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ABSTRACT A mammalian fetus expresses a variety of antigens potentially unknown to the immunologically competent mother. Presented here are the results of investigations of maternal immune reactivity to paternally derived antigens of fetoplacental unit, detected at various levels: 1) spleen and distant lymphatic organs, 2) regional lymph nodes draining uterus, and 3) materno-fetal interface. The results suggest that the mother's immune system reacts differently in semiallogeneic pregnancies than in syngeneic ones. The type of the systemic immune response depends on the stage of pregnancy. Increased percentage of $CD8^+$ cells and decreased $CD4^+/CD8^+$ cell ratio was found in distant and regional lymphatic organs during pregnancy. The paternal class I MHC antigens expressed on the trophoblast cells are nonpolymorphic molecules which can have a role in immunotrophism of the placenta and in fetal allograft protection.

KEY WORDS: *reproductive immunology, immune response, decidua, placenta, MHC antigens*

Introduction

The feto-placental unit (FPU) in mammals expresses during pregnancy a variety of paternally inherited and embryonic antigens potentially unknown to the immunologically competent mother. The question why, in spite of these differences, the fetus in mammals is not rejected as an allograft, remains the most intriguing question not only in reproductive immunology but in transplantation immunobiology as well. A variety of potential mechanisms have been proposed to explain the inefficiency of maternal rejection reaction of the fetal allograft (Rukavina and Silobricic, 1980). According to today's knowledge there is no single mechanism able to explain this phenomenon. The role of facilitating antibodies, immunosuppressive antigen–antibody complexes, various kinds of suppressor cells and suppressive humoral factors and specific suppression of maternal alloreactivity have been proposed as preventing immune destruction of the fetus by the mother (Rukavina, 1985; Beer 1988). Alloantigens of FPU are constantly being shed into maternal circulation or being deported by regional lymphatics and so being presented to the centers of maternal immune response (Clark, 1990). Maternal sensitization to paternally inherited fetal antigens has been proven (Rukavina *et al.*, 1979; Rukavina and Matejcic, 1980) but simultaneously with maternal sensitization, another type of immune reaction – counterbalancing the former and protecting the fetus – develops. The immune agents of this reaction are enhancing antibodies and specific suppressor cells (Voisin, 1975).

Maternal antifetal immune reactivity could be studied at various levels: 1) systemic (spleen and distant lymph nodes), 2) regional (lymph nodes draining uterus), and 3) feto-maternal interface (the place where maternal and fetal tissues and cells come into direct and intimate contact).

Systemic reactivity during pregnancy

Systemic active suppression is not necessary for successful allopregnancy. There are many results showing that the pregnant mother is able to mount all kinds of immune response to unknown antigens, including infective agents (Beer and Billingham, 1971). However, the results of our experiments, dealing with differences in reactivity between syngeneic and allogeneic pregnancies, clearly indicate that the mothers do not react immunologically in the same way at the systemic level when carrying semiallogeneic or syngeneic fetuses. It is interesting that the type of the reactivity (hypo, hyper or normo) depended on the stage of the pregnancy (Doric and Rukavina, 1987; Kapovic and Rukavina, 1990, 1991a).

In the experiments illustrated in Fig. 1, we have centered our attention on the problem of whether alloreactivity (estimated by *in vitro* mixed lymphocyte reaction – MLR) to paternal strain and third party alloantigens is unchanged or modified at both examined lymphatic organs (spleen and cervical lymph nodes – CLN) during syngeneic and allogeneic pregnancy. Female AO rats ($RT1^u$ haplotype) were mated to AO (syngeneic pregnancy) or DA ($RT1^a$ - allogeneic pregnancy) males and MLR with CLN and spleen cells was performed at days 3, 7, 11, 14, 17 and 21. The results clearly showed

Abbreviations used in this paper: FPU, fetoplacental unit; MLR, mixed lymphocite reaction; CLN, cervical lumph nodes; PALN, paraaortic lymph nodes.

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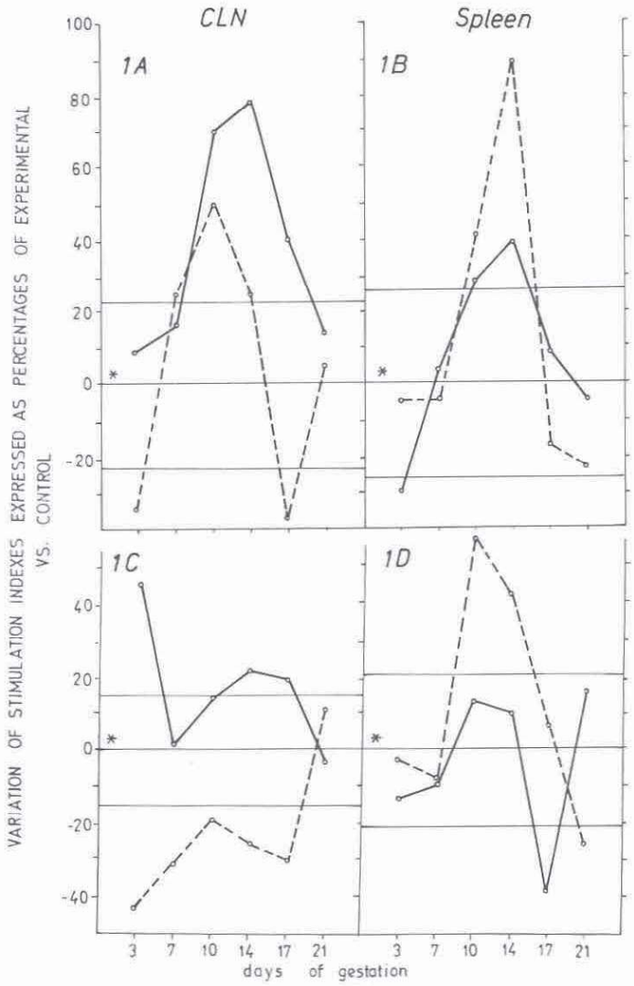

Fig. 1. Changes in specific (1A and 1B) and nonspecific (1C and 1D) MLR of cervical lymph nodes (1A and 1C) and spleen (1B and 1D) cells *in AO females ($RT1^u$) pregnant syngeneically (m–m) or allogeneically (m– – –m; males DA-$RT1^a$).*

Responding cells Stimulating cells (mitomycin treated)

AO → DA (Fig. 1A and 1B)

AO → Y-59-$RT1^c$ (Fig. 1C and 1D)

**= Virgin rats (Mean ± SE)*

decreased specific alloreactivity against DA in the early and last phases of allopregnancy in the CLN (1A) and enhanced reactivity at midpregnancy in the spleen (1B). Nonspecific alloreactivity of spleen cells harvested from allopregnant females against mitomycin treated cells of Y-59 rat ($RT1^c$) was again more pronounced at midpregnancy than the reactivity of cells from syngeneically pregnant females (1D). In the CLN, a suppressed nonspecific alloreactivity was obtained in the early phase of allogeneic pregnancy (1C). The results of the experiments reported herein show that a suppression of alloreactivity indeed exists at the level of CLN. In the same experimental model we followed CD4/CD8 cell ratio and reactivity to polyclonal mitogens in the CLN and spleen. The data showed that the cell ratio decreased as a result of the increase in the percentage of $CD8^+$ cells, and that reactivity to T-polyclonals in early and late phases of allopregnancy at the level of CLN is suppressed, but enhanced, particularly to ConA, at midpregnancy (Kapovic and Rukavina, 1991a). The observed suppression of alloreactivity could be attributed to gestational hormones and to a variety of pregnancy-associated suppressive proteins (Szekeres-Bartho *et al.*, 1985; Wheeler *et al.*, 1987; Parr *et al.*, 1988; Toder, 1988). The increase in alloreactivity in the spleen at midpregnancy coincides with the expression of class I MHC pregnancy-associated (Pa) antigen (Kanbour *et al.*, 1987). Although systemic active suppression is not necessary for a successful allopregnancy, our results clearly showed that alloreactivity in distant lymphoid organs is directly or indirectly controlled during pregnancies and must be taken into account for possible pathogenic threats or autoimmune processes (Chaouat and Monnot, 1984). The increased alloreactivity in syngeneically pregnant females at the level of CLN (Fig. 1A and C), together with increased reactivity to PWM (T-B polyclonal stimulator) observed in the same experimental model (Kapovic and Rukavina, 1991a) constitute evidence supporting this opinion.

Alloreactivity in the regional lymph nodes

Increased lymphatic drainage from the pregnant uterus may carry alloantigens of FPU to the regional (draining) lymph nodes (paraaortic lymph nodes – PALN). The manifold increase of PALN is one of the most striking changes during allogeneic pregnancies found in many mammalian species (Beer and Billingham, 1971). The response to alloantigens of the FPU at the level of PALN has many interesting pecularities, e.g. intrauterine sensitization by alloantigens induced cytotoxic cells in the spleen, but not in PALN (Searle, 1986). Facilitation of orthotopic skin allograft survival was obtained in females simultaneously implanted and transplanted. This facilitating effect vanishes if PALN are excised at the time of skin allograft implantation (Rukavina *et al.*, 1980). In primiparous and multiparous mice, pregnant either syngeneically or allogeneically, we found an increase in alloreactivity of PALN cells at midgestation but a decrease during the periimplantation period and third week of gestation (Doric and Rukavina, 1987). Detailed studies of rat PALN cells were tested during all phases of pregnancy and strong specific suppression to paternally inherited fetal alloantigens was obtained in preimplantation and periimplantation periods, but an almost double increase of alloreactivity in midpregnancy of allogeneically pregnant female rats (Kapovic and Rukavina, 1991b). The CD4/CD8 cell ratio in the PALN of allogeneically pregnant rats showed suppression during all phases of the pregnancy as a result of a permanent increase in the percentage of $CD8^+$ cells (Kapovic and Rukavina, 1990). The observed suppression of alloreactivity is in line with the results showing a dramatic increase in T-cell suppressor factor production in PALN cells during the periimplantation period, with the maximum on day 5 (Ribbing *et al.*, 1988) and maximal production of prostaglandins, especially PGE2, at the time of blastocyst implantation (Moulton, 1984). The observed enhancement at midpregnancy correlates with the time of nonclassical class I antigen expression (Pa antigen) at the basal trophoblast membrane (Kanbour *et al.*, 1987) and fits well with the immunotrophism hypothesis which claims that maternal T-cell recognition of paternally derived placental class I antigens has the effect of expanding the growth potential of the placenta and increasing its ability to

withstand microbial assault arriving via the maternal circulation (Wegmann, 1990).

Immunoregulation at feto-maternal interface

Local immunoregulatory mechanisms acting at the place where maternal and fetal tissues come into direct and intimate contact play a key role in the protection of fetal allografts. Participating in this immunoregulation are both maternal tissue (decidua) and fetal tissue (trophoblast).

Decidual cells

The immunoregulatory potential of decidual cells and leukocyte subpopulations residing in decidua (macrophages and lymphocytes) has been proven in various experimental models. The implantation of skin allografts into uterine horns of hormonally (estrogen+progesteron) pretreated rats (pseudopregnancy) or in the uteri of pregnant females have had a much longer survival time than allografts placed at nonuterine sites or in the uterine horns of nonpregnant rats (Beer and Billingham, 1971). The intensity of systemic immunity following intrauterine allograft rejection was much lower compared to sensitization after skin allografts were transplanted orthotopically (Rukavina *et al.*, 1980). Three types of cells with immunosuppressive functions have been isolated from early pregnancy decidua: first, trophoblast dependent non-T cells releasing a factor which blocks the action of IL-2; second, trophoblast independent cells producing PGE2 which blocks the production of IL-2, and the third type, hormone dependent cells isolated from the endometrium (Daya and Clark, 1990). The decidua has to account for the dual functions, the immune tolerance to the conceptus on the one hand and local immune response to infectious organisms on the other, for which an intact maternal systemic immune function is necessary. The cell composition of the decidua and its function is adapted to fulfill both functions. Besides decidual cells, macrophages, large granular lymphocytes or endometrial stromal granulocytes and nongranulated lymphocytes are found (Bulmer *et al.*, 1988). In a detailed analysis of the cell content of biopsy specimens of decidual tissue obtained from first trimester human pregnancies we found a predominance of macrophages and $CD56^+$ cells (large granulated lymphocytes). The number of $CD4^+$ and $CD8^+$ cells was much higher than the number of $CD3^+$ cells, which suggests that these markers are present on different types of cells other than T cells (Petrovic *et al.*, 1990). The differences we have observed in cell content between biopsy specimens and specimens obtained by curettage point up the importance of careful interpretation of investigations cited in the literature. Namely, material obtained by curettage can be contaminated by fetal, maternal and placental cells. Decidual cells and decidual leucocytes, together with trophoblast cells, are a potent source of immunoregulatory substances able to suppress, at the local level, maternal rejection reaction (Menu and Chaouat, 1990).

Major histocompatibility (MHC) antigen expression on the trophoblast

The cell surface expression of class I and class II MHC antigens in the placenta is under complex genetic regulation. The class II antigens are not expressed in the mouse, rat or human (Gill *et al.*, 1991). Several authors have reported non-classical characteristics of class I proteins in the placenta and also a differential expression on the trophoblast cell surface (Kanbour *et al.*, 1987). The pregnancy-associated (Pa) paternal antigen with broadly shared determinants is expressed on the cell surface in the rat placenta, whereas the classical class I transplantation antigens remain inside the cell (Kanbour *et al.*, 1987). The epitopes of the Pa antigen are not recognized by cytotoxic T lymphocytes (CTL), but they elicit alloantibody responses (Ghani *et al.*, 1984). The Pa antigen has been isolated from the cDNA library and sequenced (Radojcic *et al.*, 1989; Radojcic *et al.*, 1990) and it has the same exon organization as the other class I genes. The protein deduced from this cDNA clone has all the characteristics of a classical class I molecule: three extracellular domains (~90 amino acids long), transmembrane and cytoplasmic domains, two glycosylation sites and conserved cysteine positions (Radojcic *et al.*, 1989), but also some unique features. It is not clear yet whether some variable aminoacid regions are species specific or the unique characteristics of the rat placental antigen. The same cDNA clone was expressed *in vitro* on the surface of COS7 fibroblasts and reacts with anti Pa antibodies. A specific restriction fragment has been found for serologically Pa positive rats (Radojcic *et al.*, 1990). These results suggest a separate loci of class I gene activated in the placenta which encode broadly shared and nonpolymorphic antigen. The mechanism by which this fine control of class I expression is achieved is still unknown.

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