

Numerical Changes of Immunoglobulin Containing Cells in the Lymph Nodes Draining the Uterus of Pregnant Mice

Douk Ho Hwang, Kwang Ho Lee, Ka Young Chang
Sang Ho Baik, Key June Seoung and Byung Lan Lee

Department of Anatomy, College of Medicine, Seoul National University, Seoul 110, Korea

=Abstract=This experiment was performed to detect which IgG subclasses are stimulated by the fetal antigens. The experimental animals were mated syngeneically (female ICR X male ICR) or allogeneically (female ICR X male DDY), and the renal and lumbar lymph nodes draining the uterus were dissected out on the 9th and 18th days of pregnancy. The lymph nodes were stained with methyl green pyronin(MGP) and treated with a immunohistochemical reaction. Then each group was compared with the virgin control group.

The following results were obtained:

1. In the group of full term mice which were mated syngeneically, the numbers of the IgG1, IgG2b and IgM containing cells were significantly greater than those of virgin.
2. In the group of mid term mice which were mated allogeneically, the number of the IgG2b containing cells was significantly greater than that of virgin.
3. In the group of full term mice which were mated allogeneically, the numbers of the IgG1, IgG2b, IgG3 and IgM containing cells were significantly greater than those of virgin.
4. In the group of full term mice which were mated allogeneically, the numbers of the IgG1, IgG2b and IgG3 containing cells were significantly greater than those of syngeneic mating.

On the basis of the above results, it is suggested that the IgG1, IgG2b and IgG3 were stimulated during pregnancy in mice.

Key words: *Pregnancy, Immunoglobulins, Lymph nodes, Immunohistochemistry*

INTRODUCTION

The fetuses of all outbred animals present a set of paternal antigens which are foreign to their mother. And the fetus survives during pregnancy without rejection. During the normal course of pregnancy, both cellular and humoral immune response to paternal antigens develop in the maternal side. Anti-Rh antibodies in Rh-negative women and anti-HLA antibodies in maternal side, are mediated by the fetal antigens (Goodlin and Herzenberg 1964; Maroni and Parrot 1973). Maternal cellular immune responses in vitro are lymphocyte

proliferation and cytotoxicity against paternal antigens (Youtananukorn *et al.* 1974; Baines *et al.* 1976).

Tofoski and Gill(1977) described that removal of the lymph nodes draining the uterus decreases the reproductive capacity in allogeneic matings. And immune response has been defined morphologically by the enlargement of the lymph nodes draining the uterus, by the proliferation of blast cells, and by the large numbers of plasma cells in the medullary cords (Maroni and de Sousa, 1973; Ansell *et al.* 1978; Shaya *et al.* 1981; Carter and Dresser 1983). A direct immunofluorescence assay by utilizing antisera to mouse Thy-1 showed a marked

increase in the number of T cells in the lymph nodes draining the uterus during the later half of a first allogeneic pregnancy (Carter *et al.* 1983). Hirahara *et al.* (1980) reported that suppressor T cells were increased and helper T cells were decreased in the peripheral blood during pregnancy. Carter and Dresser (1983) reported that there was a considerable increase in the numbers of IgG- and, to a lesser extent, IgM-secreting cells in the paraaortic lymph nodes of the pregnant mice. However it is unknown which IgG subclasses are stimulated by the fetal antigens.

This study was designed to detect the numerical changes of the IgG1, IgG2a, IgG2b, IgG3, IgM and IgA containing cells in the lymph nodes draining the uterus of the pregnant mice as a humoral immune response to fetal antigens.

MATERIALS AND METHODS

1. Animals

ICR female mice aged between 8 and 10 weeks were used in this experiment. Five groups, each consisting of five mice, were mated with ICR (syngeneically) or DDY (allogeneically) males. The day when the vaginal plug was found in the morning, was denoted as day 0 of pregnancy.

Group I : Virgin

Group II_m : Mid term (9th day of pregnancy)
X ICR male

Group II_f : Full term (18th day of pregnancy)
X ICR male

Group III_m : Mid term
X DDY male

Group III_f : Full term
X DDY male

2. Processing of tissue

Each mouse was anesthetized with 0.04 mg of sodium pentobarbital by the intraperitoneal injection. And then to visualize the lymphatic drainage of the uterus, mice were injected with 11% solution of patent blue violet through the wall of the uterine horns. The lumbar and renal lymph nodes were dissected out, and fixed in 6% formalin-60% ethyl alcohol solution during 1-2 days. After fixation, dehydration and clearing of tissues were carried out in the usual method, finally embedded in paraffin. Tissues were serially cut at 6 micrometers. Each section was stained with methyl green pyronin (MGP). For the immunohistochemical reaction, deparaffinized sections were treated with a fresh

0.3% hydrogen peroxide in methanol for 30 minutes to remove the endogenous peroxidase. Sections were rinsed in 3 changes of pH 7.2, 0.02 M phosphate buffered saline (PBS) for 10 minutes. Sections were incubated with 5% bovine albumin in PBS for 10 minutes in the moist chamber and drained. Then sections were covered with the primary antibodies, which were titrated previously in optimal dilution, at the room temperature for 30 minutes. The primary antibodies were rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3 (Zymed Laboratories Inc., South San Francisco, U.S.A.), rabbit anti-mouse IgM, IgA (Miles Laboratories, Elkhart, U.S.A.). After incubation, sections were washed in 3 changes of PBS for 10 minutes. Next the washed sections were incubated with the secondary antibody in the procedure as previous. The secondary antibody was goat anti-rabbit IgG peroxidase conjugate (Sigma Chemical Co., Saint Louis, U.S.A.). To stain horseradish peroxidase, the substrate solution containing 30 mg 3,3'-diaminobenzidine tetrahydrochloride and 50 microliter 3% hydrogen peroxide in 40 ml, pH 7.6, 0.05 M TRIS-buffer, was used for 10 minutes. After several washing of tissues, these were counterstained by using 1% methyl green. After staining, slides were dehydrated, cleared and mounted by using a synthetic mounting medium.

The number of the immunoglobulin containing cells was counted by using an eye piece graticule delineating an area of 0.04 mm² X 5 areas in the random selected medulla. Because each section was done serially, each class and subclass of the immunoglobulin containing cells could be counted in the same region.

3. Statistical analysis

Wilcoxon rank sum test was used to determine the significance.

RESULTS

1. IgG1 containing cells

In the renal nodes, the number of the IgG1 containing cells was 8.2 in group I, 5.8 in group II_m, 49.2 in group II_f, 21.2 in group III_m and 336.6 in group III_f. In the group II_f and III_f, the number of the IgG1 containing cells was significantly greater than that of group I ($p < 0.05$) (Table 1, Fig. 1).

In the lumbar nodes, the number of the IgG1 containing cells was 6.6 in group I, 15.0 in group II_m, 47.8 in group II_f, 22.8 in group III_m and 463.6 in group III_f. In the group II_f and III_f, the number of the IgG1 containing cells was signifi-

Table 1. The average numbers of IgG1, IgG2a, IgG2b, IgG3, IgM and IgA containing cells per unit area in the renal nodes

Groups	IgG1	IgG2a	IgG2b	IgG3	IgM	IgA
I	8.2	1.0	10.6	13.4	6.6	11.2
II _m	5.8	0.8	29.8	47.8	17.8	10.8
III _f	49.2*	5.2	81.4*	72.4	35.2	11.0
III _m	21.2	2.6	45.0*	34.6	18.6	29.2
III _f	336.6*	4.8	258.6*	492.0*	49.6*	16.0

*: Significant differences from virgin control($p < 0.05$)

Table 2. The average numbers of IgG1, IgG2a, IgG2b, IgG3, IgM and IgA containing cells per unit area in the lumbar nodes

Groups	IgG1	IgG2a	IgG2b	IgG3	IgM	IgA
I	6.6	1.4	4.2	4.0	4.0	4.6
II _m	15.0	1.2	6.4	17.8	19.8	4.8
III _f	47.8*	5.2	61.8	40.8	25.0*	10.6
III _m	22.8	6.4	7.2	9.0	16.6	7.2
III _f	463.6*	19.0	427.8*	292.8*	31.8*	14.8

*: Significant differences from virgin control($p < 0.05$)

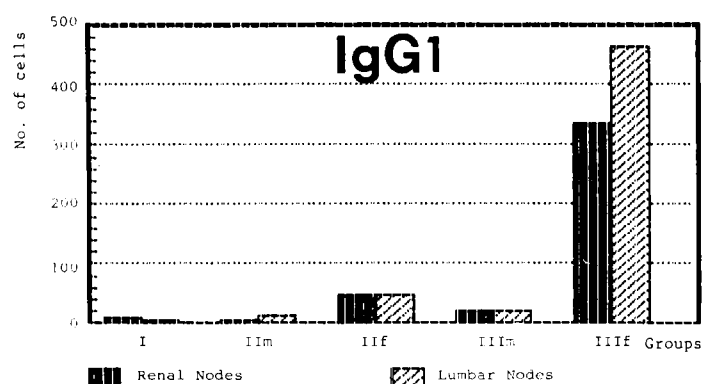


Fig. 1. The average numbers of IgG1 containing cells per unit area in the renal and lumbar nodes.

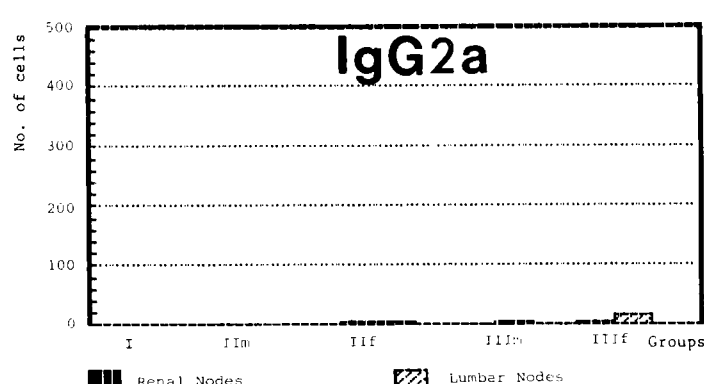


Fig. 2. The average numbers of IgG2a containing cells per unit area in the renal and lumbar nodes.

cantly greater than that of group I ($p < 0.05$) (Table 2, Fig. 1).

2. IgG2a containing cells

In the renal nodes, the number of the IgG2a containing cells was 1.0 in group I, 0.8 in group II_m, 5.2 in group III_f, 2.6 in group III_m and 4.8 in group III_f (Table 1, Fig. 2).

In the lumbar nodes, the number of the IgG2a containing cells was 1.4 in group I, 1.2 in group II_m, 5.2 in group III_f, 6.4 in group III_m and 19.0 in group III_f (Table 2, Fig. 2).

3. IgG2b containing cells

In the renal nodes, the number of the IgG2b containing cells was 10.6 in group I, 29.8 in group

II_m, 81.4 in group III_f, 45.0 in group III_m and 258.6 in group III_f. In the group III_m, III_f and III_f, the number of the IgG2b containing cells was significantly greater than that of group I ($p < 0.05$) (Table 1, Fig. 3).

In the lumbar nodes, the number of the IgG2b containing cells was 4.2 in group I, 6.4 in group II_m, 61.8 in group III_f, 7.2 in group III_m and 427.8 in group III_f. In the group III_f, the number of the IgG2b containing cells was significantly greater than that of group I ($p < 0.05$) (Table 2, Fig. 3).

4. IgG3 containing cells

In the renal nodes, the number of the IgG3 containing cells was 13.4 in group I, 47.8 in group

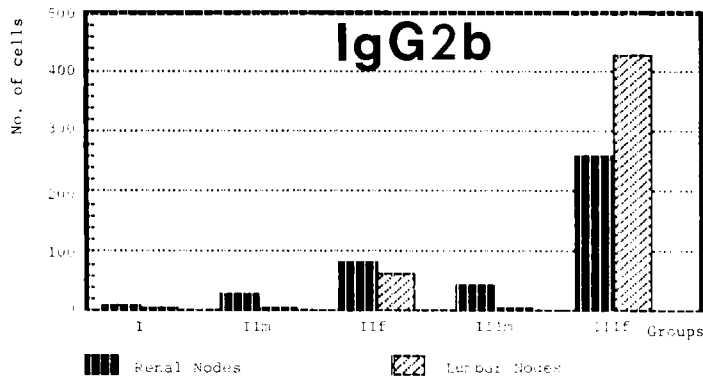


Fig. 3. The average numbers of IgG2b containing cells per unit area in the renal and lumbar nodes.

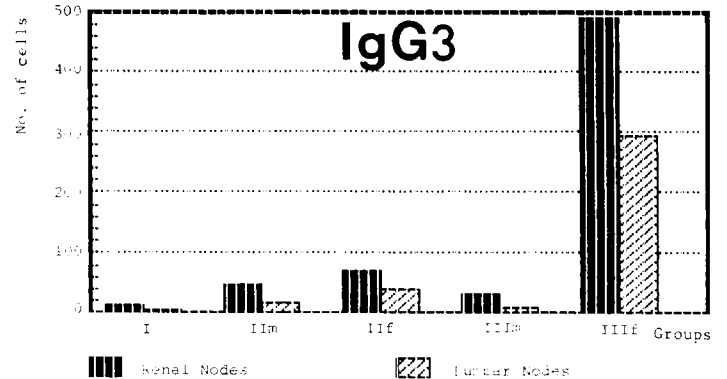


Fig. 4. The average numbers of IgG3 containing cells per unit area in the renal and lumbar nodes.

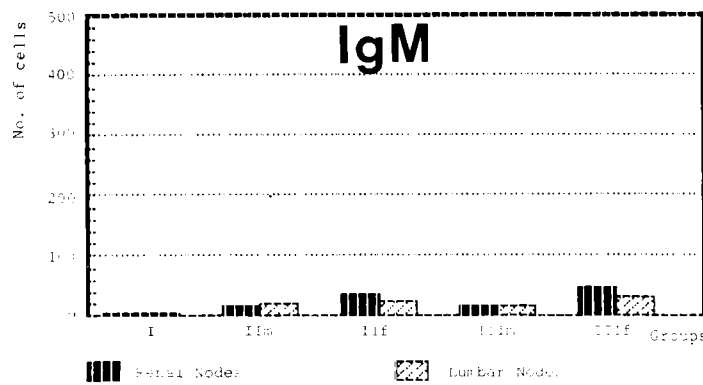


Fig. 5. The average numbers of IgM containing cells per unit area in the renal and lumbar nodes.

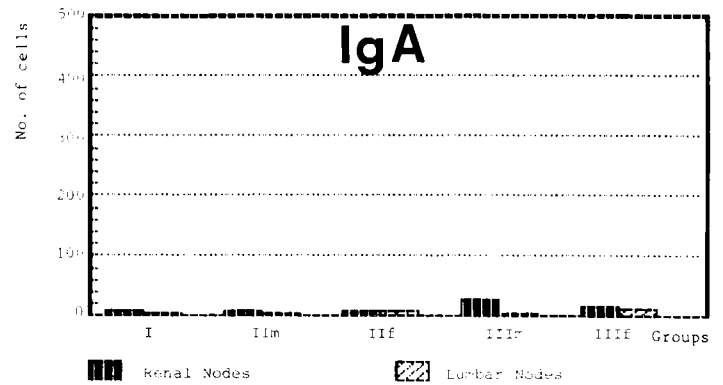


Fig. 6. The average numbers of IgA containing cells per unit area in the renal and lumbar nodes.

IIIm, 72.4 in group IIIf, 34.6 in group IIIIm and 492.0 in group IIIIf. In the group IIIIf, the number of the IgG3 containing cells was significantly greater than that of group I ($p < 0.05$) (Table 1, Fig. 4).

In the lumbar nodes, the number of the IgG3 containing cells was 4.0 in group I, 17.8 in group IIIm, 40.8 in group IIIf, 9.0 in group IIIIm and 292.8 in group IIIIf. In the group IIIIf, the number of the IgG3 containing cells was significantly greater than that of group I ($p < 0.05$) (Table 2, Fig. 4).

5. IgM containing cells

In the renal nodes, the number of the IgM containing cells was 6.6 in group I, 17.8 in group IIIm, 35.2 in group IIIf, 18.6 in group IIIIm and 49.6 in group IIIIf. In the group IIIIf, the number of the IgM containing cells was significantly greater than that of group I ($p < 0.05$) (Table 1, Fig. 5).

In the lumbar nodes, the number of the IgM containing cells was 4.0 in group I, 19.8 in group IIIm, 25.0 in group IIIf, 16.6 in group IIIIm and 31.8 in group IIIIf. In the group IIIf and IIIIf, the number of the IgM containing cells was significantly greater than that of group I ($p < 0.05$) (Table 2, Fig. 5).

6. IgA containing cells

In the renal nodes, the number of the IgA containing cells was 11.2 in group I, 10.8 in group IIIm, 11.0 in group IIIf, 29.2 in group IIIIm and 16.0 in group IIIIf (Table 1, Fig. 6).

In the lumbar nodes, the number of the IgA containing cells was 4.6 in group I, 4.8 in group IIIm, 10.6 in group IIIf, 7.2 in group IIIIm and 14.8 in group IIIIf (Table 2, Fig. 6).

7. Histological observation

In the virgin control group, the size of renal and lumbar nodes was small. The cortex had small numbers of lymphatic nodules. A few plasma cells were localized in some medullary cords, but not in all medullary cords (Fig. 7).

In the groups of mid term, a slight increase of plasma cells were observed in medullary cords (Fig. 8). Other findings were similar to the virgin control group.

In the groups of full term, the size of lymph nodes was large. The cortex and medulla were so developed that many lymphatic nodules, postcapillary venules and medullary cords were easily identi-

fied. Many plasma cells were densely packed in the medullary cords (Fig. 9). In the medullary cords, each class and subclass of the immunoglobulin containing cells were mixed (Fig. 10 & 11, 13 & 14). In the group of full term mice which were mated allogeneically, the numbers of the IgG1, IgG2b and IgG3 containing cells were significantly greater than those of syngeneic mating.

DISCUSSION

In this experiment, the lumbar and renal lymph nodes were visualized in green color by injecting 11% patent blue violet through the wall of the uterine horns. The previous observation that hypertrophy of the lymph nodes draining the uterus occurred in interstrain mating (McLean *et al.* 1974; Shaya *et al.* 1981) was confirmed in this experiment. Ansell *et al.* (1978) and Chambers and Clarke (1979) reported that the lumbar lymph nodes draining the uterus changed in weight with lymphocyte accumulation and cellular proliferation during syngeneic and allogeneic pregnancies in the mice. These findings suggest that the immune response initiated in both lumbar and renal lymph nodes.

However, it is unknown which IgG subclasses are stimulated by the fetal antigens. This experiment was performed to detect the numerical changes of the IgG1, IgG2a, IgG2b, IgG3, IgM and IgA containing cells in the lymph nodes draining the uterus of the pregnant mice as a humoral immune response to fetal antigens. The results in this experiment showed the increases in the numbers of some IgG subclasses and IgM containing cells during pregnancy. The most striking increase was found in the group of full term mice which were mated allogeneically (Table 1 and 2). And there was a significant difference between allogeneic and syngeneic mating in the group at full term. This difference may be due to a different fetal antigenicity. The changes in the group of full term mice were more prominent than those of mid term mice, were due to a size of fetal antigens. It is quite natural that an increase in the number of the IgM containing cells was found during pregnancy in this experiment. Because an increase in the number of the IgM containing cells precedes that of the IgG containing cells in both primary and secondary immune responses. Mosley *et al.* (1975) observed that an increase in the large pyroninophilic cells in the thymus-dependent areas of the cortex and an increase in the plasma cells in the medullary cords

of the allogeneic pregnant rat. Newport and Carter (1983) assayed the level of T and B lymphocytes in the lymph nodes draining the uterus during pregnancy, and they found an increase in the level of B cells. Carter *et al.* (1983) reported the percentages of T and B lymphocytes in the peripheral blood, spleen and lymph nodes of CBA syngeneic and allogeneic pregnancies. At 19 days of syngeneic pregnancy, the level of T cells in the blood had decreased and that of B cells had increased. In the spleen, a decrease in both T and B cells occurred earlier in pregnancy and had returned to control levels by 19 days. During the last few days of pregnancy, the paraaortic lymph nodes draining the pregnant uterus of syngeneically mated mice showed a 1.7-fold increase in the level of B cells over that in virgin controls. There was no change in T or B cell status in the brachial nodes taken from the same animal. All these results were consistent with the conclusion that the fetal antigens elicit immune responses mediated by T and B cells.

Interestingly, a variety of studies have shown that certain antigens elicit antibody responses largely restricted to a single or two subclasses of IgG. The four subclasses of IgG are IgG1, IgG2a, IgG2b and IgG3 in the mouse, whereas IgG1, IgG2, IgG3 and IgG4 are the subclasses of IgG in the human. Antibody responses to bacterial carbohydrates, that is, dextran and groups A and C streptococcal carbohydrate, were restricted to IgG3 subclass in the mouse (Perlmutter *et al.* 1978). Polyclonal stimulation of mouse splenic B lymphocytes with lipopolysaccharide, T-independent antigen, resulted in the selective expression of IgG2 and IgG3 plaque forming cells in addition to IgM plaque forming cells, whereas IgG1 and IgG2 plaque forming cells with helper cell-dependent activation (Martinez *et al.* 1980). IgG1 and IgG3 subclasses were increased to Rh factor in the human (Devey and Voak 1974). In this experiment, the pregnancy induced the increase of all the subclasses of IgG, except IgG2a, containing cells in the lymph nodes draining the uterus of the mice. Nonsignificant change of IgG2a containing cells was due to a small number of experimental animals.

Rocklin *et al.* (1973) found that maternal serums blocked the production of a migration inhibitory factor when maternal cells reacted with fetal cells. This blocking factor was isolated in the IgG fraction by Pence *et al.* (1975). Moreover, the administration of IgG which obtained from retroplacental

blood of multiparous women increased graft survival by Riggio *et al.* (1978). So it is necessary to design the experiment that each subclasses of IgG act as blocking antibodies during pregnancy in the mice. Besides the blocking antibodies (Currie 1970; Stimson and Blackstock 1976; Smith 1978; McIntyre and Faulk 1979), the impairment of T cell activity (Putilo *et al.* 1972; Clark and McDermott 1978 & 1981; Shridama *et al.* 1982) have been postulated as a mechanism of nonrejection phenomena during pregnancy. The concentrations of serum IgG, IgM and IgA during pregnancy were controversial (Gudson 1969; Maroulis *et al.* 1971; Amino *et al.* 1978). Thus it is necessary to observe the numerical changes of the immunoglobulin containing cells in the spleen during pregnancy.

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= 국문초록 =

임신한 생쥐의 자궁유출 임파절에서 면역 글로불린 함유세포 수의 변화

서울대학교 의과대학 해부학교실

황덕호 · 이광호 · 장가용 · 백상호 · 성기준 · 이병란

본 실험은 태아 항원에 의해 자극되는 IgG의 아강을 탐지하고자 시행하였다. 실험동물은 유전적동계(암 ICR X 수 ICR) 또는 동종이인자형(암 ICR X 수 DDY) 교배를 시켜서 자궁유출 임파절인 신임파절과 요임파절을 임신 제9일, 제18일에 적출하였다. 임파절을 methyl green pyronin 및 면역조직화학 염색을 시행하여 실험군과 실험대조군인 처녀군과 비교 관찰하여 다음과 같은 결론을 얻었다.

1. 유전적동계 교배를 시킨 임신말기의 생쥐군에서 IgG1, IgG2b 그리고 IgM 함유세포의 수가 처녀군보다 증가하였다.
2. 동종이인자형 교배를 시킨 임신중기의 생쥐군에서 IgG2b 함유세포의 수가 처녀군보다 증가하였다.
3. 동종이인자형 교배를 시킨 임신말기의 생쥐군에서 IgG1, IgG2b, IgG3 그리고 IgM 함유세포의 수가 처녀군보다 증가하였다.
4. 임신말기의 생쥐군에서는 동종이인자형 교배를 시킨 군이 유전적동계 교배를 시킨 군보다도 IgG1, IgG2b 그리고 IgG3 함유세포의 수가 증가하였다.

이상의 결과는 IgG1, IgG2b 그리고 IgG3가 임신한 생쥐에서 자극된 IgG의 아강이었다.

LEGENDS FOR FIGURES

- Fig. 7. Medulla of the renal node in the virgin control mouse. A few plasma cells are localized in some medullary cords. MGP stain, X400.
- Fig. 8. Medulla of the lumbar node in the mid term mouse which was mated syngeneically. A slight increase of plasma cells is seen. MGP stain, X400.
- Fig. 9. Medulla of the renal node in the full term mouse which was mated allogeneically. Many plasma cells are densely packed. MGP stain, X400.
- Fig. 10. Adjacent section of figure 9. Many IgG1 containing cells are seen with its cytoplasm which is positive reaction. Immunohistochemical reaction, X400.
- Fig. 11. Adjacent section of figure 10. Many IgG2b containing cells are seen. Immunohistochemical reaction, X400.
- Fig. 12. Another field of figure 9. Many IgG3 containing cells are seen. Immunohistochemical reaction, X400.
- Fig. 13. Another field of figure 9. Several IgM containing cells are seen. Immunohistochemical reaction, X400.
- Fig. 14. Adjacent section of figure 13. Several IgA containing cells are seen. Immunohistochemical reaction, X400.

