the suppressor/ cytotoxic T lymphocytes, decreased in the deep cortex from the 8th day of pregnancy. However, from the 14th day of pregnancy, the density increased (compared with that of the pregnancy 8th day group), and there was a peak number at the term of pregnancy. Lee and his colleagues (1990) reported the same results in the spleens of pregnant mice. Where have the Lyt-2 positive cells in the spleen or uterus-draining lymph node moved? This is a very important question, and there is some speculation about it. However, in order to investigate the above phenomenon clearly, the proportions of T cell subtypes in the peripheral blood, thoracic duct, and interface between the uterus and placenta must be calculated simultaneously through well-devised experiments.

We have obtained the following conclusions based on the facts observed and discussed in this experiment. First, after implantation, the maternal immune system is challenged continuously by the fetal antigen or pregnancy-associated antigen. Second, the fetal antigens evoke the local as well as systemic immune responses. Third, Lyt-2 positive cells play a key role in the early stage of pregnancy, and IgG positive plasma cells (including L3T 4 positive cells) play a key role in the late stage of pregnancy in preventing the fetus from being rejected by the maternal immune mechanism.

REFERENCES

Baines MG, Speers EA, Prossa H, Miller KG. Characteristics of the maternal lymphoid response of mice to paternal strain antigens induced by homologous pregnancy. Immunol. 1976, 31: 363-369

Carter J, Newport A, Keeler KD, Dresser DW. FACS analysis of changes in T and B lymphocytes populations in the blood, spleen and lymph nodes of pregnant mice. Immunol. 1983, 48: 791-797

Clark DA, Chaput A, Tutton D. Active suppression of host-vs-graft reaction in pregnant mice. VII. Spontaneous abortion of allogeneic CAB/J x DAB/2 fetuses in the uterus of CBA/J mice correlates with deficient non-T suppressor cell activity. J. Immunol. 1986, 136: 1668- 1675

Clark DA, Chaput A, Walker C, Rosenthal K. Active suppression of host-vs-graft reaction in pregnant mice. VI. Soluble suppressor activity obtained from decidua blocks the response to IL-2. J. Immunol. 1985, 134: 1659-1668

Clark DA, McDermoff MR. Active suppression of host-vs-graft reaction in pregnant mice. III. Development kinetics, properties, and mechanism of induction of suppressor cells during first pregnancy. J. Immunol. 1981, 127: 1267-1273

Clark DA, Phillips RA, Miller RG. Characterization of the cells that suppress the cytotoxic activity of T lymphocytes. II. Physical properties, specificity, developmental kinetics of the inhibitory splenic non-T cell population. Cell Immunol. 1977, 34: 25-31

Clark DA, Slapsy RM, Croy BA, Krcek J, Rossant J. Local active suppression by suppressor cells in decidua: A review. Am. J. Reprod. Immunol. 1984 (a), 5: 78-89

Clark DA, Slapsy RM, Croy BA, Rossant J. Immunoregulation of host-vs- graft responses in the uterus. Immunol. Today. 1984 (b), 5: 11-19

Devey ME, Voak D. A critical study of the IgG subclasses of Rh anti-D antibodies formed in pregnancy and in immunized volunteers. Immunolf. 1974, 27: 1073-1079

Garcia-Rozas C, Plata A, Diaz-Espada F, Kreisler M, Martinez-Alonso C. Alkaline phosphatase activity as a membrane marker for activated B cell. J. Immunol. 1982, 129: 52-55

Goodlin RC, Herzenberg LA. Pregnancy induced hemagglutins to paternal H-2 antigens in multiparous mice. Transplantation. 1964, 2: 357-361

Hwang DH, Lee KH, Chang KY, Baik SH, Seoung KJ, Lee BL. Numerical changes of immunoglobulin containing cells in the lymph nodes draining the uterus of pregnant mice. Seoul J. Med. 1986, 27:

201-209

Kim GS, Kim SW, Lee WJ, Lee KH, Chang KY. Immunocytochemical study on the changes of the percentage and absolute numbers of T lymphocytes and their subsets in the peripheral blood of Korean pregnant women. Korean J. Obstetrics & Gynecology. 1987, 30: 1625-1634

Lee WJ, Lim CW, Hwang DH, Lee KH, Chang KY. Immunohistochemical study on changes of T-and B-cell populations in mouse spleens during pregnancy. Seoul J. Med. 1990, 31 (3): 167-181

Lee WJ, Kim YK, Hwang DH, Chang KY, Lee KH. Morphological characterization of T cell dependent B cell activation process in the mouse spleen. Korean J. Anat. 1991a, 24 (2): 226-241

Lee WJ, Chang KY, Lee KH. Immunohistochemical study on the changes in the distribution of T & B lymphocytes by administration of mitogens and T cell dependent antigen in the mouse spleen. Korean J. Anat. 1991b, 24 (4): 422-437

Maroni ES, Parrot DMV. Progressive increase of immunity against paternal transplantation antigens after multiple pregnancies. Clin. Exp. Immunol. 1973, 31: 253-262

Martnez-A C, Coutinho A, Augustin A. Immunoglobulin C-gene expression. I. The commitment to IgG subclass of secretory cells is determined by the quality of the nonspecific stimuli. Eur. J. Immunol. 1980, 10: 698-702

McLean JM, Mosley JG, Gibbs ACC. Changes in the thymus, spleen and lymph nodes during pregnancy and lactation in the rat. J. Anat. 1974, 118: 223-229

Mosbach-Ozmen L, Lehuen-Renard A, Gaveriaux C, Loor F. Membrane alkaline phosphatase activity: An enzymatic marker of B-cell activation. Ann. Inst. Pasteur/Immunol. 1986, 137: 109-125

Riggio RR, Saal SD, Stenzel KH, Rubin AL. Retroplacental serum factors and passive enhancement. Transplant. Proc. 1976, 8: 281-285

Rocklin RE, Kitzmiller JL, Kaye MD. Immunobiology of the maternal-fetal relationship. Ann. Rev. Med. 1979, 30: 375-404

Slapsy RM, Beeson J, Clark DA. Recruitment of a trophoblast dependent suppressor cell to the implantation site of allopregnant mice. Fed. Proc. 1985, 44: 1880-1186

Slapsy RM, Beeson J, Clark DA. The role of the trophoblast in the localization of decidua-associated suppressor cell. Am. J. Reprod. Immunol. 1984, 6: 66-72

Slapsy RM, Clark DM. Active suppression of host-vs-graft reaction in pregnant mice. V. Kinetics, specificity, and in vitro activity of non-T suppressor cells. Am. J. Repord. Immunol. 1983, 3: 65-72

Sridama V, Pacini F, Yang SL, Moawad A, Reily M, DeGroot LJ. Decreased levels of helper T cell - A possible cause of immunodeficiency in pregnancy. New Eng. J. Med. 1982, 207: 352-356

Strelkauska AJ, Wilson BS, Dray S. Inversion of levels of human T and B cells in early pregnancy. Nature. 1975, 258: 331-332

Thong YH, Steele RW, Vincent MM, Hensen SA, Bellanti JA. Impaired in vitro cell mediated immunity to rubella virus during pregnancy. New Eng. J. Med. 1973, 289: 604-606

Voisin GA. Immunological interventions of the placenta in maternal immunological tolerance to the fetus in immunology of reproduction, Wegman TG and Gill III TJ, eds. New York, Oxford: Oxford University Press, 1983, pp. 181-203

EXPLANATION OF FIGURES

Fig. 1. Immunohistochemical staining pattern of IgM positive cells in uterus draining lymph node of virgin control. Counterstained with methyl green. Numerous surface IgM positive cells (black) are clustered mainly in the outer cortex. ×40.

Fig. 2. Immunohistochemical staining of IgM positive cells in uterus draining lymph node of the pregnancy 5th day mouse. Counterstained with methyl green. Many surface IgM positive cells (blue black) are distributed over the outer cortex and corticomedullary junction. Several IgM positive plasma cells are scattered over the medulla. ×40.

Fig. 3. Immunohistochemical staining of IgM positive cells in uterus draining lymph node of the pregnancy 14th day mouse. Counterstained with methyl green. Many surface IgM positive cells (blue black) are distributed over the outer cortex in follicular pattern. The staining intensity is stronger than those of virgin control and the pregnancy 5th or 8th day groups. Much more IgM positive plasma cells than those of former groups are darkly stained in the medulla. ×40.

Fig. 4a. Double staining pattern of activated B cells (red) and Lyt-1 positive cells (blue black) in uterus draining lymph node of virgin control. Many Lyt-1 positive cells are densely present over the deep cortex. Some positive cells are also scattered over the medulla. Many activated B cells are present mainly over the medulla. ×40.

Fig. 4b. Double staining pattern of activated B cells (red) and L3T4 positive cells (blue black) in uterus draining lymph node of virgin control. Many L3T4 positive cells are distributed over the deep cortex. Few positive cells are present in the medulla. ×40.

Fig. 5a. Double staining pattern of activated B cells (red) and Lyt-1 positive cells (blue black) in uterus draining lymph node of the pregnancy 5th day mouse. Many Lyt-1 positive cells are present over the deep cortex and the medulla. Activated B cells are distributed over the deep cortex and the medulla. ×40.

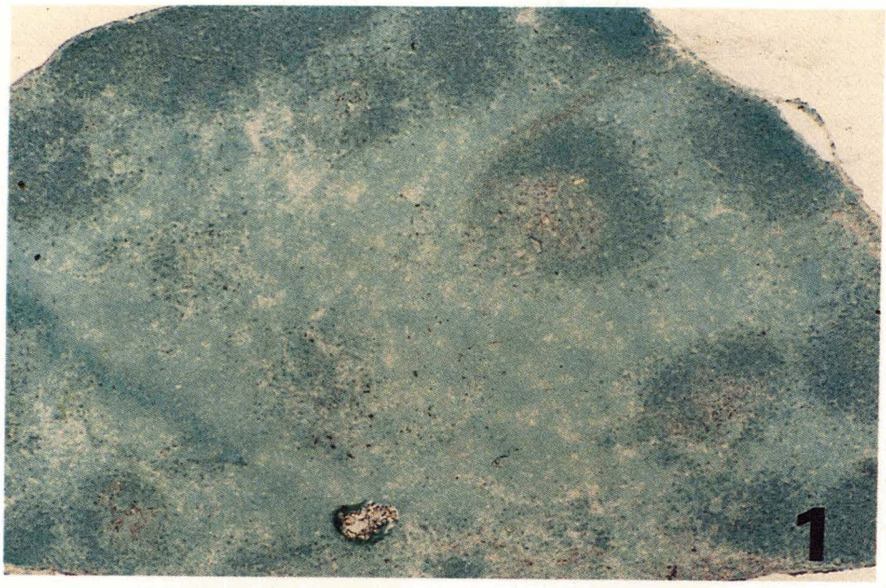
Fig. 5b. Double staining pattern of activated B cells (red) and L3T4 positive cells (blue black) in uterus draining lymph node of the pregnancy 5th day mouse. L3T4 positive cells are distributed over the deep cortex. The density of the positive cells is lower than that of virgin control. ×40.

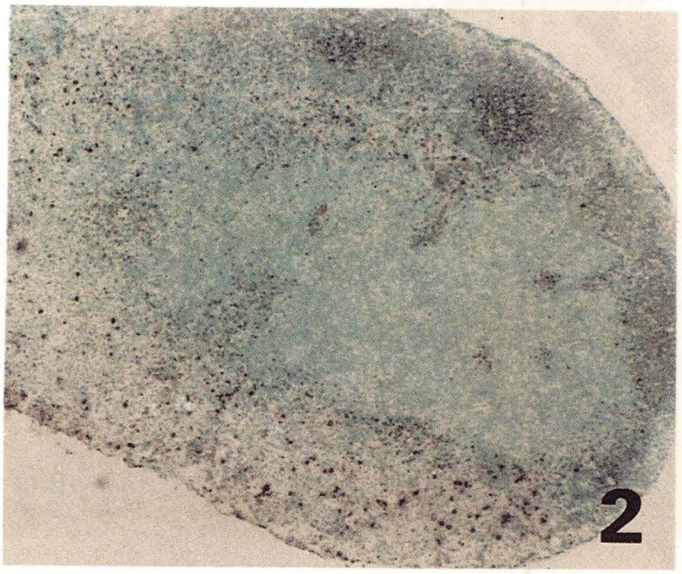
Fig. 6a. Double staining pattern of activated B cells (red) and Lyt-1 positive cells (blue black) in uterus draining lymph node of the pregnancy 8th day mouse. Many Lyt-1 positive cells are present over the deep cortex. The density of positive cells is lower than those of virgin control and the pregnancy 5th day group. Activated B cells are distributed over the deep cortex. ×40.

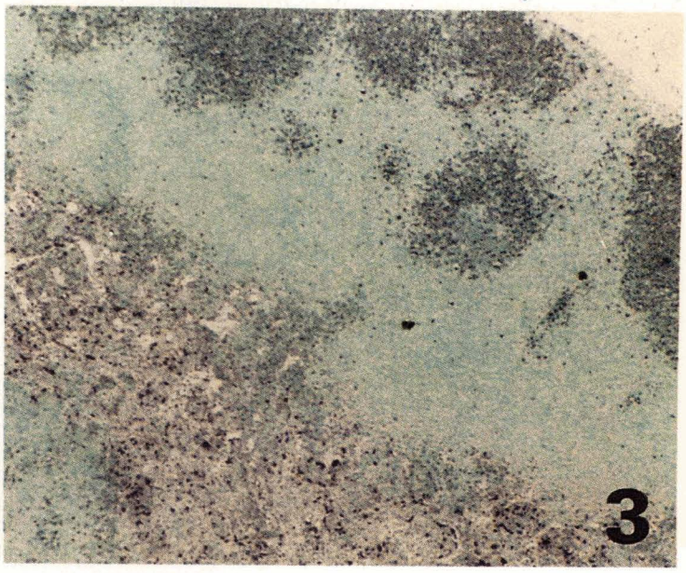
Fig. 6b. Double staining pattern of activated B cells (red) and L3T4 positive cells (blue black) in uterus draining lymph node of the pregnancy 8th day mouse. Many L3T4 positive cells are present over the deep cortex. The pattern of distribution and the density are very similar to those of Thy-1.2 positive cells. ×40.

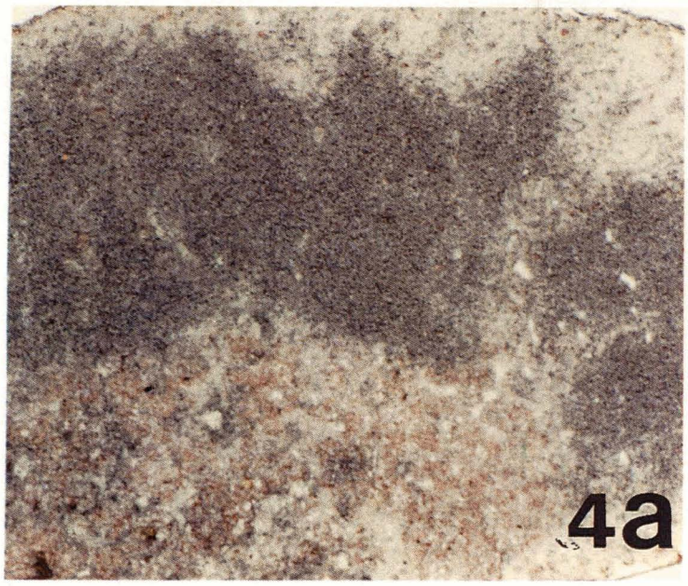
Fig. 7a. Double staining pattern of activated B cells (red) and Lyt-1 positive cells (blue black) in uterus draining lymph node of the pregnancy 14th day mouse. Counterstained with methyl green. Many Lyt-1 positive cells are distributed over the nearly all cortex. Some positive cells are present in the medulla. Activated B cells are stained red in the medulla, but are masked by Lyt-1 positive cells in the deep cortex. ×40.

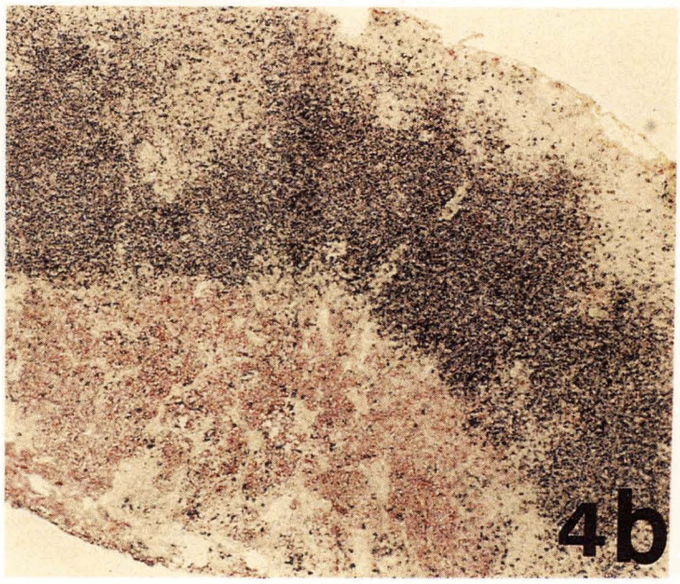
Fig. 7b. Double staining pattern of activated B cells (red) and L3T4 positive cells (blue black) in uterus draining lymph node of the pregnancy 14th day mouse. Counterstained with methyl green. Many L3T4 positive cells are distributed over the deep cortex. The density of positive cells is nearly same as that of virgin control. ×40.

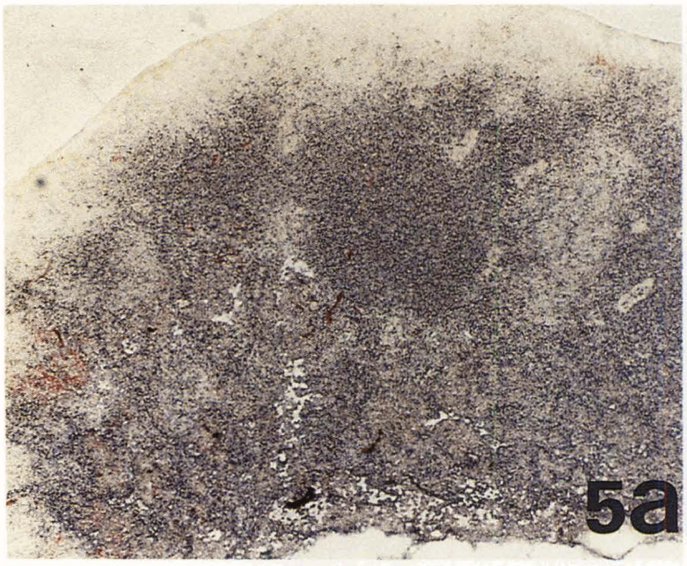

1

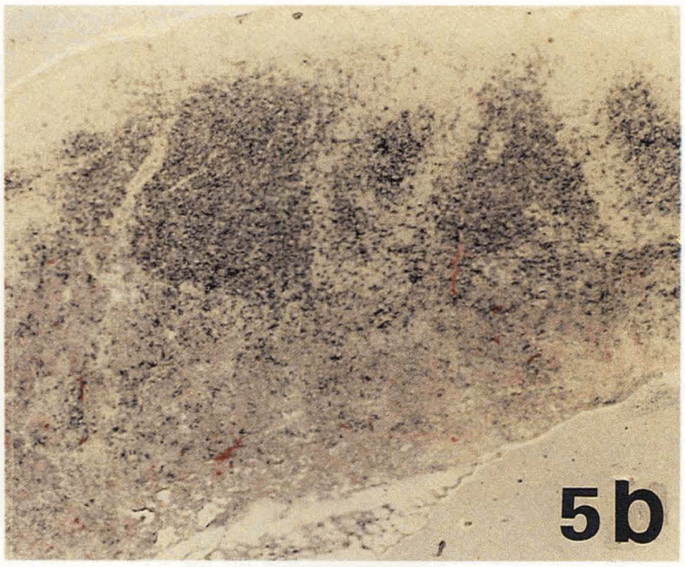

2

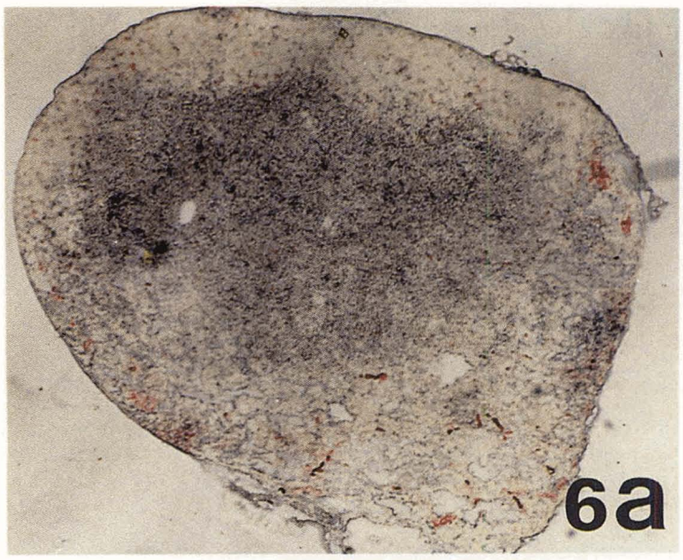

3

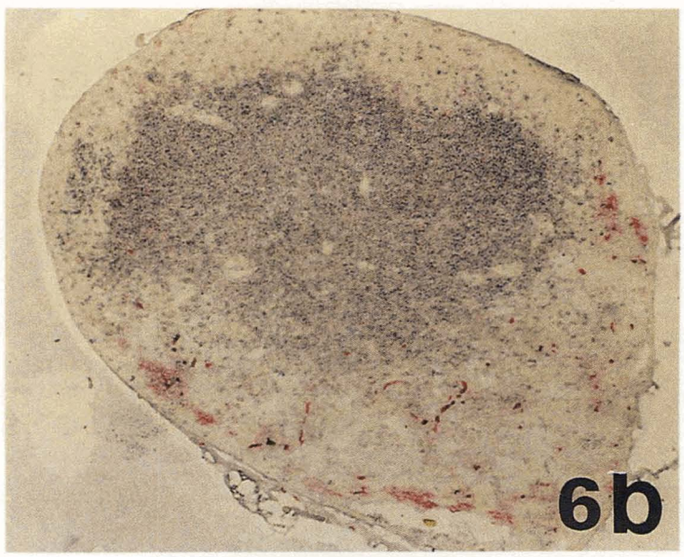

4a

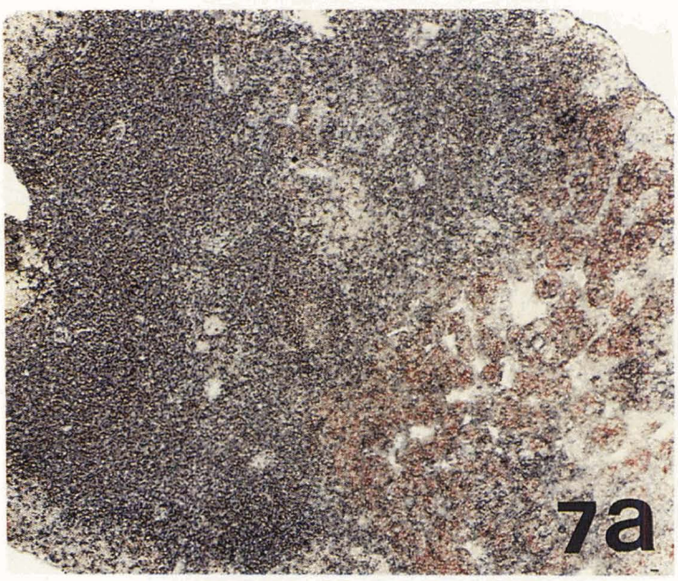

4b

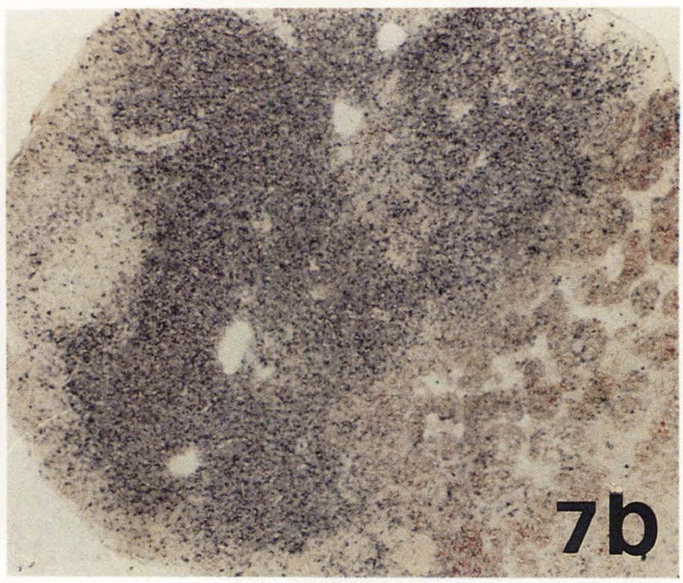

5a


5b


6a


6b


7a


7b

=국 문 초 록=

자궁유출림프절에서 T 및 B 세포계의 임신기간에 따른 변화에 관한 면역조직화학적 연구

서울대학교 의과대학 해부학교실 및
경상대학교 의과대학 내과학교실*

이왕재 · 황영실* · 황덕호 · 이광호 · 장가용

임신기간에 따른 생쥐의 자궁유출림프절에서의 면역반응의 변화를 관찰하기 위해 ICR계의 생쥐를 이용하여 다음과 같은 실험을 수행하였다. 실험동물은 처녀군, 임신 제 2 일군, 임신 제 5 일군, 임신 제 8 일군, 임신 제14일군, 임신 제19일군 등 6개의 군으로 나누었다.

관찰대상은 자궁유출림프절에 존재하는 T 세포계와 B 세포계의 시기별 변화에 대한 것으로서 B 세포계통의 변화를 관찰하기 위해서는 활성 B 세포, IgM 및 IgG 양성형질세포, 표면 IgG 양성세포들의 시기별 출현수와 분포양상을 관찰하였고, T 세포계통의 변화를 관찰하기 위해서는 L3T4 양성세포, Lyt-2 양성세포들의 수적 및 분포변화를 관찰하였다.

실험방법으로는 활성 B 세포의 경우는 membrane alkaline phosphatase(이하 mAP로 약함) 활성도를 측정하는 조직화학염색방법을, 나머지 세포들은 각각의 항체를 이용한 avidin-biotin-peroxidase complex, alkaline phosphatase anti-alkaline phosphatase 등의 면역조직화학염색법을 이용하였다. 이 실험을 통해서 얻은 결과들을 보면 임신 말기에 L3T4 양성세포는 심피질에서, IgM 양성형질세포와 IgG 양성형질세포는 수질에서 증가되어 관찰되었다. 반면 Lyt-2 양성세포는 임신 제 5 일에 수질 혹은 수질피질 경계부위에서 증가되어 관찰되었다. 이 결과들로부터 다음과 같은 결론을 얻었다.

첫째 임신 소산물은 전신적뿐만 아니라 현저한 지역적 면역반응을 일으키는 것으로 생각되고, 둘째 임신 초기에는 Lyt-2 양성세포들의 수적, 분포적 변화가 일어나고 임신 중기 이후에는 B 세포계의 형질세포들과 L3T4 양성세포들의 수가 증가하는 것으로 보아 전자의 세포는 임신 초기에, 그리고 후자는 임신 후기에 각기 중요한 역할을 하는 것임을 암시하고 있다.