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Maternal T cells of immunized pregnant mice induce immune suppression in their offspring

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SUMMARY

The present study focused on the influence of maternal immunity during pregnancy on the responsiveness of the immune system(s) in offspring. Maternal immunization of pregnant mice with T-dependent foreign antigen sheep red blood cells (SRBC) induced suppression of anti-SRBC plaque-forming cell (PFC) responses in their offspring. We attempted to identify the cell species among the maternal lymphoid cells of the immunized pregnant mice that induced this suppression in their offspring, by separating the maternal cells into T cells, B cells and macrophages, or T-cell subsets, and then adoptively transferring them into other normal pregnant mice. The results demonstrated the following: first, maternal $CD4^+$ T cells of immunized pregnant mice induced immune suppression in their offspring. Second, maternal T cells could be activated during pregnancy in the same fashion as in non-pregnant mice. The T-cell factor(s) for the immune suppression in offspring is produced not only by maternal T cells of immunized pregnant mice but also by T cells activated in non-pregnant mice. Third, cellular organization was required for maternal T cells to induce this immune suppression in their offspring.

INTRODUCTION

Much attention has been focused on why the immune system of the pregnant female mouse does not reject foetuses expressing different genetic phenotypes from the self since half of the genome of the zygote is derived from the paternal mouse.[1,2] This problem also concerns the influence of maternal immune responses on the immunity of offspring against foreign substances. It has been observed that maternal immunization of pregnant mice induces immune suppression in their offspring and that this suppression persisted for about 15 weeks, and is antigen specific.[3-5] In this paper, we report that the maternal T cells of pregnant mice are responsible for suppression in their offspring and that this phenomenon depends on the interactions through the placenta between the maternal immune system and the developing ones of foetuses.

Abbreviations: Eagle's MEM, Eagle's minimal essential medium supplemented with 0·3 g/l L-glutamine, 15 mM HEPES buffer, 1 mM sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, 10 μg U/ml streptomycin, 100 U/ml penicillin; FCS, foetal calf serum inactivated at 57° for 30 min; mAb, monoclonal antibody; mT, maternal T cells; PBS, phosphate-buffered saline; PEC, peritoneal exudate cells; PFC, plaque-forming cells; PMSF, phenylmethyl-sulphonylfluoride; RPMI-1640, RPMI-1640 supplemented with 0·3 g/l L-glutamine, 15 mM HEPES buffer, 1 mM sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, 10 μg U/ml streptomycin, 100 U/ml penicillin; SPC, spleen cells; SRBC, sheep red blood cells.

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MATERIALS AND METHODS

Animals

C57BL/6J mice were purchased from Japan CLEA Inc. (Tokyo, Japan) and bred at the Animal Laboratory of Kanazawa Medical University (Uchinada, Japan) under specific pathogen-free conditions.

Immunization

Female pregnant mice 8–10 weeks old were immunized intraperitoneally with sheep red blood cells (SRBC) at 2×10^8 cells/body at about 10 days of gestation. Non-pregnant female mice 8–10 weeks old were immunized intraperitoneally at SRBC 2×10^8 cells/body.

Cell preparations

Spleen cells (SPC) or peritoneal exudate cells (PEC) were obtained from pregnant or non-pregnant mice 5 or 6 days after immunization. SPC or PEC were suspended in 5 ml of 10% foetal calf serum (FCS)–RPMI-1640 and cultured for 2 hr at 37° in 5% CO_2 and moist air. Macrophages were recovered as adherent cells to FCS-coated dishes (FCS; Cell Culture Laboratory Inc., lot no. 00939-01) and purified by treatment with anti-Thy-1.2 mAb (Cedarlane Ltd, Ontario, Canada, CL8600, lot no. 1214), anti-mouse μ-chain monoclonal antibody (mAb)

(Cedarlane; CL6005-A, lot no. 7112) and complement (Cedarlane; Low-Tox M rabbit complement, CL3051, lot no. 4449). Viable cells obtained were washed three times at 4°, 400 ***g*** for 10 min in RPMI-1640 and used as macrophages. SPC or PEC were depleted of adherent cells as described above and the rest of the SPC or PEC (non-adherent cells) were suspended in 10% FCS–Eagle's MEM and used for obtaining T and B cells. The T-cell-rich population was obtained from non-adherent cells by incubation within nylon wool (Wako Pure Chemical Industries, Ltd, Osaka, Japan) columns for 60 min at 37°, 5% CO_2 in moist air and collected as eluted cells. Then T cells were purified from these cells by treatment with anti-mouse μ-chain and complement. After the treatment with anti-mouse μ mAb + C′, viable cells were washed three times at 4°, 400 ***g*** for 10 min in RPMI-1640 and used as T cells. The B-cell-rich population was obtained from the cell population that was trapped by the nylon wool column on T-cell purification from non-adherent cells, and then treated with anti-Thy-1.2 and complement. After the treatment with anti-Thy-1.2 mAb + C′, viable cells were washed three times at 4°, 400 ***g*** for 10 min in RPMI-1640 and used as B cells.

The subset-depleted T-cell populations were obtained as follows. T cells purified with nylon wool column were incubated with anti-L3T4 mAb (Cedarlane; CL012A, lot no. C91D) or anti-Lyt-2.2 mAb (Meiji Health Institute, Tokyo, Japan; lot no. 020–421) at 4° for 1 hr. Then, anti-mouse μ-chain mAb was added and incubated for 1 hr at 4°, and finally, the complement was added at 37° for 45 min. After this treatment, the cells were washed three times and centrifuged at 400 ***g***, 4° for 10 min. Viable cells were recovered and used as the $L3T4^+$ cell-depleted T cells (L3T4-depleted T cells) or $Lyt\text{-}2.2^+$ cell-depleted T cells (Lyt-2.2-depleted T cells) in the following experiments. The lymphoid cells were suspended in RPMI-1640, except in the cultures for the adherence of macrophages to dishes and the purification of T cells with nylon wool columns, where the cells were suspended in 10% FCS–RPMI-1640 (Nissui Corp., Tokyo, Japan, code no. 05918) and 10% FCS–Eagle's MEM (Nissui Corp. code no. 05900), respectively.

Adoptive transfer

The SPC, PEC or T cells, B cells and macrophages separated from SPC or PEC of the immunized female mouse were suspended in RPMI-1640 and then transferred into the normal non-immunized pregnant mice by intravenous injection. The recipient mice were in Days 10–13 of gestation. The dose for the adoptive transfer was $1\text{–}2 \times 10^4$ cells/mouse. In the transfer of the lymphocytes and macrophages obtained from the immunized non-pregnant mouse, the same procedures were carried out.

T-cell lysate preparation

T cells were resuspended in phosphate-buffered saline (PBS) containing 10 μg/ml phenylmethyl-sulphonylfluoride (PMSF) at pH 7·2, and lysed by repeated freezing and melting in liquid nitrogen and warm water (37°) 10 times and then by ultrasonication. The lysate obtained was used for the adoptive transfer.

Anti-SRBC PFC responses

The offspring of the immunized or recipient pregnant mice were reared for about 6 weeks, and then immunized intraperitoneally with SRBC at 2×10^8 cells/animal. Anti-SRBC PFC were detected on Day 5 or 6 after immunization with a modified Jerne plaque assay.[6] Direct plaque-forming cells were estimated as IgM PFC. Indirect PFC were developed by adding rabbit anti-mouse IgG, and IgG PFC were estimated as the difference between the plaque numbers of direct and indirect PFC. Anti-SRBC PFC responses of each animal group were compared by Student's *t*-test.

RESULTS

Maternal immunization and adoptive transfer of lymphoid cells derived from the immunized pregnant mice

Figure 1a shows the basic schedules for the maternal immunizations of pregnant mice and PFC assay in the offspring. When the pregnant female mouse had been immunized intraperitoneally with 2×10^8 SRBC, the production of anti-SRBC PFC in offspring was completely suppressed (Fig. 1b).

To determine which maternal lymphoid cells are responsible for this suppression of anti-SRBC PFC responses, adoptive transfer experiments were carried out (Fig. 2a). The pregnant female mouse was injected intraperitoneally with 2×10^8 of SRBC on Day 10 of gestation. Five or 6 days after injection, the maternal PEC or SPC were obtained from this pregnant mouse. In the first, the maternal SPC were transferred into other non-immunized pregnant mice on Days 10–13 of gestation. The offspring of the recipient pregnant mice were reared for about 6

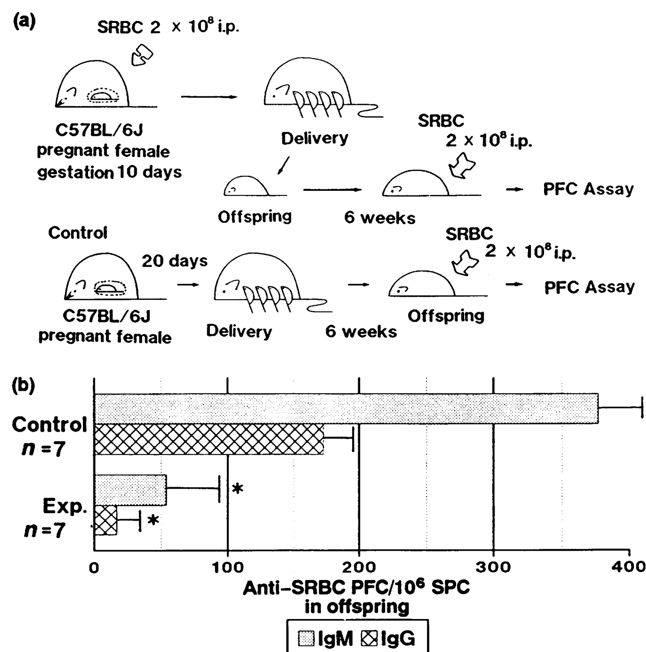

Figure 1. Maternal immunization of the pregnant mouse and PFC responses to SRBC in offspring. (a) Schedule for maternal immunization and detection of PFC produced by the offspring. (b) PFC responses to SRBC. Experiment shows the PFC responses of the offspring derived from the pregnant mouse that had been immunized intraperitoneally with 2×10^8 of SRBC during pregnancy. Control, mother was normal. See text for details. Bars are SE of PFC values for the groups. $*P < 0.001$.

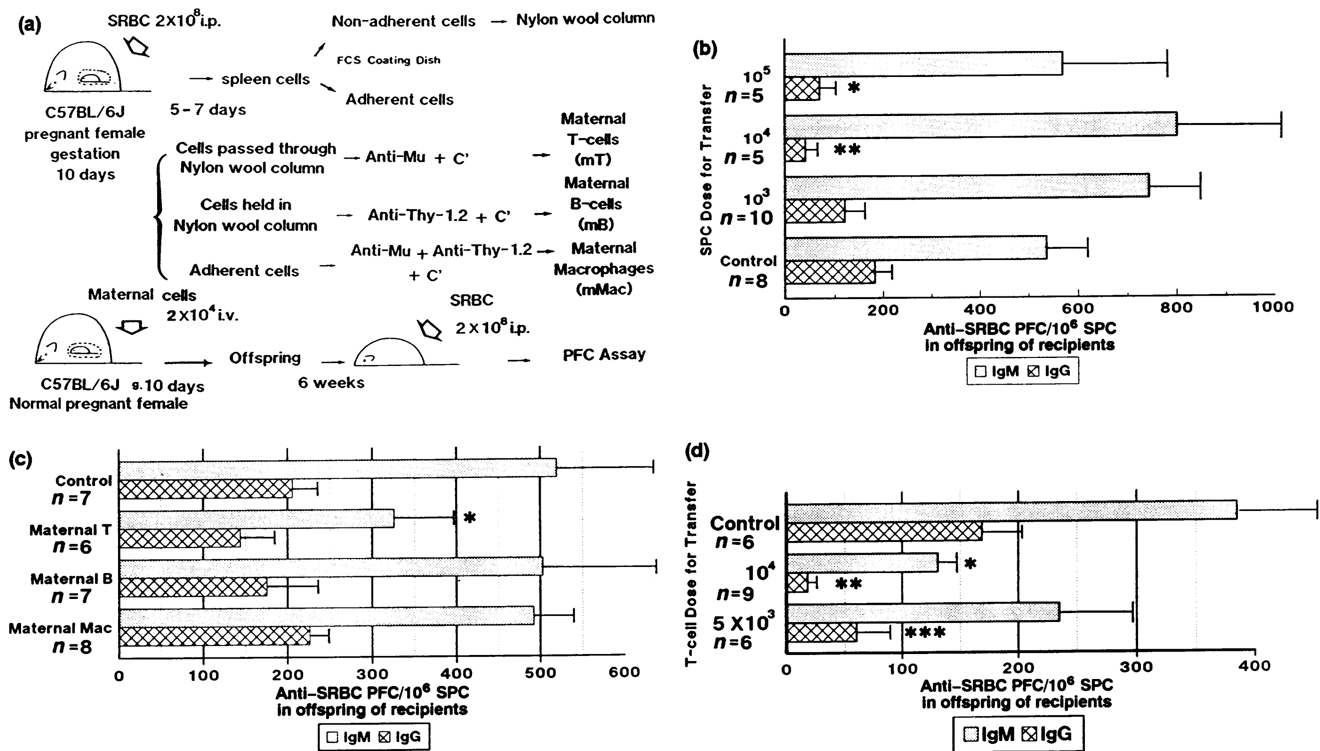

Figure 2. (a) The experimental schedules for maternal immunization of the pregnant mouse and the adoptive transfer of the maternal lymphoid cells. (b) SPC doses for transfer into the recipient pregnant mice (y-axis). PFC responses of the offspring from the recipient pregnant mice (x-axis). SPC were obtained from SRBC-immunized pregnant mouse. $*0{\cdot}01 < P < 0{\cdot}02$; $**0{\cdot}001 < P < 0{\cdot}01$. (c) Cells for transfer were obtained from PEC of SRBC-immunized pregnant mouse. Cell species for transfer into the recipient pregnant mice (y-axis). The cell dose for transfer was 2×10^4 cells/mouse. $*0{\cdot}02 < P < 0{\cdot}05$. (d) The titration for the adoptive transfer of the maternal T cells collected from SPC of the immunized pregnant mouse. Cell doses for transfer (y-axis). $*0{\cdot}001 < P < 0{\cdot}01$; $**P < 0{\cdot}001$; $***0{\cdot}02 < P < 0{\cdot}05$. Bars are the SE of PFC values for the groups. Controls show the PFC production in offspring from normal pregnant mice.

weeks and tested for their anti-SRBC PFC responses (Fig. 2b). The suppression was observed only in the IgG PFC when the recipient mice received 10^4 or more SPC from the immunized pregnant mice. At the dose of 10^6 SPC, similar results were obtained in other experiments [one example is as follows: 1067 ± 64 IgM PFC and 194 ± 25 IgG PFC/10^6 SPC in the offspring of the normal pregnant mouse ($n=5$), 1107 ± 110 IgM PFC and 56 ± 22 IgG-PFC/10^6 SPC in the offspring of the recipient mouse ($n=7$); $0{\cdot}001 < P < 0{\cdot}01$ in IgG PFC]. (For the maternal PEC of the immunized pregnant mice, the same experiments were carried out and similar results obtained; data not shown.)

Furthermore, the maternal cells of the immunized pregnant mice were separated into T cells, B cells and macrophages, and transferred to other normal pregnant mice. Thus, the pregnant femal mouse was injected intraperitoneally with 2×10^8 SRBC on Day 10 of gestation. Five or 6 days after injection, the maternal PEC or SPC were obtained from this pregnant mouse. The maternal SPC or PEC of immunized pregnant mice were separated into T cells, B cells and macrophages. Each type of maternal cells was transferred intravenously into other non-immunized pregnant females on Days 10–13 of gestation. The offspring of the recipient pregnant mice were reared for about 6 weeks and tested for their anti-SRBC PFC production.

Figure 2c shows the results in offspring of the pregnant mice that had received T cells, B cells and macrophages separated from the maternal PEC of the immunized pregnant mouse. The suppression of anti-SRBC PFC was observed only in the offspring of the recipient pregnant mice that had received T cells of the immunized pregnant mice by adoptive transfer. Such suppression was not observed in offspring from the recipient pregnant mice that had received other types of cells. Thus neither maternal B cells nor macrophages induced the suppression of PFC responses in the offspring of the recipients. Figure 2d shows the titration for the transfer of the maternal T cells obtained from the SPC of the immunized pregnant mouse. 5×10^3 or more maternal T cells were required to obtain a suppressive effect on the PFC responses in the offspring; no such effect was observed with lower doses. In other experiments, maternal B cells were obtained from the SPC of the 2×10^8 SRBC-immunized pregnant mice and then transferred into the normal pregnant mice at the dose of 2×10^4 according to the same procedure as described in Fig. 2a. One example of the results is as follows: 521 ± 71 IgM PFC and 190 ± 60 IgG PFC ($n=5$) in the offspring of the recipient, 539 ± 66 IgM PFC, 174 ± 29 IgG PFC ($n=9$) in offspring of the normal pregnant mouse. No suppression was observed in the experiment of B-cell transfer derived from SPC. These experiments were carried out

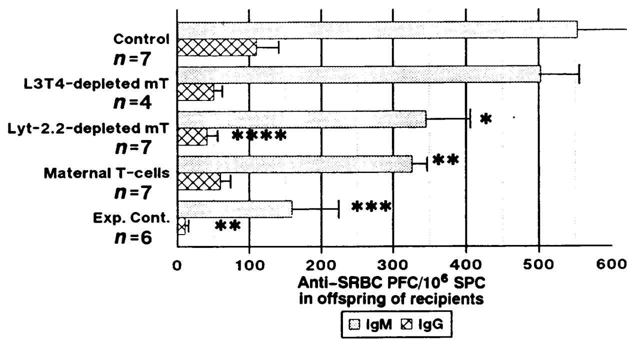

Figure 3. The adoptive transfer of the maternal T-cell subsets derived from the SRBC-immunized pregnant mouse into the other normal pregnant mice. The schedule for the transfer experiments was the same as in Fig. 2. Drawn left to the columns are the T-cell subsets for the transfer into the recipient mice, prepared by treatment with monoclonal antibodies. The cell dose for transfer was 2×10^4 cells/mouse. Data shown are the PFC production of offspring from recipient pregnant mice. Control shows the PFC production in offspring from the normal pregnant mice. EXP-CONT shows the PFC producion of offspring from the pregnant mouse immunized i.p. with 2×10^8 SRBC. $^*0{\cdot}01 < P < 0{\cdot}02$; $^{**}0{\cdot}001 < P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; $^{****}0{\cdot}02 < P < 0{\cdot}05$. Bars are the SE of PFC values for the groups.

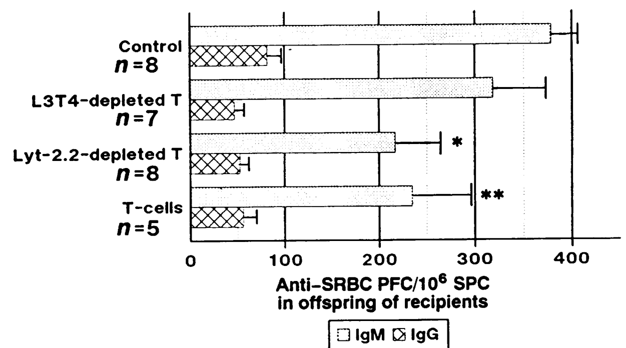

Figure 4. The adoptive transfer of T-cell subsets derived from the non-pregnant, but SRBC-immunized female mouse into the normal pregnant mice. The schedule for the transfer experiments was as in Fig. 2, except that the donor female mouse was not pregnant. T-cell subsets for the transfer into the recipient pregnant mice (y-axis). The cell dose for transfer was adjusted as 2×10^4 cells/mouse in all groups. Data shown are the PFC production of offspring from recipient pregnant mice. Control shows the PFC production in offspring from normal pregnant mice. $^*0{\cdot}01 < P < 0{\cdot}02$; $^{**}0{\cdot}02 < P < 0{\cdot}05$. Bars are the SE of PFC values for the groups.

three times and similar results were obtained. As a result, the maternal T cells of immunized pregnant mice are predominantly responsible for the suppression of PFC responses in offspring.

Maternal T-cell subsets

We also investigated which subset of the maternal T cells was responsible for the suppression of PFC production in offspring (Fig. 3). The pregnant mouse was intraperitoneally immunized with the same dose of SRBC as above on Day 10 of gestation. On Day 6 after immunization, nylon wool-purified T cells were obtained from the maternal spleen cells, and separated into maternal L3T4-depleted T cells, maternal Lyt-2.2-depleted T cells and whole population of T cells. Each population of T cells was adoptively transferred into other normal pregnant mice in the same way. The production of anti-SRBC PFC was examined in offspring of recipient pregnant mice in the same way as described above. No suppression of anti-SRBC PFC was observed in offspring of the recipient pregnant mouse into which the L3T4-depleted T cells were transferred. On the other hand, suppression of PFC was observed in offspring of recipient pregnant mice into which the maternal Lyt-2.2-depleted T cells or whole population of maternal T cells were transferred, suggesting that the maternal L3T4$^+$ T cells are responsible for the immune suppression induced by the maternal immunization. The same experiment was repeated twice and similar results were obtained.

Adoptive transfer of the T cells derived from the immunized but non-pregnant female mouse

A non-pregnant female mouse was immunized intraperitoneally with 2×10^8 SRBC. On Day 6 after immunization, T cells, L3T4-depleted T cells and Lyt-2.2-depleted T cells were prepared from the spleen as described above, and then, adoptively transferred into normal pregnant mice, adjusting the cell number as 2×10^4 cells/mouse in all the groups. The offspring of the recipient pregnant mice were examined for the production of anti-SRBC PFC in the same way as described above. The production of anti-SRBC PFC was significantly suppressed in offspring of the recipient pregnant mice that had received Lyt-2.2-depleted T cells or the whole population of T cells (Fig. 4). However, the L3T4-depleted T cells of the donor failed to induce the suppression of PFC responses in offspring of the pregnant recipient. The same experiment was carried out three times and similar results were obtained.

Adoptive transfer of the maternal T-cell lysate of the immunized pregnant mouse

A pregnant mouse was intraperitioneally immunized with 2×10^8 SRBC on Day 10 of gestation. On Day 6 after immunization, the maternal T cells were purified with nylon wool column and by treatment with anti-mouse μ-chain and complement. T cells were then lysed as described in the text. This lysate was diluted appropriately with PBS and then transferred intravenously into other normal pregnant mice at the dose corresponding to 10^5 T cells/mouse. The offspring of the recipient pregnant mice were examined for the production of anti-SRBC PFC in the same way. As shown in Fig. 5, no suppression of IgM PFC production was observed in offspring of recipient pregnant mice into which the T-cell lysate was adoptively transferred (no suppression was observed at the dose corresponding to 2×10^4 T cells; data not shown), despite the fact that transfer of maternal T cells of the same origin induced suppression of anti-SRBC PFC in offspring of recipient pregnant mice. Only the suppression of IgG PFC was observed. We repeated this experiment three times and similar results were obtained in all.

DISCUSSION

We examined the influence of the maternal immunity of the pregnant mouse on the immune reactivity of offspring. Maternal immunization of the pregnant mice with T-dependent

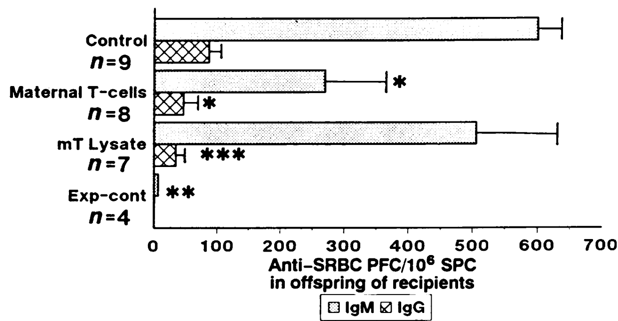

Figure 5. The adoptive transfer of the maternal T cells and their lysate into other normal pregnant mice. The schedule for the transfer experiments was as in Fig. 2. Cells or lysate adoptively transferred into the normal pregnant mice (y-axis). The cell dose for transfer was 2×10^4 cells/mouse. The lysate of maternal T cells were transferred at the dose corresponding to 2×10^5 T cells/mouse. Data shown are PFC responses to SRBC in offspring of recipient pregnant mice. *$0{\cdot}001 < P < 0{\cdot}01$; **$P < 0{\cdot}001$; ***$0{\cdot}02 < P < 0{\cdot}05$. Brackets are the SE of PFC values for the groups.

foreign antigen SRBC induced suppression of the PFC responses in their offspring. Similar results were obtained for cell-mediated immunity (Y. Fujii and N. Yamaguchi, unpublished data) and other T-dependent antigens.[5] The suppression of PFC responses in the offspring that we studied is not due to the down-regulatory effect of the maternal immunoglobulins that had been transmitted through placenta and/or by early milk.[3,7] First, the suppression of PFC responses in offspring is not influenced by the exchange of foster-nursing mouse (milk provider mouse) as reported by one of the authors,[3] excluding the possibility that the maternal immunoglobulins transmitted by early milk are responsible for the suppressions of PFC responses in offspring. Second, the transfer of maternal B cells of immunized pregnant mice failed to induce the suppression of PFC responses in offspring of recipient pregnant mice. If maternal immunoglobulins were responsible for the suppression in offspring, the transfer of maternal SPC at a higher dose ($\geq 10^5$) may induce the suppression in the offspring of the recipient pregnant mice; this was not so. It is, therefore, improbable that the final products of activated maternal B cells (e.g. IgM, IgG, etc.) are responsible for the suppression of PFC responses in offspring of immunized pregnant mice. Third, in this study it was demonstrated in the experiments of adoptive transfer that the maternal $CD4^+$ T cells of immunized pregnant mice were predominantly responsible for the immune suppression of offspring. Furthermore, when T cells activated in non-pregnant female mice were adoptively transferred into normal pregnant mice, these T cells also induced the suppression of PFC responses in offspring of pregnant recipients. Thus the immune suppression described in this paper depends on the physiological and/or antatomical environment that has developed uniquely during pregnancy. However, it seems unlikely that the maternal environment of pregnant mice affects the functions and/or phenotypes of the activated T cells. Rather, it is speculated that some T-cell factor(s) would be produced on activation both during pregnancy and in the non-pregnant state and then induce the immune suppression in offspring only when transmitted into them through placenta. This conception is consistent with the observation that maternal immunizations were effective only during pregnancy on the suppression of PFC responses in offspring.[5]

Interestingly, when the maternal T-cell lysate was obtained from immunized pregnant mice and adoptively transferred into normal pregnant mice, it failed to induce suppression of the IgM PFC responses in their offspring. This suggests that the cellular organization of maternal T cells was essential to the induction of the immune suppression in their offspring, although it does not necessarily exclude the possibility that the soluble factor(s) are involved in these phenomena.

The present findings suggest two possibilities for the matenal T-cell factor(s) that induce immune suppression in their offspring. The first is that the factor(s) might be produced solely by maternal $CD4^+$ T cells on activation and directly transmitted through the placenta into the foetuses. The second possibility is that the helper T cells included in the maternal $CD4^+$ T cells have an indirect effect on the suppression in their offspring. Thus, the suppressor T cells and/or other type of suppressive cells might be induced by helper T cells activated and produce suppressive factor(s) such as TsF_1.[8] Those maternally produced suppressive factors might be transmitted to the offspring through the placenta. Cellular organization might be necessary for the interaction between the maternal helper and suppressor T cells that are required for the production of 'soluble factor(s)'. In any case, the biological properties of the maternally transmitted factors inducing immune suppression in offspring remain to be clarified. It should be emphasized that in our experimental systems described here, the maternal immunity of pregnant mice was positively activated to respond to foreign antigens (SRBC and other T-dependent antigens) and that the antigenic determinants, at least the antigens themselves were then unlikely to be transmitted directly into the foetuses (offspring) through the placenta. Thus the immune suppression reported here was basically different from the tolerance induced by prenatal treatment in conditions where antigenic determinants are directly transmitted into foetuses.[9,10]

Data reported by one of the authors show that maternal immunization of pregnant mice induces suppressor T cells in offspring.[11] It seems likely that maternal immunity of pregnant mice has some influence on the immune repertoire of offspring. But the biological and immunological significance of our data remains to be defined. From the view point that vertebrate immune systems have evolved to protect the host from infectious agents,[12] the phenomena reported here are apparently disadvantageous to the maintenance of the mouse population of the species, at least, the subspecies of *Mus musculus domesticus* to which our laboratory mice belong. Further studies are needed to understand better the significance of these findings.

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