THE ACCUMULATION OF A NOVEL TYPE OF SUPPRESSOR CELL IN THE UTERUS OF ALLOPREGNANT MICE DURING SUCCESSFUL PREGNANCY

By

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ABSTRACT

Successful pregnancy involves the accumulation of non-T, FcR-positive cells at the implantation site of syngeneic allogeneic pregnant mice. The decidua-associated cells sediment at 3 ± 0.5 mm/h, contain cytoplasmic granules, and can inhibit the generation of CTL (cytotoxic T cells) in vitro and in vivo by blocking the response to interleukin 2. It has been shown that xenogeneic embryos can be gestated successfully if enveloped in the trophoblast genotypically compatible with the pseudopregnant recipient (Rossant et al., 1982, J. Emb. Exp. Morph., 69: 141). We have recently demonstrated that the trophoblast plays a critical role in the localization of the decidua-associated suppressor cell in pseudopregnant mice. We now show that supernatants generated from trophoblast cell cultures and day 9.5 ectoplacental cone cultures were successful in the recruitment of the non-T, granulated suppressor cell which sediments at 3 ± 0.5 mm/h. These results suggest that the trophoblast elaborates a factor(s) which plays an important role in the accumulation of the decidua-associated suppressor cells in the decidua which may protect the antigenic fetus from maternal immune rejection.

To my husband, Vitas

for his love, patience and unfailing encouragement

to my mother

for her loving support

and

in memory of my beloved father

I dedicate this thesis.

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CTL	Cytotoxic T Lymphocyte
HLA	Human Leukocyte Antigen
GVH	Gravt-vs-Host
NK	Natural Killer
CML	Cell Mediated Lympholysis
MLC	Mixed Lymphocyte Culture
FBS	Fetal Bovine Serum
PBS	Phosphate Buffer Saline
BSA	Bovine Serum Albumin
PLN	Peripheral Lymph Nodes (auxillary + brachial + inguinal)
SRBC	Sheep Red Blood Cell
GI	Gastrointestinal
IL-2	Interlenkin-2

Chapter 1

Introduction

1 Introduction

In an outbred population, such as man, spontaneous abortion is the most common complication of pregnancy. Although the percentage of pregnancies which fail is unclear, Roth (1963) has calculated that 15% of diagnosed pregnancies resulted in abortion, while Miller et al (1980) reported 43% and Edmonds et al (1982) has suggested as many as 60% of conceptuses are lost before 16 weeks gestation. Several reasons have been given for the failure of these pregnancies. Chromosomal abnormalities are a well established cause of fetal death in humans (Khudr, 1974; Boue et al 1973; Byrd et al, 1977) mice (Bennett 1975; Klein and Hammerberg, 1977) and rats (Gill and Repetti, 1979; Kunz et al 1980). Other possible causes of abortion may be infection, anatomical and/or hormonal abnormalities. Recently Johnson et al (1984) has suggested that a majority of spontaneous abortions are immunological in that the woman fails to make an appropriate response to the pregnancy and thus she fails to circumvent the allograft rejection leading to the elimination of the fetus. The impact of recurrent spontaneous abortions has provided a great momentum for many highly motivated investigators into basic biomedical research in immune regulation during pregnancy. This momentum is driven by the hope that in the not too distant future immunological approaches will be exploited in fertility control. The following section will briefly review the evolution of reproductive immunology to the important and fascinating discipline that it is today. The review will highlight the current state of knowledge in reproductive immunology,

particularly the paucity of information on immune regulatory mechanisms within the uterus during pregnancy.

In 1953 Medawar proposed that the semi-allogeneic fetus is not rejected as a foreign graft for the following reasons. (1) The conceptus may not be immunogenic and therefore does not evoke an immunological response. (2) Pregnancy may alter the maternal immunological response. (3) The uterus may provide an immunological pivileged site. (4) The placenta may be an effective immunological barrier between the mother and the fetus. Over the last 30 years, since Medawar's proposals, an enormous amount of knowledge has been generated to delineate the immunological mechanisms of successful pregnancy.

In all mammalian species the placental trophoblast is the only fetal element in direct contact with maternal tissues. It is therefore important to understand the structure and immunobiology of the placental-trophoblast to determine the potential susceptibility to maternal immune attack.

1.1 Organization of the Placenta

The organization and immunobiology of the placenta are very restricted and almost solely derived from information on the proliferating trophoblast and organized placenta of the mouse, and the early villous trophoblast and full-term placenta of man (reviewed by Billington, 1975). In brief, there are two tissue types of placenta; the chorion-vitelline placenta where the vascularized yolk sac fuses with the trophoblast or the chorion, and the chorio-allantoic placenta where the chorion is vascularized. The human and mouse placenta are of the latter type. The chorio-allantoic placenta shows great diver-

sity of form. The classification is dependent upon the degree of invasiveness of the trophoblast into the maternal tissue. In the pig, the trophoblast wall does not invade maternal tissue but simply lies in intimate contact with the uterine epithelium, and is referred to as an epithelio-chorial placenta. Where there exists a slight invasion of the uterine wall, with the trophoblast in contact with connective tissue, the placenta is referred to as syndesmochorial. When the trophoblast erodes through to the endothelium of the maternal blood vessels in the uterus this establishes an endothelio-chorial placenta. Where the degree of trophoblast invasiveness involves the breaching of uterine capillaries to bath the trophoblast in maternal blood is designated the hemochorial placenta. Such is the placenta type of man, other primates and rodents (see review, Billington, 1975).

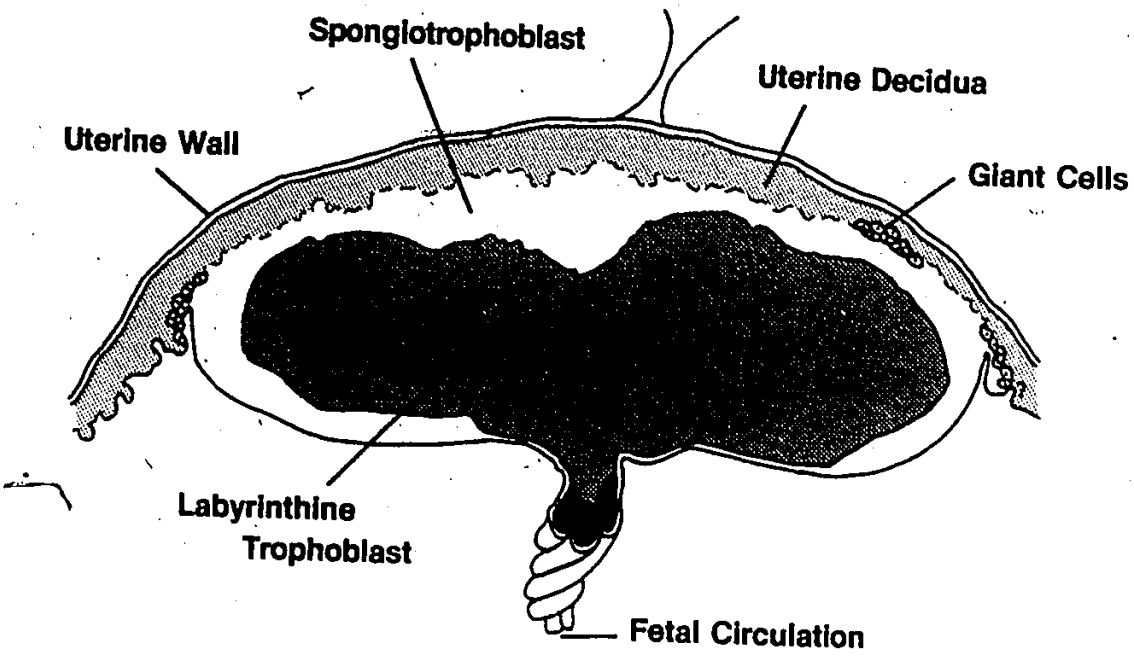
1.1.1 Mouse Placental Development:

The primary trophoblast giant cells arise from the trophectoderm in the abembryonal lateral areas of the blastocyst. Their function is not clearly defined, but an anchoring mechanism during implantation has been suggested. The trophectoderm overlying the inner cell mass at the embryonic pole of the blastocyst undergoes rapid proliferation to form the ectoplacental cone which is composed of highly invasive trophoblast cells. The centre of the ectoplacental cone proliferates and differentiates to form the labyrinthine and spongiotrophoblast. The composition of the mature murine placenta is illustrated diagrammatically in Figure 1. All three types of trophoblast cells are in contact with maternal tissue. The giant cells and spongiotrophoblast cells abut uterine decidual tissue with close

Figure 1

Diagrammatic representation of the mouse placenta

Figure 1


Spongiotrophoblast
Uterine Decidua
Uterine Wall
Giant Cells
Labyrinthine
Trophoblast
Fetal Circulation

cellular contact. The labyrinthine trophoblast is bathed by maternal blood in placental sinuses.

1.1.2 Human Placental Development:

After implantation of the human blastocyst, the trophectoderm gives rise to two forms of trophoblast, an inner cellular cytotrophoblast, and an outer syncytiotrophoblast.. The syncytial trophoblast is derived from the differentiation and breakdown of cytotrophoblast cell membranes, and progressively increases during pregnancy. At an early stage of placental development columns of cytotrophoblast push through the syncytium forming the chorionic villi, and spreads out at the ends to form a "shell" around the embryo, referred to as the cytotrophoblastic shell. As the placenta matures the villi develop with an outer covering of syncytium and an underlying layer of cytotrophoblast which in the later stages of pregnancy becomes discontinuous. The villous syncytium is bathed in maternal blood and the cytotrophoblast shell or the basal plate is in direct contact with the uterine decidual tissue. Figure 2 shows a diagrammatic illustration of the human placenta (Billington, 1975; Moore, 1977).

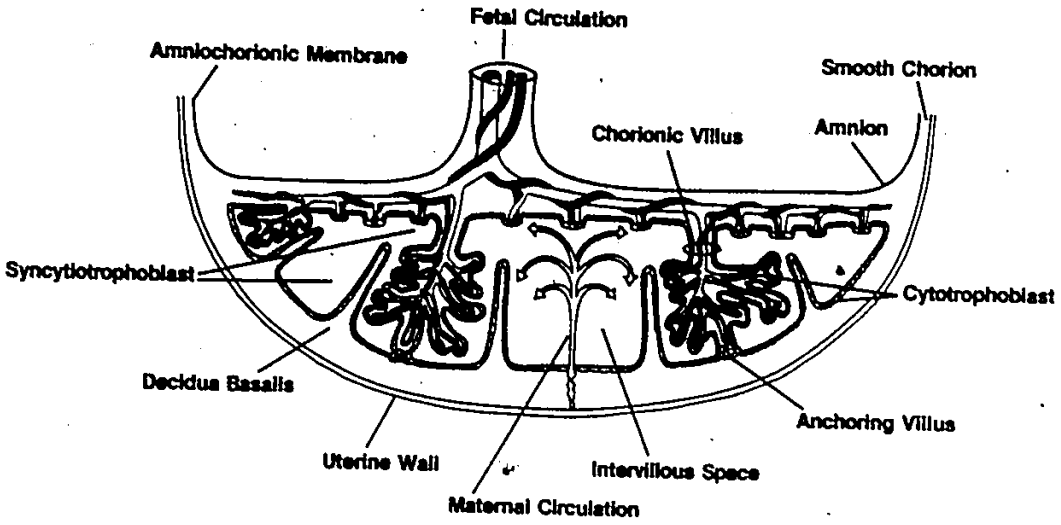
1.2 Trophoblast Antigenicity

According to the classical view, for a host to undergo a maximal allograft rejection response, major histocompatibility (MHC) antigens of both Class I and Class II need to be recognized by allore-active T cells of the host (Cerottini and Brunner, 1974). Cytotoxic T cell precursors, bearing the receptors for Class I MHC molecules respond to the alloantigen and undergo clonal expansion and maturation into effector CTL with help provided by T helper (T_H) cells. Pre-

Figure 2

Diagrammatic representation of the human placenta

Figure 2


Fetal Circulation
Amniochorionic Membrane
Smooth Chorion
Chorionic Villus
Amnion
Syncytiotrophoblast
Cytotrophoblast
Decidua Basalis
Anchoring Villus
Uterine Wall
Intervillous Space
Maternal Circulation

cursors of T_H recognize allo Class II MHC molecules and undergo clonal expansion, leading to the generation of T_H which provides help by elaborating interleukin 2 (McDevitt, 1980). And finally by virtue of recognizing class I MHC molecules, CTL destroy the target cells (Alter and Bach, 1979). In 1981, Loveland et al proposed an alternate hypothesis based on studies in T cell depleted rats, which stipulated that the T cells which respond to foreign MHC antigens and mediate graft rejection are not the conventional CTL but are T cells phenotypically identical to those mediating delayed type hypersensitivity and upon stimulation they elaborate lymphokines to recruit macrophages and other effector cells which may destroy the graft (Loveland et al., 1981).

What ever the mechanism of graft rejection, MHC antigens serve as principle allo recognition molecules on the allografted cells. Thus the alloantigenic status of the trophoblast cells is a key determinant of the immune interactions between the mother and her fetus.

The opinion about trophoblast immunogenicity is divided. Simmons and Russell demonstrated that some cells from the ectoplacental cone transplanted under the kidney capsule were not rejected, and therefore suggested that this trophoblast is devoid of transplantation antigens (Simmons and Russell, 1962). However, other trophoblast cell populations such as giant cells were not examined in this manner. For instance, Kaneko et al have shown that in certain strains of mice, transplanted trophoblast cells may be subject to rejection (Kaneko and Nishimura, 1978). Using serological techniques, it has been demonstrated that the murine blastocyst expresses only low levels

of paternal histocompatibility antigens prior to implantation (Billington et al., 1977; Webb et al., 1977). At the time of implantation, both H-2 and non-H-2 transplantation antigens disappear from the ectoplacental cone which will give rise to the trophoblast (Searle et al, 1976). Using a sensitive hemadsorption technique, Sellens et al. were unable to detect H-2 alloantigens on the trophoblast giant cell outgrowths cultured from 7 1/2 day ectoplacental cones (Sellens et al., 1978). Human trophoblast studies have shown that little, if any, HLA or β2 microglobulin are present on the surface of trophoblast lining the placenta (Faulk and Temple, 1976; Goodfellow et al., 1976; Faulk et al., 1977). Unlike trophoblast cells, the cells of a growing fetus transplanted into extrauterine sites were successfully rejected implicating the presence of MHC antigens on their surface (Woodruff, 1958; Simmons and Russel, 1962; Gill and Repetti, 1979). Taken together, these observations suggest that the trophoblast cells protect the antigenic fetus by acting as a quarantining barrier between the fetus and maternal tissues.

On the other hand, Kirby and his colleagues developed the idea that the trophoblast is antigenic but that it is enveloped in a pericellular layer of placental fibrinoid, an amorphous mucopolysaccharide rich in sialic acid, which protects it from immunological damage by masking the antigenic sites (Kirby et al., 1964). Currie and Bagshawe (1967; Currie et al, 1968) have reported human and mouse in vitro studies that trophoblast cells stripped of its covering sialomucin by enzyme treatment undergo cytolysis in the presence of allogeneic lymphocytes, whereas the control cultures of trophoblasts with the

fibrinoid coat intact were not destroyed.

Recent observations using a sensitive radioautographic technique Chatterjee-Hasrouni and Lala, (1981) have shown that MHC antigens of both parental type are present on mouse trophoblast cells at densities equivalent to or higher than those on adult thymocytes. In vivo localization studies using radioiodinated monoclonal antibodies or labelled Fab_2 against paternal H-2 antigens have shown that the majority of the antibodies localized in the spongiotrophoblast region, which is in direct contact with the maternal circulation at the fetal-maternal interface and in the yolk sac venous plexus in the lateral areas of the placenta (Raghupathy et al., 1981). Taken together, these data does not support the negative results on the expression of transplantation antigens as previously discussed in mouse (Webb et al., 1977) and human placenta (Faulk et al., 1977). This disparity may be explained by the presence of several types of trophoblast cells in the placenta (Billington, 1976), only some of which express the paternal MHC antigens. Taken together, these data indicate that while trophoblast cells that are in direct contact with maternal blood in the labyrinthine/syctiotrophoblast may express only low levels of Class I paternal antigens in vivo (Jenkinson and Owen, 1980; Sunderland et al., 1981; Wegmann, 1981), those trophoblast cells that directly contact the maternal decidual cells in the spongiotrophoblast which serve to anchor the placenta to the uterus do appear to express significant quantities of paternal MHC antigens (Jenkinson and Owen, 1980; Raghupathy et al., 1981; Sunderland et al., 1981).

Contrary to the observation of the presence of Class I antigens (MHC) it has been observed that murine trophoblast cells lack Class II (Ia) antigens (Chatterjee-Hasrouni et al., 1981; Raghupathy et al., 1981; Jenkinson and Searle, 1979) which are postulated to serve as potent helper determinants in allogeneic reactions (McDevitt, 1980).

Several additional lines of evidence now reveal problems with the inert trophoblast barrier theory, suggesting that the putative lack of paternal transplantation antigens on the syncytiotrophoblast is not sufficient to explain the survival of the fetal-allograft. Trophoblast-specific TA1 and TA2 antigens have been detected on human trophoblast cells (Faulk et al., 1979) and rat trophoblast cell which is designated the pregnancy-associated (Pa) antigen (Ghani et al., 1985). In addition, the presence of allotypic trophoblast-lymphocyte cross-reactive (TLX) antigens have been described on the syncytiotrophoblast cell membrane (McIntyre et al., 1983). Some investigators claim that maternal recognition of TLX antigens is essential for successful pregnancy (McIntyre et al., 1983).

Data from horse-donkey matings describes a situation where some trophoblast cells detach from the placenta and form cellular islands in the maternal decidua. During the pregnancy these island clusters regress in a manner resembling a typical allograft rejection with massive lymphocytic infiltration (Allen, 1979). Thus, a putative lack of MHC antigens on the trophoblast does not necessarily render the trophoblast inert. It has been previously shown that non-MHC antigens can elicit a strong graft rejection response (Halle-Pannenko

et al., 1978). Taken together, these data suggest that the trophoblast is not necessarily immunologically invulnerable.

The trophoblast barrier theory is weakened by the evidence that there exists cellular and molecular traffic between the mother and fetus. Fetal cells may be found in maternal blood (Herzenberg et al., 1979), and although it has been shown that the passage of significant numbers of maternal lymphoid cells into the fetus is rare (Hunziker et al., 1984) some have shown that maternal WBC may enter the fetus in sufficient numbers to produce the occasional chimera (Barnes and Holliday, 1970; Gill, 1977). There have been reports that intensive active immunization of the mother with tissues bearing paternal or embryonic antigens can lead to infertility, resorption or neonates which subsequently develop a runting syndrome similar to a graft-vs-host reaction (Parmiani and Della Porta, 1973; Milgrom et al., 1977; Hamilton et al., 1979; Webb, 1980). These observations suggest that immunity to paternal or embryonic antigens can overcome the putative trophoblast barrier and potentially damage the fetus.

1.3 The Uterine Response

1.3.1 Decidualization

During early mammalian pregnancy the mesenchymal cells of the uterine stroma undergo a local proliferation referred to as decidualization. The mechanisms which elicit the decidual reaction remain unclear. It is known that progesterone is critical in preparing the uterus for decidual induction (Yochim, 1984). Induction signals include the implanting blastocyst and a variety of artificial stimuli such as, trauma to the endometrial epithelial or oil injected into the

uterine lumen of hormonally primed mice (Miller and Emmens, 1969). Recently, it has been shown that factors such as prostaglandins, PGE_2, $PGF_{2\alpha}$, (Ohta, 1983), histamine (Hatanaka et al., 1982; Wordinger et al., 1985) and pyridine nucleotide metabolism (Yochim 1984; Cummings and Yochim, 1984) are important in the cytodifferentiation of the uterine stroma.

The fundamental role of decidual tissue remains undefined although many functions have been proposed, including, nutrition of the embryo (Krehbiel, 1937), protection of the maternal tissues against invading trophoblast cells of the placenta (Kirby, 1968) and immunoregulation (Kirkwood and Bell, 1981; Slapsys and Clark, 1982).

Kearns and Lala (1982) claim the decidual matrix to be derived from the bone marrow on the merit that it possessed immunoregulatory properties and upon examination of the decidual cell H-2 haplotype of radiation chimeras. At present, this idea has been challenged and refuted by a number of groups who have shown by isozyme marker methods that the decidual cell proper is not derived from the bone marrow (Gambel et al., 1985a; Fowlis and Ansell, 1985). The bone marrow derived cells in the decidua may be accounted for by the lymphoid cells, monocytes and macrophages which infiltrate the decidua in early pregnancy (Padykula et al., 1978; Bulmer and Johnson, 1985; Kabawat et al., 1985). It is therefore apparent that decidua tissue is comprised not only of classically defined decidual cells but of a spectrum of cell types.

One explanation proposed for the survival of the fetal-allograft is that the uterus provides a "privileged site", similar to

the anterior chamber of the eye and cornea. However, unlike the chamber of the eye where the donor tissue is not rejected due to the absence of vascularization, the murine endometrium has been shown to be richly vascularized (Kirby et al., 1967). Skin isografts placed in a non-decidualized uterus have been shown to be promptly rejected (Beer et al., 1971; Beer and Billingham, 1974). In contrast, skin isografts survived significantly longer in a decidualized uterus (Beer et al., 1971; Beer and Billingham 1974; Dodd et al., 1980). Not only does decidualization protect the graft from immunological rejection but it also protects the mother against the invasion of the myometrium by trophoblastic cells. Kirby (1968) demonstrated that ectoplacental cones transplanted in the uterus of ovariectomized mice resulted in trophoblast cell invasion into the myometrial layer which was prevented in decidualized mice. It is therefore apparent that the development of a decidual layer between the maternal endometrial layer and the fetal placental layer is important in normal gestation. In recent years much interest has been focused on the properties of the decidua, in particular immunoregulatory, which will be discussed in greater detail in the following sections.

1.4 The Maternal Immunological Reaction to the Fetus

1.4.1 Cell Mediated Sensitization

To understand successful pregnancy it is important to determine whether fetus specific effector cells are generated by the mother. A number of assays have been employed to address this issue which unfortunately present conflicting results. It is known that after *in vivo* alloimmunization the sensitized individual's cells re-

spond in the mixed lymphocyte reaction (MLR) to cells from the immunizing donor with a heightened response (Oppenheim et al., 1965). This provides a means of studying pregnancy sensitization, in that if the mother has become sensitized to her fetus, her lymphocytes should give a heightened MLR on contact with fetal (paternal) MHC in vitro. This assay has not detected a primed-type of secondary response by maternal cells with neonatal or paternal stimulatory cells (Carr et al., 1974; Sargent et al., 1982; Moore et al., 1983; Vanderbeeken et al., 1984). In contrast, the release of a lymphokine-macrophage migration inhibition factor (MIF) has been detected in short term cultures of maternal lymphocytes with neonatal and paternal lymphocytes which is absent from nulliparous women and males, suggesting that maternal cellular hypersensitivity is detected (Rocklin et al., 1973; Youtananukorn et al., 1974).

Circulating primed effector cells may be detected in vivo by means of skin grafting. If a mother has been sensitized, then one would see an accelerated rate of graft rejection of paternal or infant skin (Ward et al., 1978). Maroni and Parrot (1973) reported such an accelerated rejection in mice against paternal skin grafts. However, this phenomena was usually seen after the second pregnancy (Maroni and Parrot, 1973). In contrast, Andersen and Monroe (1962) showed that post partum women grafted with their children's skin, the graft survived much longer than skin grafted between other family members.

The appearance of circulating primed cytotoxic T cells measured against fetal cells could not be detected by some investigators (Vanderbeekan et al., 1984; Sargent et al., 1985) but have been demon-

strated in some (but not all) multiparous women (Chardonnens and Jeannet, 1980; Genetet et al., 1982) and in the spleens of some allo-pregnant mice (Smith, 1983). Thus, a minority of women may possess specific cytotoxic cells, although the success of pregnancy does not appear to be threatened.

Another indication of the mother's cellular response to her allogeneic fetus is the enlargement and weight gain of the paraaotic lymph nodes draining the uterus (DLN) in rats (Beer and Billingham, 1979; McLean et al., 1980) and in mice (Baines et al., 1977; Ansell et al., 1978). This observation is common place in the inductive phase of transplantation immunity to allografts such as skin and bone (Parrott 1967; Burwell 1962). DLN enlargement has been attributed to increased cellularity due to T blast cell expansion (Maroni and DeSousa 1973; Foster et al., 1979) and is more pronounced in allo-geneic compared to syngeneic matings (Maroni and DeSousa 1973). It is of interest that the extent of stimulation observed in the DLN of allogeneic is not as striking as the node draining the site of skin allografts (Scotherne and McGregor, 1955). This may be due to the separation of the mother from the conceptus by the decidual cells, or the antigenicity of the trophoblast and/or to the poor lymphatic drainage of the endometrium in mice (Head and Seeling, 1984). Contrary to the observations of DLN enlargement during pregnancy, Chambers and Clarke (1979) showed no difference in DLN enlargement between allogenic and syngenic matings. This may be explained by the fact that the size of DLN may change during pregnancy and therefore the time chosen to make these measurements may reflect variability

(Maroni and DeSouza 1973; Chambers and Clarke 1979; McLean et al., 1980).

Surgical removal of DLN appears to impair fertility (Beer and Billingham 1977; Gill and Kunz, 1979). Therefore, with this evidence it has been postulated that DLN enlargement may represent a positive stimulating effect in allogeneic pregnancies and may be associated with a generation or accumulation of regulatory suppressor cells which inhibit transplantation immunity against fetal and trophoblast antigens.

1.4.2 Humoral Sensitization

It is now clearly established that the maternal immune system develops a humoral response against antigens present on the semi-allogeneic conceptus. These antibodies have been reported in the sera of pregnant mice (Mishell et al., 1963; Goodlin and Herzenberg 1964), rats (Smith and Sternlicht 1982), horses (Allen 1979; Antczak 1980) and humans (Terasaki et al., 1970; Nymand et al., 1971). This response is not unique to mammals, humoral factors have been detected in the amphibian salamander (Chateaureynand et al.; 1979). There exists much variability on the kinetics of this response. In some women the first pregnancy elicits only a weak antibody response of variable titre. It is not until subsequent allopregnancies that a strong allo-antibody response is detected (Voisin and Chaouat 1974; Smith and Sternlicht, 1982; Ghani et al., 1985). In contrast, others have shown a maternal antibody response to be maximum in the first trimester of gestation (Bell and Billington 1980; Power et al., 1983; Davies and Browne 1985). This discrepancy might be explained, in that, the

detection of alloantibody is identified primarily against paternal MHC specificities. The appearance of antibodies early in first pregnancies is usually against non-MHC specificities on trophoblast cell preparations (Power et al., 1983; Davies 1985; Davies and Browne 1985). This may suggest that the kinetics and strength of the antibody response may be due to antigen processing at the fetal maternal interface. Ghani et al. (1985) have shown that pregnancy induced antibodies against MHC reacted with a Class I selective haplotype differing from the haplotype eliciting antibody response from solid organs, suggesting a unique immunological difference at the placenta interface.

It is apparent that pregnancy-induced antibodies are primarily non-complement fixing in nature (Voisin and Chaouat 1974; Bell and Billington 1981; Smith and Sternlicht, 1982; Power et al. 1983; Ghani 1985) although low levels of C'-dependent cytotoxicity have been reported in the mouse (Baines et al., 1976) and human (Nymand et al., 1971) with multiparity. Further investigation into this area showed no correlation of the presence of cytotoxic antibody and the frequency of abortions (Nymand et al., 1971). The pregnancy-induced alloantibody has been shown to be predominantly of the IgG isotype and IgG_1 subclass in mice (Voisin and Chaouat 1974; Bell and Billington 1980) and humans (Taylor and Hancock 1975; Power et al., 1983; Singal et al., 1984; Davies 1985). It is known that non-complement fixing antibodies, especially of IgG subclass, to be the main humoral component of the facilitation reaction that may lead to the enhanced growth of a correspondng allografted tumor threatened by a rejection

reaction, provided the alloantibodies are directed against the alloantigen molecules (Voisin and Chaouat, 1974). In transplantation medicine it has been known for many years that simultaneous grafting of an allogeneic organ and inoculation of alloantibody against the graft antigens can delay graft rejection (Hellstrom and Hellstrom, 1970). "Blocking Antibodies" are thought to prevent contact between cells of the graft and circulating "sensitized" cytotoxic cells of the host by sterically blocking or masking the alloantigens involved in the rejection reaction (Hellstrom and Hellstrom 1970; Chang and Sugarbaker 1980). In humans, the IgG fraction of sera from pregnant women has been claimed to protect trophoblast cells from lysis by maternal lymphocytes (Taylor and Hancock, 1975). Voisin and Chaouat (1974) and Wegmann et al. (1979) have eluted IgG from the placenta which shows paternal leukocyte specificity (Wegman et al. 1981). Since the IgG eluted from the placenta is non-C' fixing it is postulated to be a "blocking" or an "enhancing antibody" (Voisin and Chaouat, 1974) which may have immunological significance in the protection of the conceptus. Studies have shown that sera from pregnant women can inhibit antipaternal activity in mixed lymphocyte cultures in vitro (Voisin and Chaouat 1974; Singal et al., 1984). Therefore, in addition to the "blocking antibody" theory, the alloantibody may block the functioning of maternal lymphocytes, for example, in the form of immune complexes.

Immune complexes may activate regulatory mechanisms such as suppressor T cells (Rao et al., 1980). Circulating immune complexes have been detected during human pregnancy (Masson et al., 1977;

Davies, 1985) and in rodents (Tung 1974). In addition, Singal and his associates (1984) have shown the generation of an antiidiotypic blocking antibody which binds to the antipaternal specificity idiotype on maternal sensitized lymphocytes. A lack of alloantibody blocking factor has been observed in abortion prone women (Rocklin et al., 1976; Stimson et al., 1979; Power et al., 1983). This does not exclude the possibility of their presence in early pregnancy and subsequent disappearance as pregnancy fails. It has been shown that the enhancing action are alloantibodies directed against class II antigens in mice (Segal et al., 1979) and humans (Rocklin et al., 1976).

There are some problems that weaken the validity of immunological enhancement as an explanation for the success of allogeneic pregnancy. For example, agammaglobulinemic women undergo successful pregnancy despite the fact that they would not be expected to produce any IgG enhancing antibody (Holland and Holland 1966; Kobayashi et al., 1980). In mice, there are stains which are "non-responders" and produced no alloantibody in spite of multiple pregnancies with H-2 and non-H-2 incompatible strains (Bell 1984). Thus a lack of alloantibody in women with recurrent abortion may be a result rather than a cause of the failure of successful pregnancy. Furthermore, it has been shown that lysis of target cells by activated CTL occur rapidly and is only temporarily delayed by precoating the targets with alloantibody (Faanes et al., 1973). Therefore, one would not expect that alloantibody in placenta would offer significant protection against the local action of sensitized CTL.

Taken together, these data suggest that immunological enhance-

ment by alloantibody against paternal antigens may not be sufficient to account for the survival of the fetal allograft, and that it now appears more likely that regulation of the cytotoxic cells involved in graft rejection (Fernandez-Cruz et al., 1980) may play a key role in fetal protection.

1.5 Regulatory Cells

As discussed previously, a number of different types of cells appear to participate in allograft rejection (Roberts and Hayry 1976; Storm et al., 1977; Hayry et al., 1979; Loveland et al., 1981). In many experimental systems, it has been confirmed that there is an accumulation of an enriched population of specifically sensitized cytotoxic T effector cells at the site of allograft rejection (Ascher et al., 1981; Von Willebrand et al., 1979; Hayry et al., 1979). Recent evidence has shown that resorbing (aborting) xenogeneic and allogeneic blastocysts are infiltrated with cytotoxic T lymphocytes following implantation in pseudopregnant mice (Croy et al., 1982; Chaouat et al, 1986). Thus the regulation of CTL may be an important mechanism that may impair the major effector population of fetal-allograft rejection.

It is well known that suppressor T cells play an important role in the regulation of immune responses (Benacerraf, 1980). In vivo generation of CTL has been shown to be regulated by a suppressor T cell population (Rollinghoff et al., 1977). In addition, Rich et al. (1979) and Truitt et al. (1978) have shown that H-2 antigens stimulate suppressor T cells which inhibit the generation of CTL in the local nodes draining the site of allogeneic challenge. Therefore it is postulated that the generation of suppressor T cells in allo-

geneically pregnant females could act to prevent rejection of the fetus.

Suppressor T cells are generated in the mother as a result of allogeneic pregnancy, both in women (McMichael and Sasazuki, 1977) and in mice (Chaouat and Voisin 1979; Chaouat and Voisin, 1980; Chaouat, et al., 1982). However, in these situations, in order to detect the suppressor T cell activity multiparous allopregnant mice are required (Chaouat and Voisin, 1979; 1980; Chaouat et al., 1982) since suppressor T cells are not detected until the 3rd-4th parity. Experimental studies have shown that suppressor T cells generated during allopregnancy do not afford protection to intrauterine or subcutaneous grafts of paternal tumors (Nagarkatti and Clark, 1983). These observations suggest that suppressor T cells may be an epiphenomena of pregnancy and do not contribute to the success of the fetal allograft. If suppressor T cells played a significant role in the success of pregnancy one would expect to see a high frequency of paternal haplotype sharing among children in large families. Such a situation would contradict the Darwinian theory of maximizing the genetic variation established with mating if haptotype selection occurred.

On account of the evidence demonstrating the generation of suppressor cells in the local nodes draining the site of antigenic challenge (Rich et al., 1979; Truitt et al., 1978), a number of studies were done to investigate the local uterine draining nodes (paraaortic lymph nodes) in allogeneically pregnant mice.

Cells harvested from the paraaortic lymph nodes from allogeneic and syngeneic pregnancies, demonstrated a depressed immune

response *in vivo*, in terms of their ability to react against paternal alloantigens in a GVH mortality assay (Clark and McDermott 1978; Nicklin and Billington 1982) and *in vitro* in terms of the number of cytotoxic cells generated during mixed lymphocyte culture (Hamilton and Hellstrom 1977; Clark and McDermott, 1978; Smith 1981). Kastner et al. (1977) have reported that immune suppression may be induced by non-H-2 histocompatibility antigens where H-2 homology is present. This observation may explain the suppression observed in the DLN of syngeneically pregnant mice. For example, the suppression may have been triggered by maternal recognition of embryonic and/or placental-specific antigens. Although a reduced immune response is observed in both, allogeneic and syngeneic combinations, the greatest reduction is observed in allogeneic matings (Nicklin and Billington 1982). In strain combinations where there exists a high tendency to resorb their fetuses, a lack of suppressor activity was found in the DLN whenever resorption (abortion) occurred (Clark and McDermott 1978; Smith 1981). Although some investigators have found systemic antibody responses to be suppressed in mice during the latter half of pregnancy (Baines et al., 1977; Sasaki and Ishida, 1975) there are a number of reports which have found normal or increased antibody responses in pregnant mice (Kenny and Diamond 1977; Merrit and Galton 1969; Clark et al., 1980) and women (Murray et al., 1979). Taken together, these data suggest that there is a localized selective impairment of CTL generation in the DLN during pregnancy which may bear *in vivo* significance in ensuring the survival of the fetal allograft.

Many varieties of suppressor cells have been described to sup-

press by elaborating soluble suppressor factors (Rich et al., 1979; Truitt et al., 1978; Rich and Pierce 1974). Truitt et al. (1978) have described such a factor produced by H-2 specific suppressor T cells. Although the suppressor T cell was H-2 specific, the factor produced inhibited the generation of CTL to unrelated H-2 determinants. Thus, it appears that the regulation of CTL generation can have both a specific and a non-specific component. It has been demonstrated that specific soluble suppressor factors (Chaouat et al., 1982) and non-specific soluble suppressor factors (Clark et al., 1980; Maraz et al., 1974) are generated during allogeneic pregnancies.

The non-specific suppression of CTL generation in the DLN of pregnant mice has been shown to be associated with a non-T suppressor cell (Clark and McDermott, 1981; Smith 1981). A variety of non-T suppressor cells have been described in a number of systems. For example, suppressor B cells have been described in murine cell-mediated autoimmunity (Russell et al., 1974) and in the activation of suppressor T cells in tumor associated immunosuppression (Ninneman 1978). Suppressor cells without conventional T cell or B cell surface markers, are called "null cells" and have been described in spleens of neonate mice (Rodriguez et al., 1979) ageing mice (Roder et al., 1977) and in murine trypanosomiasis (Pearson et al., 1979). Recently, NK cells have been shown to possess B cell immunoregulatory functions in vivo (Abruzzo and Rowley 1983; Brieva et al., 1984).

1.6 Local and Systemic Suppressor Mechanisms

1.6.1 Systemic Suppression

Systemic weakening of the maternal immunological system may be

one explanation for the survival of the fetal allograft. Non-specific immunological modifications during gestation have been demonstrated. Certain autoimmune disorders have been noted to decrease during pregnancy, such as rheumatoid arthritis (Persellin 1977) and autoimmune experimental allergic encaphalomyelitis (Keith 1978). There also appears to be an increased susceptibility to certain bacterial and viral infections (Young and Gomez 1979; Van Zon and Eling, 1980; Taina et al., 1985) and enhancement of certain malignant neoplasms (Gustafsson and Kottmeier 1962; Shiu et al., 1976).

It has been known for some time that dramatic thymic involution occurs in murine (Persike 1940; taken from Phuc et al., 1981; Pepper 1961; Maroni and DeSousa 1973) and human pregnancy (Nelson et al., 1967, 1973). With the above mentioned information many investigators have examined changes in lymphocyte subpopulations and various functional aspects of cell mediated immunity during pregnancy (Strelkauskas et al., 1978; Birkland and Kristoffersen 1980; Lucivero et al., 1983; Tallon et al., 1984; Bailey et al., 1985). These investigations have yielded interesting and conflicting results. The general conclusions drawn from these studies are that although pregnant women appear to have a decreased proportion of T helper cells, they do have adequate T helper function activity (Lucivero et al., 1983; Tallon et al., 1984; Bailey et al., 1985) and therefore the changes that might be observed in lymphocyte subpopulations are not primarily responsible for the immunodeficiency of pregnancy.

Several investigators have demonstrated a suppressive activity in the serum of pregnant mice (Hellstrom et al., 1960; Harrison 1976)

and pregnant women (Kasakura 1971; Pence et al., 1975). Although the nature of the suppressor molecules in pregnancy serum are unknown, some suppression may be due to "Early Pregnancy Factor" (EPF) which appears in serum shortly after fertilization and disappears in women and animals who are spontaneously aborting (Noonan et al., 1979). However, rejection of paternal skin grafts is only slightly delayed by allogeneic pregnancy (Beer and Billingham 1974). In addition, Woodruff (1958) described an experiment where fetal tissue grafts, transplanted intramuscular, were successfully rejected by the mother while developing fetuses in utero were unharmed. It is therefore difficult to imagine how non-specific systemic suppression could prevent fetal allograft rejection without presenting a serious threat to the mother by reducing her ability to resist infectious agents. For this reason local suppressor mechanisms at the fetal-maternal interface have been proposed.

1.6.2 Local suppression

A potential suppressor substance, produced by the fetus and localized in the feto-placental unit is alpha-fetoprotein. It is known that alpha-fetoprotein suppresses antibody production and the generation of cytotoxic T cells (Dattwyler and Tomasi, 1975; Peck et al., 1982). Hooper and Murgita (1981) have shown that the primary target for alpha-fetoprotein mediated immunosuppression is a Ly 1^+ 23^- Ia^+ T cell which serves as a helper cell for antibody synthesis and collaborates with CTL precursors in CML. However, despite a high concentration of alpha-fetoprotein in fetal serum, the fetus is capable of rejecting skin grafts while in utero (Silverstein 1977)

thereby demonstrating that alpha-fetoprotein does not prevent allograft rejection.

Recent studies have focused on suppressive factors produced by trophoblast cells. It has been demonstrated by coculture techniques, that trophoblast cells suppress in vitro generation of CTL activity without affecting the antibody response (Barg et al., 1978), a pattern typical of that seen in the DLN of allopregnant mice (Clark et al., 1980). It has been reported that soluble suppressor factors produced by trophoblast cells, in particular the syncytiotrophoblast, inhibit mitogen and allogeneic cell stimulation in the MLC reaction. (Duc et al., 1985; Degenne et al., 1985; Remacle-Bonnet et al., 1985; Drake and Rodger 1985). The exact nature and immunoregulatory mechanism(s) which mediate suppression are yet unclear. It is known that trophoblast cells produce the hormones chorionic gonadotropin, progesterone and estrogen which play an important endocrine role in the success of pregnancy. Progesterone in local high concentrations can suppress graft rejection (Beer and Billingham 1979; Siiteri et al., 1977) without altering the magnitude of the antibody response (Fabris et al., 1977; Fabris 1973; Beer and Billingham 1979). Although these data taken together would argue that progesterone may be responsible for the maternal immunosuppression and protection of the fetal allograft in pregnancy, there is evidence to suggest that the mechanism of progesterone action is indirect. For example, recent experiments have demonstrated that within one horn of a uterus, transferred xenogeneic M. caroli blastocysts are rejected after implantation while the adjacent allogeneic or syngeneic M. musculus transferred blastocysts

are unharmed (Croy et al., 1982). It was shown that the resorbing xenogeneic blastocysts are infiltrated by CTL at day 9.5 of gestation, just prior to enzyme synthesis for progesterone production by the placenta (Chew and Sherman, 1975). Therefore, one would expect that the local uterine progesterone concentration at the time of the observed CTL infiltration to be similar for adjacent blastocysts within one uterine horn. It therefore appears unlikely that progesterone is the direct suppressive agent involved in pregnancy. Furthermore, the concentrations of progesterone which must be added to in vitro MLC cultures to produce suppression (Clark and McDermott 1981) are 5-10 times greater than the highest concentration of progesterone that has been observed in the studies of placenta and trophoblast tissue (Smith and Bush, 1978). Thus, the mechanism by which progesterone suppresses may be to facilitate the production of immunosuppressive proteins by trophoblast such as described by Degenne et al., (1985) and Remacle-Bonnet et al. (1985). Progesterone may also stimulate the generation or local accumulation of suppressor cells in the genital tract. This idea is consistent with observations described by Wira et al. (1980) and McDermott et al. (1980) where gestational hormones appeared to influence the localization of lymphocytes in the genital tract.

A number of studies have shown the development of local suppressor activity in the uterine decidua of pregnant mice (Kirkwood 1981; Slapsys and Clark 1982; Badet et al., 1983) and women (Golander et al., 1981; Daya et al., 1985; Nakayama et al., 1985). We have conducted intensive studies in the allopregnant mouse uterus and have

shown decidual suppressor activity to be associated with a novel type of suppressor cell which is lymphocytic in morphology, bears no conventional T cell markers ("null cell") and contains eosinophilic cytoplasmic granules (Slapsys and Clark 1982). This suppressor cell prevents the generation of CTL in vitro and in vivo (Slapsys and Clark, 1982; 1983) by a soluble factor that blocks the proliferation response of T cells to interleukin 2 (Slapsys and Clark 1983; Clark et al., 1985). Chatterjee-Hasrouni et al. (1980) and Bernard et al. (1978) have described the accumulation of non-T cells or "null cells" in the uterine decidua of pregnant mice. It was therefore our hypothesis that the decidua-associated suppressor cells localize in the uterus during allopregnancy under the influence of trophoblast.

1.7 Purpose of this Study

The studies described in this thesis, continued the investigation of localized immuno-suppression in the uterine decidua of allopregnant mice. Monoclonal antibodies allowed further characterization of the decidua-associated suppressor cell. A xenopregnancy model demonstrated the absence of the decidua-associated suppressor cell in failing pregnancy. And lastly, the role of fetal tissue components in the recruitment of the decidua-associated suppressor cell to the uterus was examined. These studies will provide ground work for future studies on the specific soluble factors elaborated by the trophoblast which are important in the recruitment and triggering of the decidua-associated suppressor cell for successful pregnancy.

Chapter 2

MATERIALS AND METHODS

2. Materials and methods

2.1 Animals

Female mice of various inbred strains were obtained from the Jackson Laboratory, Bar Harbor, Maine, and mated to DBA/2J males (Jackson Laboratory). Mus caroli mice were provided by Dr. J. Rossant, Brock University, St. Catharine's, Ontario, Canada. Ha (ICR) mice were provided by Dr. V. Chapman, Roswell Park, Buffalo, New York. Pregnant mice were produced in a controlled breeding situation. The morning that a vaginal plug was sighted was designated day 0.5 of gestation [Theiler, 1972]. C3D2F1 mice were bred in our own colony. All animals were housed in a 12h light/12h darkness regime and allowed food and water ad libitum. Cell suspensions were isolated from age-matched virgin mice or 10-20 wk old animals undergoing their first allogeneic pregnancy or pseudopregnancy. For some specific experiments CD1 nu/nu female mice were employed which were obtained from McMaster University Medical Centre's breeding colony, Hamilton, Ontario.

2.2 Media

Hanks' balanced salt solution (HBSS) (Gibco Ltd., Grand Island, NY) (Mg^{2+} and Ca^{2+} free) was used in the initial preparation of the uterine decidual cell preparations. α-Minimum Essential Media (α-MEM) (Grand Island Biological Co., Grand Island, NY) was supplemented so as to contain 10% (v/v) fetal calf serum (FCS; Grand Island Biological Co., 200 iu/ml of penicillin and 200 μg/ml streptomycin (Gibco Ltd.) was used for all cell washings and cell cultures. RPMI similarly supplemented cultured media obtained from the Culture Media

Centre Resources (Dr. W. Rawls, McMaster University, Hamilton, Ontario) was used in the maintenance of the in vitro trophobast cell cultures.

2.3 Preparation of Cell Suspensions

Animals were sacrificed by cervical dislocation and cell populations were obtained from primigravid allopregnant mice between 12.5 - 19.5 days post mating, from age matched pseudopregnant mice and virgins.

The PLN and spleens were aseptically removed and placed in ice cold α-MEM, and were disrupted by pressing through a 60-mesh stainless steel wire sieve into ice cold α-MEM. The cell suspensions were centrifuged at 200 x g for 10 min. The pellet was resuspended in cold α-MEM and viable nucleated cells were enumerated in a hemocytometer using 0.4% (w/v) trypan blue.

2.4 Preparation of Uterine Decidual Cell Suspensions

2.4.1 Mechanical isolation

Decidual and deciduoma lymphocytes were obtained from pregnant and pseudopregnant mice using aseptic technique. The procedure involved peeling the fetoplacental unit from the decidua (in pregnant mice) and scraping the remaining underlying decidua or deciduoma from the uterine wall. The decidua was pressed gently through a 60-mesh stainless steel sieve, and the lymphoid cells were isolated by centrifugation over 2 ml of Lympholyte M (Cedarlane Laboratories, Hornby, Ontario). The number of viable cells were enumerated in 0.4% trypan blue.

2.4.2 Enzymatic Isolation

In some experiments, decidual and deciduoma lymphocytes were isolated by cutting the uterus into small pieces (after removing the fetoplacental unit) and stirred in a collagenase preparation for 90 min. at 37°C (25 units of collagenase from Clostridium histolyticum [Boehringer Mannheim]) in 100 ml of Hanks' solution supplemented with 5% FBS and 200 iu/ml of penicillin and 200 µg/ml streptomycin (Gibco Ltd., Grand Island, N.Y.)). After 90 min., the suspension was poured through a sterile gauze filter, centrifuged, resuspended in media and viable cells enumerated in 0.4% trypan blue.

2.5 Cell Separation

2.5.1 Cell separation by velocity sedimentation

Separation of cells on the basis of size was achieved by velocity sedimentation at unit gravity at 4°C in the Sta-Put system (O.H. Johns Scientific Co. Ltd. Toronto, Ontario, Canada) as originally described by Miller and Phillips (1969). Briefly, cell populations were prepared as discussed in section 2.4.1. and raised as a thin band on a layer of PBS in a Sta-Put sedimentation chamber (diameter, 11.5cm). Beneath the cell layer a discontinuous step gradient, ranging from 0.35% (w/v) to 2% (w/v) BSA in PBS was established. The cells were allowed to sediment through the gradient for 3.5 - 4.0 h. Twenty-five ml fractions were collected and centrifuged at 200 x g for 10 min at 4°C. Cell pellets were resuspended in ice cold α-MEM and enumerated in 0.4% trypan blue. The mean sedimentation velocity of each fraction was computed by a programme entitled SVAL3 (for use in a Texas Instrument SR52) as prepared by Dr. David A. Clark, Department of Medicine, McMaster University.

2.6 Enumeration of Granulated Lymphocytes

To determine the percent granulated cells in a uterine decidual cell suspension the cell populations were prepared as described in section 2.4.1 and 2.4.2. Cell smears were prepared by cytocentrifugation at 5000 rpm for 5 min (Cytospin Centrifuge, Shanden Southern Instruments, Camberly, England) and stained with Diff-Quick (Harleco, Gibbstown, N.J.).

2.7 Mixed Lymphocyte Cultures

To test the ability of cells from the uterine decidua of pregnant and pseudopregnant mice to suppress the generation of CTL *in vitro*, washed PLN from strain matched virgins were co-cultured with test cells in 17 x 100 mm polystyrene tubes (Falcon Plastics, #2057, Oxnard, CA) in 3.2 ml of culture medium supplemented so as to contain $5x10^{-5}$M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.) and 1×10^6 irradiated (1500 rads ^{60}Co) C3D2F1 stimulatory spleen cells, and incubated at 37°C in 6-7% CO_2 for 5 days.

2.8 ^{51}Cr Release Assay

The cytotoxic activity of each culture was quantitated using a modified Brunner ^{51}Cr release assay (Brunner et al., 1968).

2.8.1 Target cells

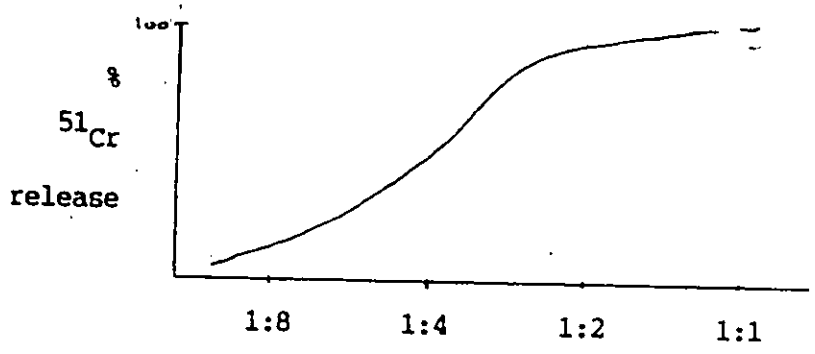
Two tumor lines were used in the cytotoxic assay. P815X2, a DBA/2 ($H-2^d$) mastocytoma line which was maintained in serial ascites transfer in DBA/2 mice, and Yac-19, a lymphoma line, kindly provided by Dr. John C. Roder, which was maintained in tissue culture in RPMI plus 10% FCS. For use as CTL target cells, 2×10^7-4×10^7 P815 cells in α-MEM were incubated with 500 μCi of ^{51}Cr-labelled sodium chromate

(New England Nuclear, Boston, MA) for 90 min then washed 3 times with tissue culture medium. Cells were then counted by trypan blue exclusion and adjusted to the desirable concentration. For use as NK target cells, Yac-19 cells were prepared as outlined above for P815 cells.

2.8.2 CTL cytotoxic assay

After a 5-day culture period, 2.0 ml of medium were carefully removed from each tube without disturbing the cell pellet. The pellet was then resuspended in the remaining 1.2 ml and 100 μl from each tube was serially diluted in microtiter trays (Cat. No. 76-002-OS, Flow Laboratories Inc., McLean, VA) and incubated with 2 x 10^4 ^{51}Cr-labelled P815 target cells for 4 h at 37°C. After 4h the ^{51}Cr release reaction was stopped by cooling the trays to 4°C. One hundred microliters of supernatant was carefully removed, transferred to a 12 x 75 mm glass tube and counted for 2 min in a Beckman Gamma 8000 Radiation Counter (Beckman Instrumentation, Inc., Fullerton, CA).

A single numerical value for cytotoxic T cell content ($N\alpha t$ x 10^3 ± SEM) was determined from titration curve of percent ^{51}Cr specific release (P) vs Target/Effector ratio



by computer fitting of the model P (percent specific ^{51}Cr-release) =

$100\ (1- e^{N\alpha te-N\alpha})$ where N=total number of sensitized cells, α is a constant proportional to CTL frequency, t represents CTL-target interaction time, and α corrects for the inhibitory effect of bystander cells, and accounts for the bend in the curve at higher cell concentrations.

2.8.3 NK cytotoxic assay

NK activity was tested by incubating effector and targets at appropriate ratios (most normally starting at 100:1 and serially diluting to 1:1) in microtitre trays. (The targets being cells ^{51}Cr-labelled (Yac-19)) for 6 hours at 37°C. After 6h the ^{51}Cr release reaction was stopped by cooling the trays to 4°C. One hundred microlitres of supernatant was carefully removed, transferred to a 12x75 mm glass tube and counted for 5 min in a Beckmann Gamma 8000 Radiation Counter. The parameter $N\alpha tx10^3$ ± SEM was determined from the titration curve discussed above in section 2.8.2.

2.9 Antiserum Treatments

Test suspensions of lymphocytes at 1 x 10^6 per ml in α-MEM, without FBS but containing 0.2% BSA were incubated for 45 min at 4°C with various antisera, centrifuged, and resuspended in a 1/20 solution of Low-Tox rabbit complement (Cedarlane Laboratory, Hornby, Ontario) in PBS for 45 min at 37°C. The antibody treatments included monoclonal antibody Thy 1.2 (New England Nuclear, Cambridge, MA) used at a dilution of 1/100, monoclonal anti-IgG-FcR (New England Nuclear used at 1/100), anti-$GM_{1.2}$ (New England Nuclear, used at 1/100), anti-Mac1 (Hybritech, San Diego, CA. used at 1/100) and anti-asialo GM_1 which was kindly produced by Dr. K. Okumura and used in a final dilution of

1/50. The dilutions used represent that which produced optimal cytotoxicity on peripheral lymphoid cells.

2.10 FcR-positive Cell Isolation

Rosette separation technique was carried by Mr. Carl D. Richards as described by Gauldie et al. (1983). Briefly, SRBC were treated with TNP (trinitrophenol) as described by Strober (1978), and the TNP-SRBC were incubated with rabbit polyclonal IgG anti-TNP. Equal volumes (100 μl) of Ab-TNP-SRBS 2% v/v in PBS and uterine decidual cell suspension were mixed and centrifuged at 200 x g at 4°C. The cells were then gently resuspended and centrifuged over Lympholyte M (Cedarlane Laboratory, Hornby, Ontario). Cells were harvested from the interface and pellet. The pellet containing the rosetted cells was designated as the FcR positive (FcR^+) population and the interface population was designated as the FcR negative (FcR^-) population.

2.11 Sponge Graft Assay

Cellulose sponge (Kongsors Fabrikker, Oslo, Norway) cut into 3 - 4 mm^2 cubes was implanted in the peritoneal cavity of DBA/2 mice for 5 days to allow fibroblast colonization as described by Roberts and Hayry (1976). The spenge matrix grafts were then removed from the mice and injected with 20 μl of medium or medium containing test cells. Test cells were obtained from uterine decidua were separated according to size as outlined in section 2.5. The test cell populations were treated with Thy 1.2 antisera plus C' as described in section 2.9, and then incubated at 37°C with 50 μg/ml of Mitomycin C (Boehringer Manneheim W. Germany) for 20 min. After 3 washes in culture medium the pellet was resuspended in α-MEM and enumerated by

trypan blue exclusion.

The treated sponges were implanted subcutaneously into virgin C3H recipients and 7 days later removed and placed in tissue culture medium. The infiltrating cells were isolated by squeezing the sponges and were tested for CTL activity at several dilutions in V-bottom microtrays (Linbro) using 2×10^3 P-815 target cells.

2.12 Uterine Suppressor Cell Characterization in Multiparous Animals

Cell suspensions were prepared from monoparous mice as described in section 2.4.1 and treated with monoclonal anti-Thy 1.2 (New Cambridge, MA.) as outlined in section 2.9. Mice were considered monoparous 15 days after parturition, and were then allowed to mate with DBA/J males. The first litters were removed at birth so that these mice were not lactating.

2.13 The Anatomical Location of the Decidua-Associated Suppressor Cell

For detection of the decidua-associated suppressor cell within the uterus, the uterus was excised and the feto-placental units removed. The decidual capsule was then cut away from the uterus and placed in ice cold α-MEM. The remaining uterus was dissected into two components, the tissue below the decidual capsule and the tissue between the decidual capsules. All tissues (capsule, below capsule and between capsule) were then prepared in the manner described in section 2.4.1 and tested for suppressor activity as described in section 2.7. The percent granulated lymphocytes was determined for each tissue as outlined in section 2.6.

2.14 Pseudopregnancy

Virgin females were made pseudopregnant by two different hormonal regimes.

A. The Miller and Emmens primed (1969) regime the virgin mice intramuscularly (im) with 100 ng of 17 β-estradiol (Sigma Chemical Co., St. Louis, MO) in 0.10 ml peanut oil on days 1-3. The mice received no hormone on days 4 and 5. On days 6 through 13, the mice were injected with 6.7 ng of estradiol and 100 mg of progesterone (Sigma) in 0.1 ml peanut oil at a separate subcutaneous (sc) site. On the eighth day of the schedule 10 ul of peanut oil were administered intraluminally to the left uterine horn through the dorsal ovarian tip, using a syringe and a micrometer and a 25 gauge needle in order to stimulate formation of a deciduoma. The animals were sacrificed on the fourteenth day and the cells harvested, prepared and tested as outlined in sections 2.4.1 and 2.7.

B. In the second pseudopregnancy regime, virgin female mice received 5.0 IU pregnant mare's serum gonadotropin (Sigma) ip, 48 hours later the female mice were injected with 5.0 IU human chorionic gonadotropin (hCG) (Sigma) ip, and 6 hours later were vaginally stimulated with a wire probe. (Where Mus caroli females were used, half the dose of hormone was used to induce pseudopregnancy). Twenty-four hours after the administration of hCG, 10 ul of peanut oil was injected into the left uterine horn to stimulate deciduoma formation as described above. The animals were sacrificed 6 days later and the cells harvested, prepared and tested as outlined in sections 2.4.1 and

2.7. To determine the yield of percent granulated lymphocytes removed from pseudopregnant animals, the cell preparations were treated as described in section 2.6.

2.15 Xenopregnancy

Xenopregnancy between Mus musculus and Mus caroli was established by Dr. Janet Rossant and Dr. Ann Croy of Brock University, St. Catharine's, Ontario, Canada. Briefly, 76 h Mus caroli blastocysts and 86 h Mus musculus blastocysts (at which point they had achieved a similar stage of development) were transferred into opposite or the same horn of the Mus musculus uterus on day 2.5 of pseudopregnancy (four to eight blastocysts of each type were transferred). On day 9.5 the recipient pseudopregnant females were sacrificed and the number of viable implanted embryos in each horn counted. Previous observations demonstrated that Mus caroli embryos appear viable and morphologically normal up to day 9.5 of pregnancy in the Mus musculus uterus (Croy et al., 1982). The viable embryo and its placenta were peeled from the decidua, and the remaining decidua was scraped from the wall of the uterus, prepared and tested for suppressor activity as outlined in sections 2.4.1 and 2.8. Enumeration of granulated cells was carried out as outlined in section 2.6.

2.16 Recruitment of Decidual Suppressor Activity

2.16.1 Trophoblast Cell Line

A spontaneous cell line derived from C3H/HeJ mated C3H/HeJ placenta showed the following trophoblastic characteristics: positive staining for alkaline phosphatase (Beeson et al., 1984), progesterone synthesis (assay described in section 2.16.1.1), growth of a solid

tumor composed of trophoblast-like giant cells when injected into an irradiated mouse (300 - 400 rads), and positive stain for cytokeratin. The cell line was a gift from Dr. James Beeson (Department of Obstetrics and Gynecology, University of Oklahoma, Tulsa, OK.) and is referred to as the Be6 line.

2.16.1.1 Progesterone Synthesis - An Histochemical Assay

Some enzymatic reactions involved in the metabolic pathway of steroid synthesis provide a histological technique in assessing progesterone synthesis. The enzyme $^{3}\beta$-hydroxy-steroid dehydrogenase catalizes one reaction in the synthesis of progesterone. This enzyme with the cofactor NAD^{+}, oxidizes the 3β-hydroxy group of pregnenolone. Enzymatic reactions in which NAD serves as a co-enzyme can be coupled with tetrazolium salts (Wattenberg, 1958) which can be demonstrated by microscopic histological techniques. The histochemical assay was carried out by Dr. E.V. YoungLai as described by Hay and Moor (1975). Briefly, the Be6 cells, which adhere to glass culture wells, were washed with cold acetone and cold phosphate buffer. Pregnenolone (Sigma, St. Louis, MO.) dissolved in dimethyl formamide was used as a substrate to demonstrate the presence of $^{3}\beta$-hydroxysteroid dehydrogenase. In some instances, NAD was used as the substrate (Sigma, St. Louis, MO.) to demonstrate the presence of tetrazolium reductase (diaphorase).

2.16.1.2 Cytokeratin - An immunohistochemical assay

HistoGen PAP (BioGenex Laboratories, Dublin C.A.) is a kit which utilizes immunoperoxidase for staining cytokeratin in Beb trophoblast cells. Briefly, Be6 cells were grown in Lab-Tek tissue

culture trays (4838 Fisher, Whitby, Ontario) (10^5/0.2 ml x 48 hrs) and were then fixed with acetone. After treatment with H_2O_2, the cells were reacted with control or anti-cytokeratin followed by second antibody with peroxidase label and then the substrate solution. Development of a brown colour signified the conversion to coloured end-product by antibody that had adhered to the cellular components.

2.16.2 Trophoblast cell culture supernatants

Supernatants from the Be6 cell culture were generated by placing 10 mls of fresh RPMI medium over a monolayer of Be6 in a culture flask and incubated at 37°C for 48h. The supernatant from the flask was then clarified by centrifugation and assayed for the ability to recruit suppressor activity into the pseudopregnant uterus as discussed in section 2.16.3.

2.16.3 Effect of trophoblast cells (Be6) in utero

Virgin females were made pseudopregnant by either a progesterone-estrogen regime or the pregnant mare sera-human chorionicgonadotropin regime as described in section 2.12. One million Be6 cells were injected into the uterine lumen at the time of the oil injection. After six days the cells were harvested from the uterus as outlined in section 2.4.1 and tested for its activity to suppress CTL generation as described in section 2.7. Other cell lines were also tested and compared to the Be6 line in their ability to recruit suppressor activity into the uterus, such as P815, and P19 which is a C3H teratocarcinoma line and was supplied by Dr. M. McBurney, Ottawa. Percent granulated cells were also determined with each decidual stimulus as described in section 2.6.

2.16.4 Day 9.5 ectoplacental supernatants

Supernatants were generated from ectoplacental cones which were dissected from day 9.5 embryos. The dissected ectoplacental cones were placed in a 10 x 15mm petri dish (Falcon, Oxnard, CA, U.S.A.) in 5 mls of fresh αMEM and incubated at 37°C for 48 h. The supernatant from the petri dish was clarified by centrifugation and assayed for the ability to recruit suppressor activity into the pseudopregnant uterus as discussed in section 2.14.3.

2.17 Statistical Analysis

A comparison of CTL activity within experiments was by the Student's t test. Experimental repeats were done to protect against artifacts of multiple comparisons. Where CTL response was suppressed to "0", no SEM was determinable; therefore, $p<0.05$ applies if control $> 0 \pm$ SEM.

Chapter 3

Experimental Results

Experimental Results

3.1 Evaluating Mechanical and Enzymatic Cell Preparation Techniques

Since other laboratories have prepared uterine cell suspensions by enzymatic methods, we compared our original isolation procedure of mechanically scraping the uterus to collagenase digestion procedures. The results in Table 1 show that similar decidua-associated suppressor activity was recovered by both isolation procedures. The cell distribution profile of Figure 3 shows that cell separation by velocity sedimentation of the two isolation procedures. Although figure 3 shows a loss of larger cells by the mechanical preparation, 1/5 collagenase preparations also showed a loss of the larger cells. The larger cells are lost in 2/4 mechanical preparations, possibly due to the variation of mesh size. Nevertheless, in agreement with our previous findings, using the mechanical procedure of cell preparation, the suppressor activity was predominantly associated with small lymphocyte-like cells when decidual suspensions were prepared by collagenase digestion (see Table 2). Although collagenase digestion provided a greater cell yield per animal than the mechanical preparation (by 2X) the cell viability was lower for the enzymatic digestion compared to the mechanical preparation ($31 \pm 23\%$ vs $62 \pm 5\%$). It should be noted that the use of certain commercially available collagenase preparations eliminated the decidua-associated suppressor activity (Clark et al., 1986a) possibly by modifying the cell surface. These data indicated that our originally chosen mechanical procedure for the preparation of decidual cell suspensions was appropriate for

Table 1

Comparison of decidua-associated cell[a] preparation procedures

Test Cells	CTL Activity per Culture Nαt x 10^3 ± SEM
3 x 10^6 VPLN	1690 ± 150
+ 10^5 mechanically isolated cells	1055 ± 82[b]
+ 10^5 collagenase preparation of cells	945 ± 55[b]

a. Decidua-associated cells were obtained from allopregnant mice in the second half of pregnancy by either mechanical or enzymatic procedures as described in Materials and Methods.

b. Significant suppression by Student's t-test $P < 0.05$.

Figure 3

Sta-Put of mechanical vs collagenase isolation profiles

Decidua-associated cell preparations were obtained by either mechanical (●) or enzymatic (o) procedures and separated in fractions of different sizes by velocity sedimentation as described in Materials and Methods. The recovered viable nucleated cell profile for each procedure is illustrated in figure 3.

Figure 3

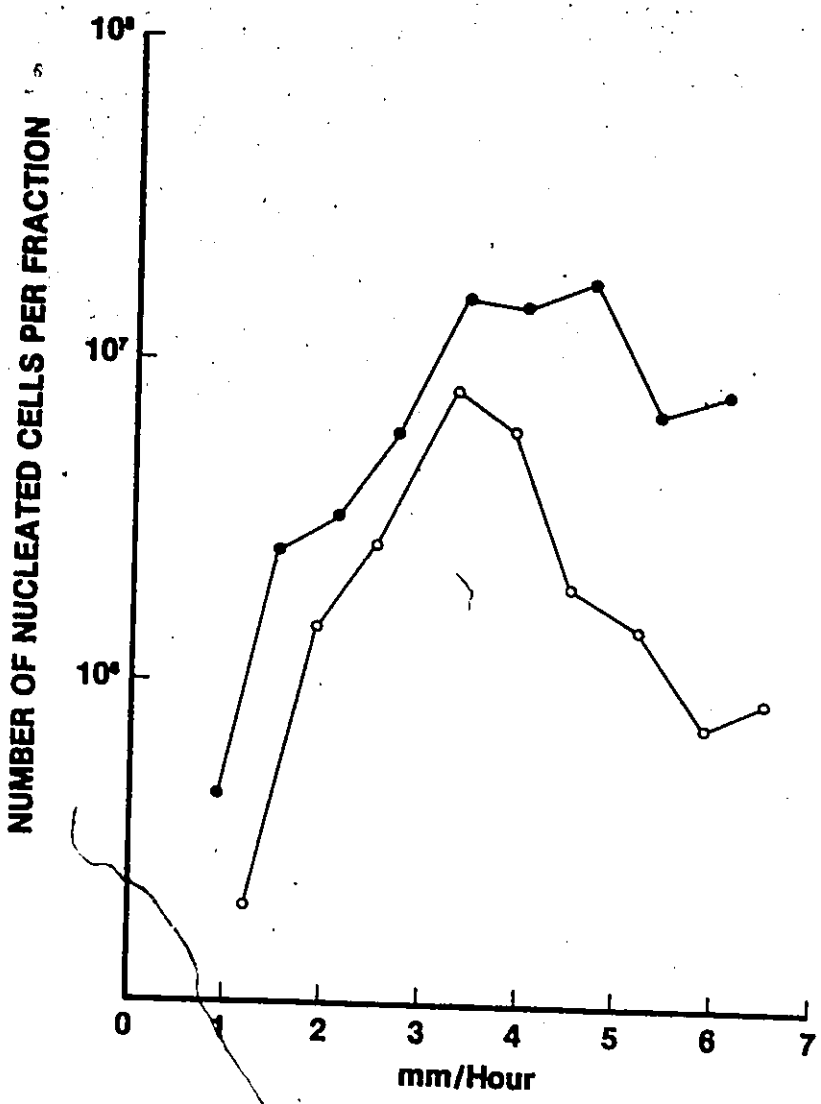

NUMBER OF NUCLEATED CELLS PER FRACTION
10⁸
10⁷
10⁶
0
1
2
3
4
5
6
7
mm/Hour

Table 2

Suppressor activity of decidua-associated[a] cells separated by velocity sedimentation

Test Cells	Cytotoxic Activity per Culture Nαt x 10^3 ± SEM
3×10^6 VPLN	1494 ± 265
+ large decidual cells (54×10^3)[c]	1770 ± 145
+ large macrophage-like cells (10^5)	1574 ± 80
+ large monocytoid (10^5) cells	997 ± 120
+ medium monocytoid (10^5) cells	1527 ± 207
+ small lymphocytes (10^5)	550 ± 42[b]

a. Decidua-associated cell suspensions from allopregnant mice were prepared by collagenase digestion and cell separation by sedimentation velocity performed as described in Materials and methods.

b. Significant suppression by Student's t-test $P < 0.05$.

c. Number in parenthesis equals the number of decidua-associated cells tested.

the study of suppressor activity within the uterus during pregnancy.

3.2 Further Characterization of the Decidua-associated Suppressor Cell: Surface Phenotype of Suppressor Cell.

3.2.1 Characterization using antisera plus complement treatment

It has previously been shown that the decidua-associated suppressor cell activity in the second half of pregnancy in allopregnant mice is resistant to destruction by anti-thy 1.2 and anti Lyt reagents (Slapsys and Clark 1983), suggesting the decidua-associated suppressor not to be a conventional T suppressor cell. Similar treatment did lower T suppressor function (Nagarkatti and Clark, 1983). In further determining the surface phenotype of the decidual cell, decidual cell preparations were treated with various antisera plus complement (C') prior to co-culturing with cells from virgin PLN. Such antisera treatment has suggested the decidua-associated suppressor activity lacks a Natural killer cell marker - asialo GM_1 (Table 3), a macrophage marker - Mac 1 (Table 4) and a granulocyte, bone marrow marker - granulocyte $GM_{1.2}$ (Table 5). However, treatment of decidual cell preparations with monoclonal antibody against IgG-Fc receptor did eliminate the suppressor activity (Table 6). Therefore, by the criteria of antibody plus complement elimination, the uterine suppressor cell appears to bear no surface markers tested except for the Fc receptor for IgG.

3.2.2 Characterization of Fc receptors on decidua-associated suppressor cells by rosetting technique

Decidual cell suspensions were rosetted with various immunoglobulin coated SRBC as described in Materials and Methods. SRBC

Table 3

Suppressor cell surface markers - anti-asialo GM_1 + C′ treatment

Test cells	CTL Activity per Culture Net x 10^3 ± SEM	
	Expt. 1	Expt. 2
$3x10^6$ VLPN	457 ± 24	4897 ± 213
" + 10^5 decidua-associated cells[a] (untreated)	390 ± 11[b]	1138 ± 57[b]
" + 10^5 decidua-associated cells (C′ only)	N.D.	724 ± 175[b]
" + 10^5 decidua-associated cells (anti-asialo GM_1 + C′)	374 ± 19[b]	699 ± 172[b]

a. Decidua-associated cells were obtained from allopregnant mice in second half of pregnancy and were untreated or exposed to C′ or anti-asialo GM_1 as described in Materials and Methods. Anti-asialo GM_1 + C′ killed approximately 10% of the cells in the decidual cell preparation. No correction was made in cell number tested for altered viabilty caused by treatment.

b. Significant suppression by Student's t test $P < 0.05$.

Table 4

Suppressor cell surface markers - anti- Mac 1 + C′ treatment

Test cells	CTL Activity per Culture $N\alpha tx10^3 \pm SEM$	
	Expt. 1	Expt. 2
$3x10^6$ VPLN	1487 ± 188	3045 ± 169
" + 10^5 decidua-associated cells[a] (untreated)	747 ± 164[b]	826 ± 85[b]
" + 10^5 decidua-associated cells (C′ only)	1125 ± 155	960 ± 76[b]
" + 10^5 decidua-associated cells (anti-Mac 1 + C′)	422 ± 52[b]	1910 ± 60[b]

a. Decidua-associated cells were obtained from allopregnant mice in second half of pregnancy and were untreated or exposed to C′ or anti Mac 1 + C′ as described in Materials and Methods. Anti Macl + C′ killed 15 ± 5% of the cells in the decidual preparation. No correction was made for altered viability caused by treatment.

b. Significant suppression by Student's t-test $P < 0.05$.

Table 5

Suppressor cell surface markers - anti-granulocyte $GM_{1.2}$ + C′ treatment

Test cells	CTL Activity per Culture $Na^{51}Cr \times 10^3 \pm$ SEM	
	Expt. 1	Expt. 2
3×10^6 VPLN	3045 ± 169	2406 ± 131
" + 10^5 decidua-associated cells[a] (untreated)	826 ± 85[b]	1572 ± 91[b]
" + 10^5 decidua-associated cells (C′ only)	906 ± 76[b]	2248 ± 411
" + 10^5 decidua-associated cells (anti-granulocyte $GM_{1.2}$ + C′)	634 ± 77[b]	1298 ± 77[b]

a. Decidua-associated cells were obtained from allopregnant mice in second half of pregnancy and were untreated or exposed to C′ or anti granulocyte $GM_{1.2}$ + C′ as described in Materials and Methods. Anti granulocyte $GM_{1.2}$ + C′ killed 14 ± 2% of the cells in the decidual cell preparation. No correction was made for altered viability caused by treatment.

b. Significant suppression by Student's t-test $P < 0.05$.

Table 6

Suppressor cell surface markers - anti-Fc Receptor + C′ treatment

Test cells ♂	CTL Activity per Culture $N\alpha t x 10^3 \pm SEM$	
	Expt. 1	Expt. 2
$3x10^6$ VPLN	947 ± 73	1685 ± 259
" + 10^5 decidua-associated cells[a] (untreated)	783 ± 50[b]	760 ± 30[b]
" + 10^5 decidua-associated cells (C′ only)	N.D.	1092 ± 51[b]
" + 10^5 decidua-associated cells (anti-FcR + C′)	877 ± 232	1490 ± 45

a. Decidua-associated cells were obtained from allopregnant mice in second half of pregnancy and were untreated or exposed to C′ or anti Fc Receptor + C′ as described in Materials and Methods. Anti-FcR + C′ killed 14 ± 2% of the cells in the decidual cell preparation. No correction was made for altered viability caused by treatment.

b. Significant suppression by Student's t-test $P < 0.05$.

coated with polyclonal IgG rosetted with 25 ± 2% (mean ± SEM) of the recovered decidual population. In contrast, preliminary studies have shown that very few cells obtained from the decidua rosette with IgA-SRBC (1.6%) and IgE-SRBC (0.6%).

Since it has previously been shown that decidual suppressor cell activity correlates with a population of small granulated lymphocytes we examined the percentage of granulated lymphocytes which rosetted with polyclonal IgG and found it to be 88%. In an attempt to separate the rosetted population from the non-rosetted population, we centrifuged the cell suspension on Lympholyte M. The population of decidua-associated cells which rosetted with polyclonal IgG or monoclonal IgG coated SRBC were collected in the pellet and designated Fc receptor positive population (FcR^+) and the non-rosetted cells collected from the interface were designated Fc receptor negative population (FcR^-). The two populations were added separately to MLC cultures to test for suppression of CTL generation. In Table 7, panel A, two representative experiments demonstrate that the suppressor activity is associated with the FcR^+ population of decidual cells. SRBC that had not been coated with antibody failed to bind the the decidua-associated suppressor cells (Table 7, panel B). Thus, in agreement with the data presented in the previous section (3.2.1), the decidua-associated suppressor cell activity appears to be associated with cells bearing FcR for IgG.

3.2.3 Characterization of in vivo suppressor cell recovered from uterine decidua

Since allogeneic pregnancy is usually successful in mothers

Table 7

FcR for IgG on decidua-associated suppressor cells

Test cells	CTL Activity per Culture Nαtx10^3 ± SEM	
A.	Expt. 1	Expt. 2
$3x10^6$ VPLN	1540 ± 115	4249 ± 212
" + 10^5 decidua-associated cells[a] (untreated)	<0[b]	2634 ± 217[b]
" + 10^5 decidua-associated cells rosetted poly-IgG-SRBC interface (FcR^-)	1583 ± 58	3972 ± 410
" + 10^5 decidua-associated cells rosetted poly-IgG-SRBC pellet (FcR^+)	850 ± 35[b]	2968 ± 47[b]
" + 10^5 decidua-associated cells rosetted mono-IgG_1-SRBC interface (FcR^-)	1306 ± 56	
" + 10^5 decidua-associated cells rosetted mono-IgG_1-SRBC pellet (FcR^+)	903 ± 43[b]	

Table 7 (continued)

FcR for IgG on decidua-associated suppressor cells

Test cells	CTL Activity per Culture Natx10^3 ± SEM
B.	
$3x10^6$ VPLN	1417 ± 63
" + 10^5 decidua-associated cells[a] rosetted - no antibody-SRBC interface	771 ± 59[b]
" + 10^5 decidua-associated cells rosetted - no antibody-SRBC pellet no antibody-SRBC pellet	1277 ± 112
" + 10^5 decidua-associated cells rosetted poly-IgG-SRBC interface (FcR^-)	1289 ± 111
" + 10^5 decidua-associated cells rosetted poly-IgG-SRBC pellet (FcR^+)	767 ± 30[b]

a. Decidua-associated cells were obtained from allopregnant mice in second half of pregnancy and were untreated or exposed to uncoated or coated SRBC with polyclonal IgG or monoclonal IgG_1 as described in Materials and Methods. The cells were then centrifuged on Lympholyte and the nonrosetted cells (FcR^-) aspirated from the interface. The rosetted cells (FcR^+) were recovered from the pellet.

b. Significant suppression by Student's t-test $P < 0.05$.

pre-immunized against paternal MHC antigens where sensitized effector cells can be circulating in the blood at the same time of implantation and initial growth of the fetus (Wegmann et al., 1979; Burton and Russell, 1981), we conducted a study to test whether the non-T suppressor cell in decidua was capable of preventing the infiltration of allografts in vivo (Slapsys and Clark, 1983). In our previous work we had demonstrated that in vitro decidua-associated suppressor cell activity is associated with small lymphocyte like cells sedimenting at 3.0 ± 0.5 mm/h at unit gravity (Slapsys and Clark, 1982). To determine if a small cell was capable of suppression of CTL development observed in sponge grafts in vivo, the decidua-associated cells were separated according to size in a StaPut apparatus as outlined in Materials and Methods Section 2.5.1. The cells recovered from the StaPut were pooled into two groups: (1) "Small" cells sedimenting at 2.0-3.9 mm/h and (2) "large" cells sedimenting at 4.0-8.0 mm/h. Sponge matrix grafts were prepared as described in Materials and Methods Section 2.11 and injected with 20 ul of medium (no test cells), "large" cells or "small" cells from pregnant mice (each sponge received an inoculum of approximately 3×10^3 cells). Prior to injection the test cells were treated with anti-thy 1.2 plus complement plus mitomycin C to eliminate T cells in the inoculum that might develop into CTL or secrete lymphokines that can act to recruit circulating CTL into grafts from the circulation (Ascher et al., 1981; Hanto et al., 1982). A second aspect of the experimental design which was important was that sponges that had received each type of treatment were transplanted together to the subcutaneous tissues of a

single recipient. Table 8 shows that significant CTL activity was present in medium treated control sponges so that the presence of putative suppressor cells in other sponges in the same animal did not prevent the immune system from generating CTL. In comparing the ability of "large" and "small" cells to inhibit CTL activity it was found that significant in vivo suppression was associated with "small" lymphocytes sedimenting at rates of less than 3.9 mm/h. Thus we conclude that, similar to the in vitro system, small, non-T suppressor cells in the decidua are capable of inhibiting CTL development in allografts in vivo.

3.3 A study to demonstrate that the uterine decidua-associated suppressor cell is distinct from decidual NK cells.

It has been documented that some types of NK cells may also lack conventional T cell markers (Herberman et al., 1979), contain cytoplasmic granules (Luini et al., 1981), and express FcR (Herberman et al., 1979). In addition, there is evidence to suggest that NK cells may exert immunoregulatory functions in vivo (Abruzzo and Rowley, 1983; Brieva et al., 1984). In view of the similarities between decidua-associated suppressor cells and NK cells, we tested whether the decidua-associated suppressor cell possessed properties of an NK cell.

3.3.1 Size of decidua-associated NK cells.

It has previously been shown that suppressor activity in the decidua and uterine venous blood of mice during the second half of pregnancy is associated with lymphoid cells that have a modal sedimentation of 3 ± 0.5 mm/hr (Slapsys and Clark, 1982). To determine if

Table 8

Size characterization of decidual suppressor cell activity in vivo

Treatment of sponge graft	CTL activity per sponge[a] Nαtx10^3 ± SEM	
Medium alone	1185 ± 225	
"large" decidual cells[b] sedimenting 4.0–8.0mm/h	938 ± 154	N.S.
"small" decidual cells[b] sedimenting 2.0–3.9mm/h	475 ± 133	P < 0.05
		P < 0.05

a CTL activity is obtained using cells pooled from 3 sponges implanted in 3 different animals. P values were obtained using a Student's t Test.

b Cells treated with anti-thy 1.2 serum + complement + mitomycin C before inoculation into sponge graft.

the natural killer cell activity might also be associated with a small lymphoid cell in decidua, decidual cell suspensions were separated by sedimentation velocity. Figure 4 shows the cytolytic activity against Yac-19 targets in the different fractions. It can be seen that NK activity was present in unseparated decidua. However, unlike the distribution of suppressor cell activity, NK cell activity was associated with a larger cell sedimenting at a modal peak of 4 mm/hr. It was unfortunately not possible to obtain enough cells to do both an NK assay and a suppressor activity assay simultaneously. However, the StaPut is sufficiently reproducible that from the repeat studies we have done, NK activity (4 ± 0.5 mm/hr N=6) and suppressor cell activity (3 ± 0.5 mm/h N=8) show a significant difference ($P < 0.05$) in the modal distribution profile.

3.3.2 Effect of NK depletion on decidua-associated suppressor cells.

Tagliabue et al (1982) have shown that the asialo GM_1 marker may be present on some types of NK cells. In treating the decidual cell preparations with anti-asialo GM_1 plus complement we were able to ablate 63% of the NK cytotoxicity recovered from the decidua. Similar treatment of a spleen cell suspension eliminated 89% of the NK activity. In contrast, as presented in section 3.2.1 Table 3, the suppressor activity in the decidua was resistant to treatment with anti-asialo GM_1 and complement.

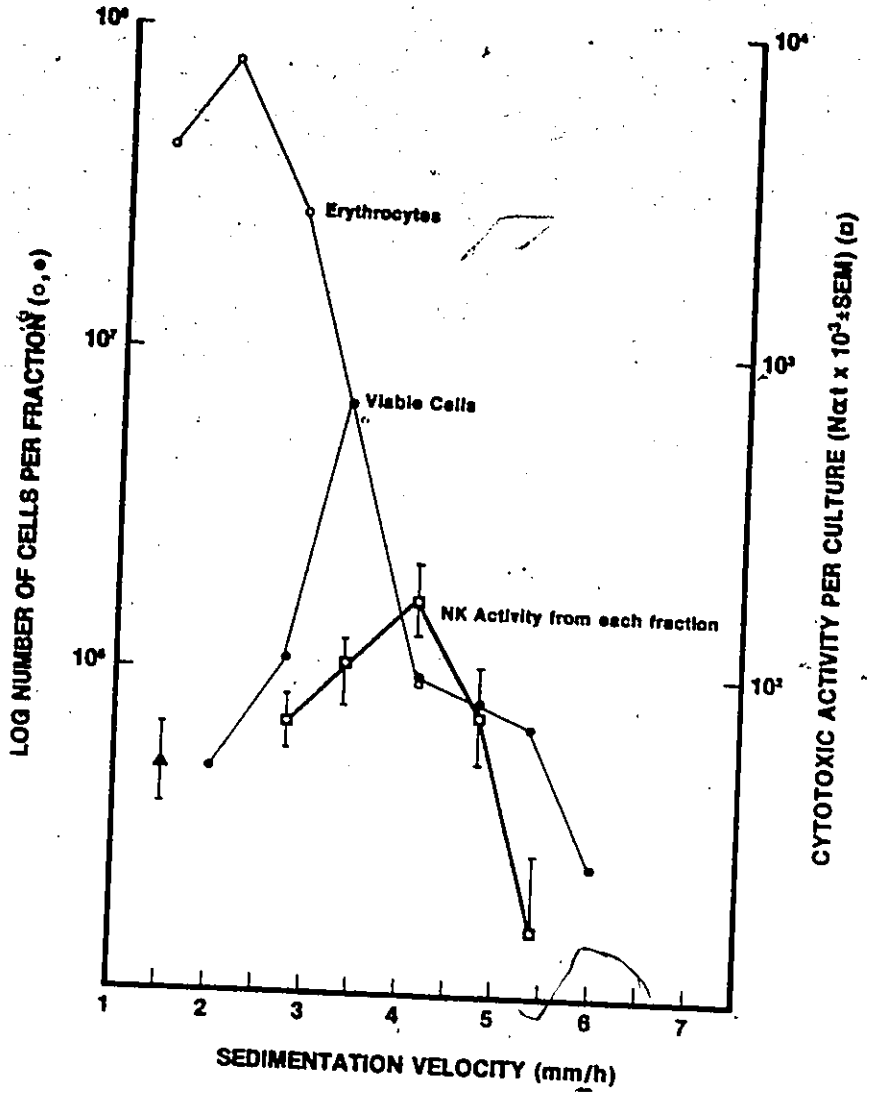
It has been previously reported that only 31% of NK cell activity associated with mucosa of the GI tract and about 83% of splenic NK activity possesses the asialo GM_1 marker (Tagliabue et al., 1982). Not surprisingly, complete elimination of decidua-associated NK acti

Figure 4

NK activity of cells recovered from the decidua

Decidua-assocoiated cells were obtained from mice 12.5 - 18.5 days after mating, centrifuged over Lympholyte M , and separated in fractions of different sizes by velocity sedimentation. NK cell activity of each fraction was assessed against YAC-19 tumor line at various effector-to-target ratios (□). The viable nucleated cell number (●) and erythrocyte number (o) per fraction is also shown. An insufficient number of cells sedimenting (>5.5 mm/hr) was recovered to permit testing.

Figure 4


Erythrocytes
Viable Cells
NK Activity from each fraction
LOG NUMBER OF CELLS PER FRACTION (o, •)
CYTOTOXIC ACTIVITY PER CULTURE (Nat x 10³±SEM) (□)
SEDIMENTATION VELOCITY (mm/h)
10⁸
10⁷
10⁶
10⁴
10³
10²
1
2
3
4
5
6
7

vity with anti-asialo GM_1 was not achievable. Therefore we tested bg/bg mice that are genetically deficient in NK cell activity (Roder et al., 1977). Table 9 Experiment 1 documents a lack of detectable NK activity in both the spleen and decidua of these mice mated to DBA/2 males in contrast to the presence of measurable NK activity recovered from the spleen and decidua of allopregnant C3H mice. Nevertheless, decidua-associated cells removed from the uteri of allopregnant bg/bg mice proved capable of suppressing CTL generation in 3 of 3 experiments. Experiment 2 in Table 9 is a representative example demonstrating the presence of suppressor cell activity in bg/bg decidua but not in the spleen. Since previous studies demonstrated a correlation between suppressor cell activity and small granulated lymphocytes (Slapsys and Clark, 1982; Slapsys and Clark, 1983), we prepared stained slides of our decidua preparation and compared the percentage of granulated cells in the decidua from NK deficient bg/bg to that in normal C3H/HeJ mice. The percentage of small lymphocytes that were granulated in allopregnant bg/bg was 33 ± 5%, a value similar to that found using allopregnant C3H/HeJ mice. Thus, both suppressor cell activity and granulated small lymphocytes were present in the pregnant bg/bg uterus in the absence of detectable NK cell activity. Thus, in taking into account all the available data, the non-T suppressor cells in the decidua appear to represent a distinct cell population and are not "regulatory" NK cells.

3.4 Suppressor cell activity of decidua during secondary allopregnancy.

To test decidua-associated suppressor cell activity of multip-

Table 9

Suppressor cell activity and NK activity in allopregnant bg/bg mice

Cells cultured	NK activity[a] Net x 10^3 ± SEM	CTL activity Net x 10^3 ± SEM
Expt. 1		
Allopregnant bg/bg spleen cells	0	
Allopregnant bg/bg decidua-associated cells	0	
Allopregnant C3H spleen cells	706 ± 261	
Allopregnant C3H decidua-associated cells	248 ± 70	
Exp. 2		
3×10^6 VPLN/bg/bg		536 ± 61
+ 10^5 bg/bg spleen cells		460 ± 116
+ 10^5 bg/bg decidua-associated cells		40 ± 10[c]

a NK activity was measured against ^{51}Cr-labelled Yac-19 target cells with a range of effector cell to target cell (E:T) ratio of 30:1 to 4:1. Cytotoxic activity was calculated as previously described.

b CTL was generated in vitro by culturing virgin PLN from bg/bg mice with 10^6 irradiated C3D2F1 stimulator cells. CTL were measured 5 days later against ^{51}Cr-labelled target cells.

c Significant suppression $P < 0.05$ by Student's t test.

arous mice, decidua was obtained from the second half of pregnancy of C3H/HeJ females undergoing their second allopregnancy and tested for its ability to suppress CTL generation of virgin PLN cells in a CML. The data in table 10, Experiment 1 shows that decidual suppressor activity was recoverable from the decidua of monoparous pregnant mice, which suppressed the in vitro generation of CTL in a dose response manner. Experiment 2 demonstrated that the suppressor cell activity was resistant to anti-Thy 1.2 plus complement treatment. Thus, similar to primary pregnancy, there appears to be an accumulation of non-T suppressor cells in the uterus of mutiparous mice which may protect the developing fetus against maternal immune rejection.

3.5 Anatomical localization of decidua associated suppressor cell activity

In an attempt to determine if the decidua-associated suppressor cell activity was concentrated at particular sites in the pregnant uterus, the uterus was initially divided into two components; 1) the implantation site and 2) the tissue between the implantation sites. Each component was then tested for its ability to suppress CTL generation in vitro. The number of viable cells recovered from each site was comparable (data not shown). However, the degree of suppression was significantly greater at the implantation site compared to the tissue tested between implantation sites (73 ± 4.5% suppression vs 53 ± 4.1% suppression respectively (mean ± SEM, $P < 0.05$ by Student's t test). We have previously reported that during successful pregnancy there is a correlation between suppressor cell activity and small lymphocytes with cytoplastic granules (Slapsys and Clark, 1982;

Table 10

Decidua-associated suppressor cell activity in multiparous female mice

Test Cells	CTL Activity per Culture Natx10^3 ± SEM
Expt. 1.	
$3X10^6$ VPLN	1823 ± 85
" + $1X10^6$ decidua-associated cells[a]	0 [b]
	P < 0.05
" + $5X10^5$ decidua-associated cells	361 ± 42[b]
	P < 0.05
" + $1X10^5$ decidua-associated cells	1201 ± 116[b]
Expt. 2.	
$3X10^6$ VPLN	2853 ± 124
" + 10^5 decidua-associated cells (untreated)	560 ± 31[b]
" + 10^5 decidua-associated cells (C' only)	2005 ± 97[b]
" + 10^5 decidua-associated cells (anti-Thy 1.2 + C')	1315 ± 38[b]

a Decidua-associated cells were obtained from the second half of pregnancy of mice undergoing their second allogeneic pregnancy.

b Significant suppression by Student's t test P < 0.05 compared to virgin PLN controls.

Slapsys and Clark, 1983). In examining the percentage of granulated lymphocytes for each uterine site, it was found that 44 ± 3% (mean ± SEM) of the small lymphocytes were granulated at the implantation site compared to 12 ± 2% in the tissue between implantation sites. Subsequent to this finding, the implantation site was further divided into 1) decidual capsule, the spongy cushion upon which the placenta rests, and 2) the tissue below the capsule representing the deeper layers of the decidua and associated myometrium. The data in Table 11 shows the suppressor cell activity to be concentrated at the implant site, but was most potent below the decidual capsule. It could also be seen that the percent granulated small lymphocytes was greatest in the deeper uterine tissues where the suppressor activity was greatest (Table 11).

3.6 Xenopregnancy

A xenopregnancy model was developed to test the possible relevance of the described local non-specific immune suppression to spontaneous abortion. This model provides a system of pregnancy failure in which fetal death consistently occurs and is associated with maternal immune reactivity. Xenopregnancy involved the transfer of Mus caroli embryos into the uteri of hormonally primed Mus musculus. The Mus caroli is a wild species of mouse from South East Asia which is genetically distinct from the laboratory mouse, Mus musculus, (Sutton and McCallum, 1972) with no interbreeding. When day 2 embryos from Mus caroli are transferred into the uteri of Mus musculus, they appear normal until 9.5 days of pregnancy but are resorbed by 15 days (Croy et al., 1982). Death is preceded by

Table 11

Suppressor cell activity at variable sites within the uterus

Test Cells	CTL Activity per culture Nαt X 10^3 ± SEM	% Granulated small Lymphocytes
3 X 10^6 VPLN	9602 ± 712	
+ decidual capsule	1910 ± 551 (80%)[a]	15%
+ below decidual capsule	541 ± 57 (95%)	31%
+ between implant sites	3493 ± 194 (63%)	12.5%

a Numbers in parentheses show percent suppression compared to virgin control

infiltration with maternal lymphoid cells that bear thy 1.2^+, $Lyt1^+2^+$ of the maternal allotype and lyse Mus caroli target cells in short term ^{51}Cr-release assay in vitro (Croy et al., 1982). It can be seen from figure 5 that Mus caroli embryos still die when viable Mus musculus embryos are present in the same uterus or even within the same uterine horn. Thus the failure of xenogeneic embryos to survive is not due to a failure of the necessary physiological conditions for pregnancy in Mus musculus (Croy et al., 1982).

3.6.1 Comparison of cell yields from interspecies and intraspecies pregnancies

Table 12 summarizes the results from six experiments in which the cell yield from the decidua in uterine horns bearing viable xenogeneic Mus caroli embryos was compared with the yield from the opposite horns bearing viable Mus musculus embryos, despite the normal morphology of the embryos at this stage. The reduction in average yield proved to be 44.2 $\pm$ 7.5% (mean $\pm$ SEM) from the Mus caroli implant sites in six experiments and was statistically significant ($P < 0.01$ by Student's t test). The reduction was not due to excess cell loss during isolation on Lympholyte M, because the cell yield before purification was 42% of the intraspecies control in experiment 5 and 48.4% in experiment 6. Because previous cell separation studies demonstrated a correlation between suppressor cell activity and the presence of small lymphocytes with cytoplasmic granules (Slapsys and Clark, 1982), stained slides were prepared of decidual cells and compared the percentage of granulated cells in the decidua associated with Mus caroli embryos with that seen in the cell population isolated

Figure 5

Uteri of C3H/HeJ mice on day 10.5 gestation.

Upper uterus contains Mus musculus embryos in left horn and resorbing Mus caroli embryos in right horn. The lower uterus containing a mixture of 2 Mus caroli embryos (arrow) and 2 Mus musculus embryos in the right.

Figure 5

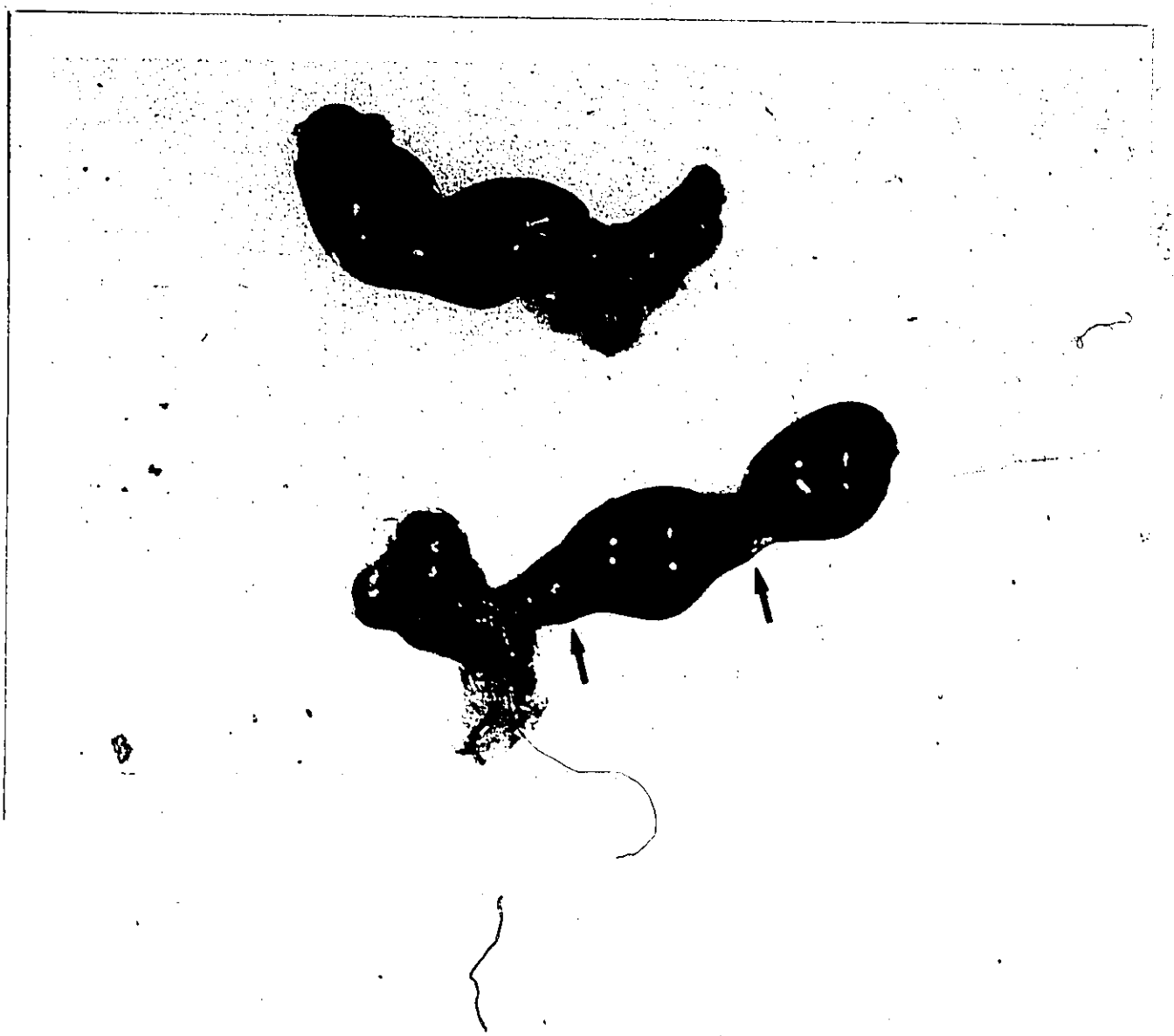


Table 12

Cell yield from decidua of uteri bearing interspecies and intraspecies conceptuses

Expt.	Genotype of Mother	Genotype of Conceptus	Number of implants	Cell yield per implant site
1	C3H/HeJ	Ha(ICR) X Ha (ICR)	5	16×10^3
		Mus caroli X *Mus caroli*	3	8×10^3
2	Ha(ICR)	Ha(ICR) X Ha(ICR)	5	40×10^3
		Mus caroli X *Mus caroli*	4	7×10^3
3	Ha(ICR)	C3H/HeJ X C3H/HeJ	5	54×10^3
		Mus caroli X *Mus caroli*	5	13×10^3
4	Ha(ICR)	Ha(ICR) X Ha(ICR)	20	47×10^3
		Mus caroli X *Mus caroli*	23	28×10^3
5	Ha(ICR)	Ha(ICR) X Ha(ICR)	24	48×10^3
		Mus caroli X *Mus caroli*	19	25×10^3
6	C3H/HeJ	Ha(ICR) X Ha(ICR)	5	46×10^3
		Mus caroli X *Mus caroli*	5	28×10^3

from the opposite horn containing Mus musculus embryos. Of the decidua-associated small lymphocytes isolated from Mus caroli only 8% were granulated lymphocytes. In contrast, the decidua cell suspension obtained from the horn containing Ha(ICR) X Ha(ICR) syngeneic embryos contained small lymphocytes of which 30% were granulated. This difference was statistically significant ($P < 0.005$) and observed in six experiments.

3.6.2 Assessment of suppressor activity recovered from the decidua of inter and intraspecies pregnancies

To examine whether the reduction of granulated cells correlated with a reduction in suppressor cell activity in the decidua associated with xenogeneic embryos, the recovered decidual cells were added to MLC cultures in vitro. Table 13 demonstrates that, in agreement with previous data, cells from decidua bearing intraspecies embryos (line 2, Expt. 1) significantly suppressed the generation of CTL in the MLC reaction. However, decidual lymphoid cells isolated from the contralateral uterine horn that contained viable Mus caroli embryos of the similar stage of development as the Mus musculus embryos failed to suppress CTL generation (line 3). Experiment 2 illustrates the result from a similar experiment in which C3H/HeJ pseudopregnant mice received allogeneic Ha(ICR) X Ha(ICR) xenogeneic Mus caroli X Mus caroli blastocysts in opposite uterine horns. As in experiment 1, decidual lymphoid cells obtained from the uterine horn bearing allogeneic embryos proved suppressive, whereas cells obtained from the opposite horn containing xenogeneic embryos failed to suppress CTL generation. Table 14 demonstrates that the decidua-

Table 13

Suppressor cell activity of cells recovered from uteri bearing allo and xeno conceptuses

Expt.	Test Cells[a]			Cytotoxic Activity Nαt X10^3 ± SEM
	Genotype of mother	Genotype of conceptus in uterine horn		
1	no test cells added			684 ± 67
	Ha(ICR)	Ha(ICR) X Ha(ICR)	(10^5)[b]	383 ± 31[c]
		Mus caroli X Mus caroli	(10^5)	626 ± 44
	C3H/HeJ	C3H X DBA/2	(10^5)	367 ± 15[c]
2	no test cells added			760 ± 82
	C3H/HeJ	Ha(ICR) X Ha(ICR)	(10^5)[b]	519 ± 83[c]
		Mus caroli X Mus caroli	($7X10^4$)	776 ± 50
	C3H/HeJ	C3H X DBA/2	(10^5)	400 ± 119[c]

a Decidua associated test cells were treated with anti-Thy 1.2 + C[1] as described in Materials and Methods to remove any allogeneic T cells that might interfere with C3H/HeJ anti DBA/2 MLC reaction. Dedicual cells from C3H/HeJ mice mated 9.5 days previously with DBA/2 mates (treated with anti-Thy 1.2 + C') were included as a positive control.

b The number in parentheses equals the number of test cells added to the MLC.

c Significant reduction in CTL yield compared with control ($P < 0.05$ by Student's t test).

Table 14

Decidua-associated suppressor cell activity recovered from Mus caroli mated to Mus caroli

Test cells	CTL Activity per culture Nαt x 10^3 ± SEM
3 x 10^6 Mus caroli Virgin spleen cells	1940 ± 69
+ 10^5 decidua-associated cells[a]	1206 ± 41[c]
+ 10^5 decidua-associated cells[b]	1006 ± 75[c]

a decidua-associated cells obtained from day 9.5 of pregnancy of Mus caroli ♀ mated to Mus caroli ♂

b decidua-associated cells obtained from day 13.5 of pregnancy of Mus caroli ♀ mated to Mus caroli ♂

c Significant suppression by Student's t test $P < 0.05$.

associated cells recovered from Mus caroli mated Mus caroli pregnancies possess suppressor activity accompanied by an accumulation of 30 ± 2% granulated small lymphocytes.

3.7 Role of the trophoblast in establishing suppressor cell activity in the decidua

Whilst the xenoblastocyst (Mus caroli) initially remains viable upon transfer into the Mus musculus uterus, it nevertheless inevitably dies and is resorbed as the Mus musculus pregnancy progresses. However, it has been demonstrated that Mus caroli can develop and gestate into viable offspring within the Mus musculus when the Mus caroli inner mass is enveloped in Mus musculus trophoblast. In contrast, Mus musculus inner cell mass enveloped in Mus caroli trophoblast and transferred into a Mus musculus pseudopregnant recipient fails (Rossant and Frels, 1980). Thus, it appears that matching the trophoblast genotype with that of decidua plays a crucial role in the outcome of pregnancy. With this information, we examined the role of trophoblast in establishing nonspecific suppressor cell activity in the decidua. With the acquisition of a C3H murine placentally-derived choriocarcinoma line (Be6 cells) (Beeson et al., 1984) we were able to test for the role of the trophoblast in recruiting or activating local uterine suppressor cells.

3.7.1. Properties of the Be6 trophoblast cells

It has been shown that in addition to the fetal component of a 13 to 15 day murine placenta, 30% of the placental cells are of maternal origin (Rossant and Croy, 1985). Descriptive studies have suggested that decidual cells and maternal macrophages may infiltrate

the spongiotrophoblast (Bell, 1983). In the absence of any tissue-specific marker which can unequivocally identify trophoblast in the murine placenta, we can only assess the trophoblastic nature of a trophoblast cell line by demonstrating known trophoblastic properties. Beeson et al. reported that the Be6 choriocarcinoma cell line is of placental origin (versus maternal origin) from the evidence that the cell line expressed a paternal X-linked enzyme isotype when congenic mice were mated, which carried different electrophoretic variants of phospho-glycerate kinase (PGK-1) (Beeson et al., 1984). In addition enzyme analysis revealed an increased level of a trophoblast marker, alkaline phosphatase (by 50X) in the Be6 line compared to two fibro-sarcoma lines RD-1024 and CR-80 (Beeson et al., 1984). Other enzyme analysis studies also strongly suggest that the Be6 cell line is tro-phoectodermal in origin in that enzymes essential for progesterone synthesis may be detected in the Be6 line. Placental progesterone is synthesized from maternal plasma cholesterol in the pathway outlined in Figure 6. One requirement for the synthesis of progesterone from pregnenolone is oxygen and reduced NAD^+ as a cofactor (Schulster et al., 1976) and the second requirement involves two enzymes: $^3\beta$-hydroxy-steroid dehydrogenase and microsomal isomerase. In enzyme histochemical assays the detection of NADH diaphorase and $^3\beta$-hydroxy-steroid dehydrogenase in the Be6 line as seen in Figure 7 as granules in the cytoplasm indicates the potential for steroid synthesis which is lacking in other tumor lines such as the P19.

Five million Be6 cells injected subcutaneously into irradiated C3H/HeJ mouse (300-400 rads) resulted in a solid tumor after several

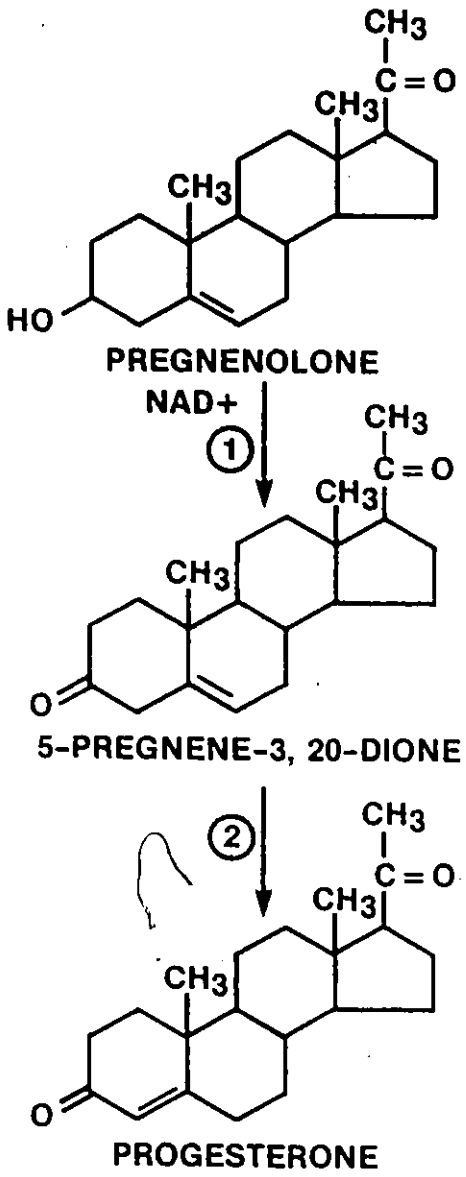
Figure 6

Biosynthesis of progesterone from pregnenolone

1. $^{3}\beta$-hydroxy-steroid dehydrogenase

2. Microsomal isomerase

Figure 6


CH3
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PREGNENOLONE
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1
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5-PREGNENE-3, 20-DIONE
2
CH3
C=O
CH3
CH3
O
PROGESTERONE

Figure 7

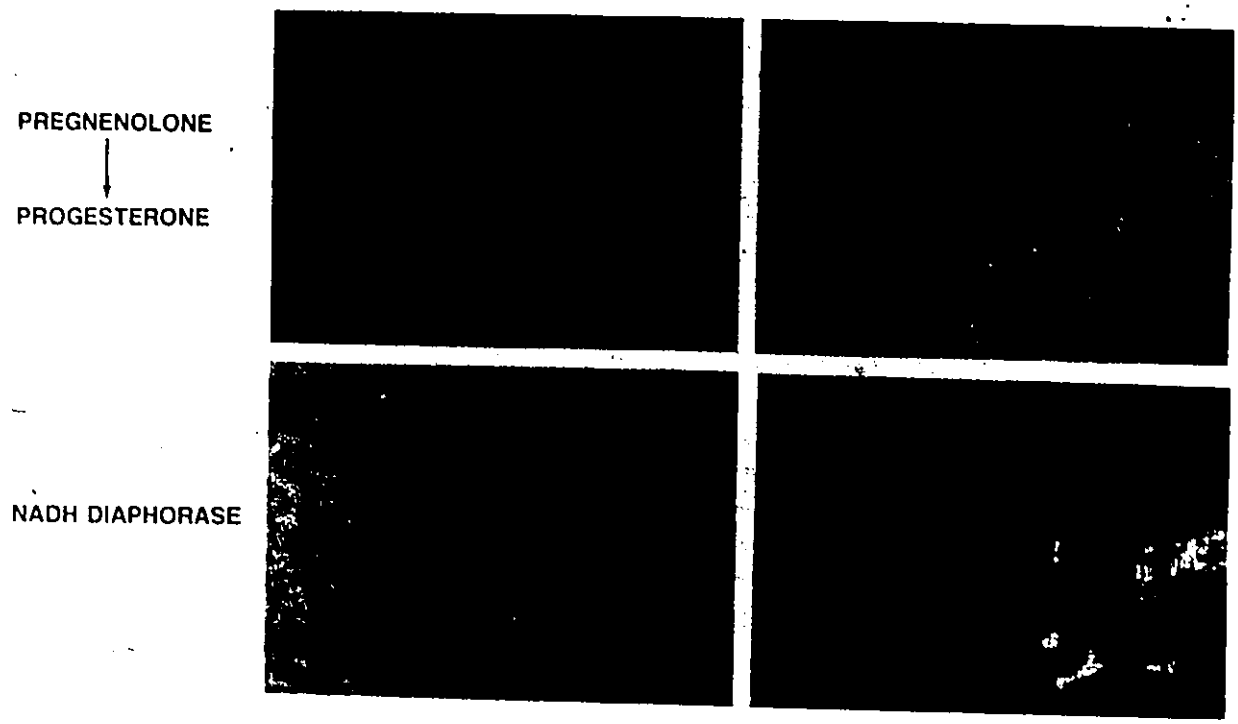
Enzyme histochemical assay for progesterone synthesis

A granules - positive stain for $^{3}\beta$-hydroxy-steroid dehydrogenase in Be6 cell line.

B $^{3}\beta$-hydroxy-steroid dehydrogenase-negative stain in P19 cells.

C Positive stain for NADH diaphorase in Be6 cell line compared to panel D in P19 cells.

Figure 7



months. Histologically the tumor was composed of small cells and giant cells (see Figure 8), similar to trophoblastic giant cells (Rossant and Tamura-Lin, 1981). In addition, Loke (1986) has shown that trophoblast cells possess an epithelial characteristic, in that it bears cytokeratin. Therefore, a cytokeratin immunohistochemical assay was used to examine the Be6 cell line (as described in Materials and Methods, section 2.16.1.2). Figure 9 demonstrates that the Be6 cell line did bind anticytokeratin antibody further demonstrating the Be6 cell line to be of a trophoblastic nature.

3.7.2 The role of the Be6 choriocarcinoma line in recruiting suppressor activity to the uterine decidua

As discussed in section 3.7 the genotype of the trophoblast appears to be critical in fetal survival. With a trophoblastic-like cell line (Be6) we examined its ability to recruit the small granulated lymphocytic suppressor cells to the uterus. As described in Materials and Methods, section 2.16.3, various stimuli were injected into the uterine lumen of pseudopregnant recipient female mice. After six days the cells were harvested from the uterus, separated according to size on a StaPut apparatus, and each fraction tested for its ability to suppress CTL generation. Figure 10 demonstrates that the recruitment of a small lymphocytic suppressor cell, sedimenting at 2-4 mm/h was achieved using the Be6 choriocarcinoma line. A similar pattern was seen in the suppressor activity recovered from the decidua of day 10.5 allopregnant C3H as seen in Figure 11. In contrast neither as seen in Figures 12, 13 and 14, oil, nor P815 nor the C3H embryonal carcinoma line P19 respectively led to the

Figure 8

Photomicrographs of Be6 tumor grown in C3H/HeJ mice.

Be6 tumor (x100) showing giant cell (A) development.

Figure 8

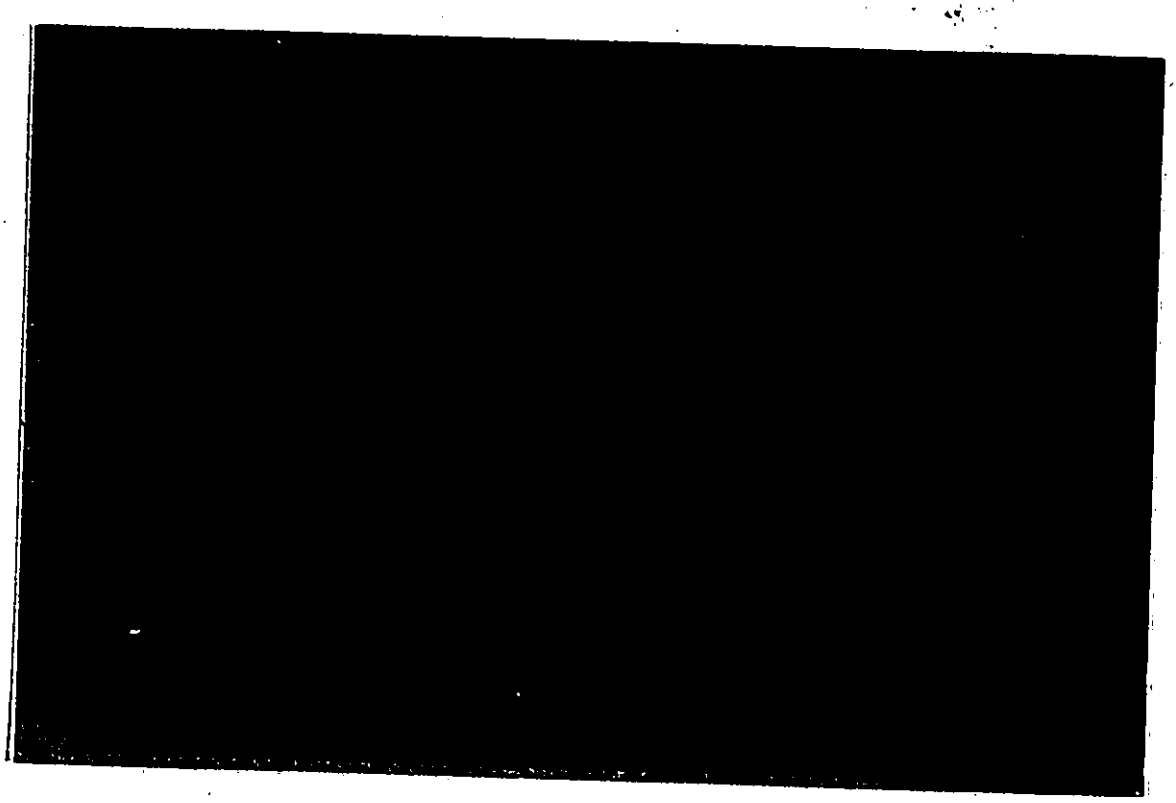


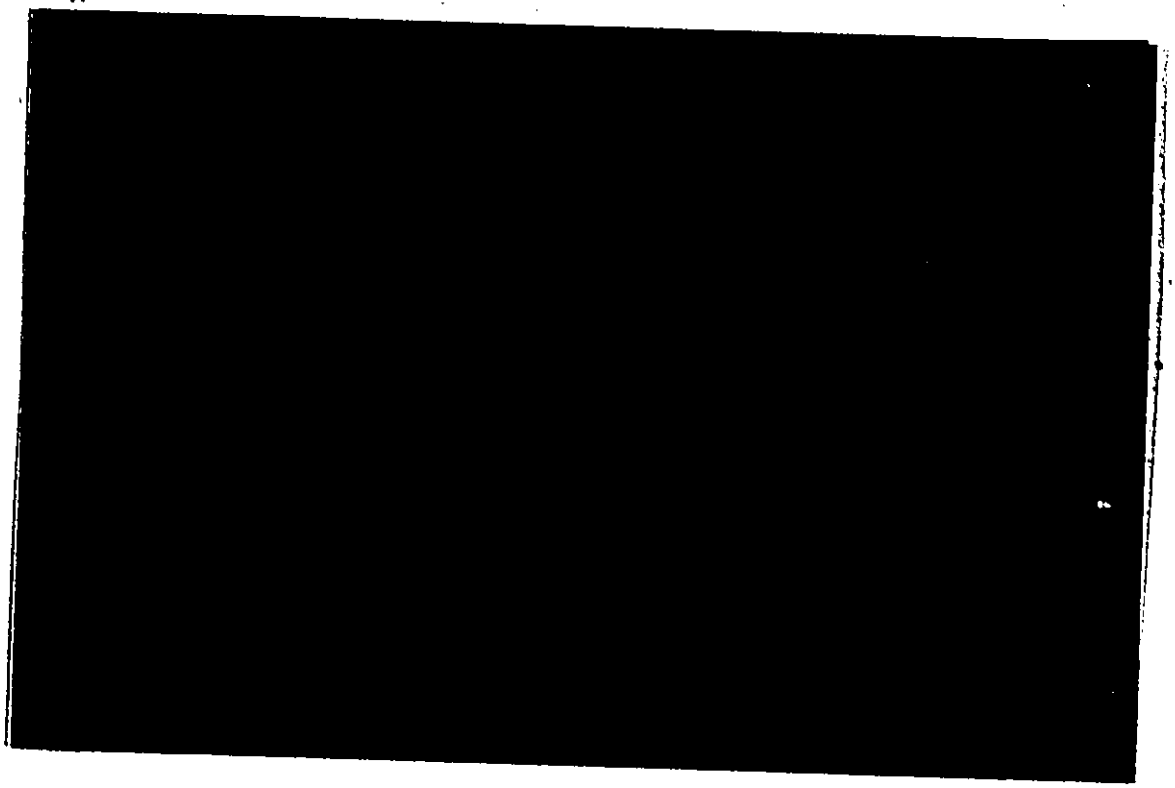
Figure 9

Immunohistochemical assay for cytokeratin in Be6 cell line

Panel A is a positive stain for cytokeratin compared to negative control, panel B.

Figure 9

A



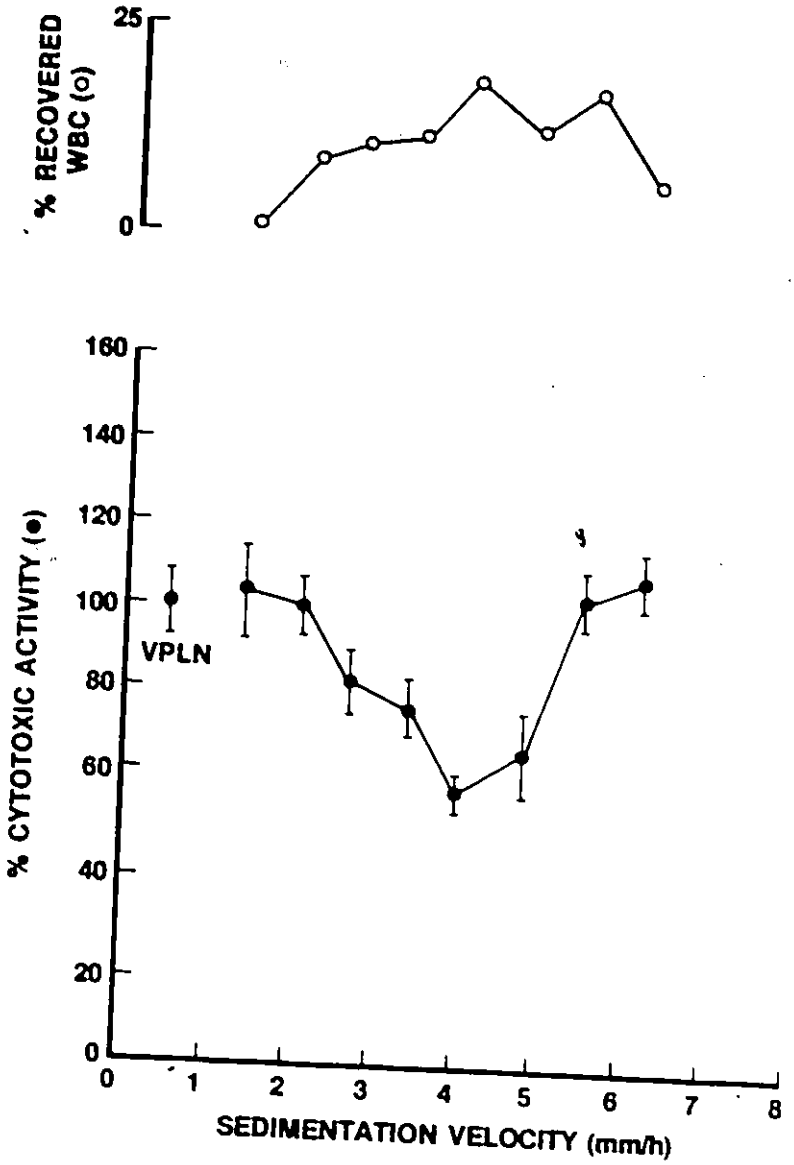
B

Figure 10

Velocity sedimentation and separation of cells from uterine decidua having received the Be6 cell line stimuli

Decidual cells obtained from pseudopregnant mice 6 days afer receiving the Be6 cell line intrauterine stimuli and separated into fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (•). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 10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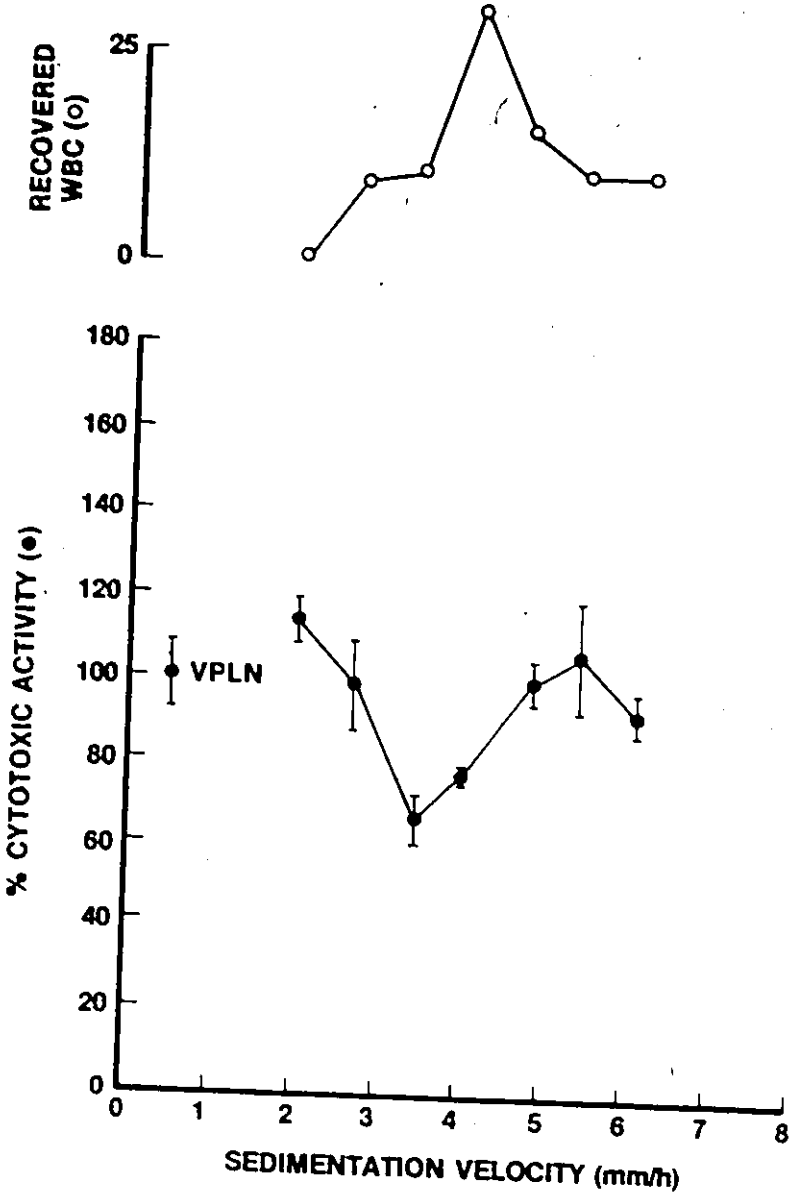
8
SEDIMENTATION VELOCITY (mm/h)

Figure 11

Velocity sedimentation and separation of cells from uterine decidua undergoing successful allopregnancy

Decidual cells obtained from day 10.5 allopregnant C3H/HeJ mated DBA/2 and separated into fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 11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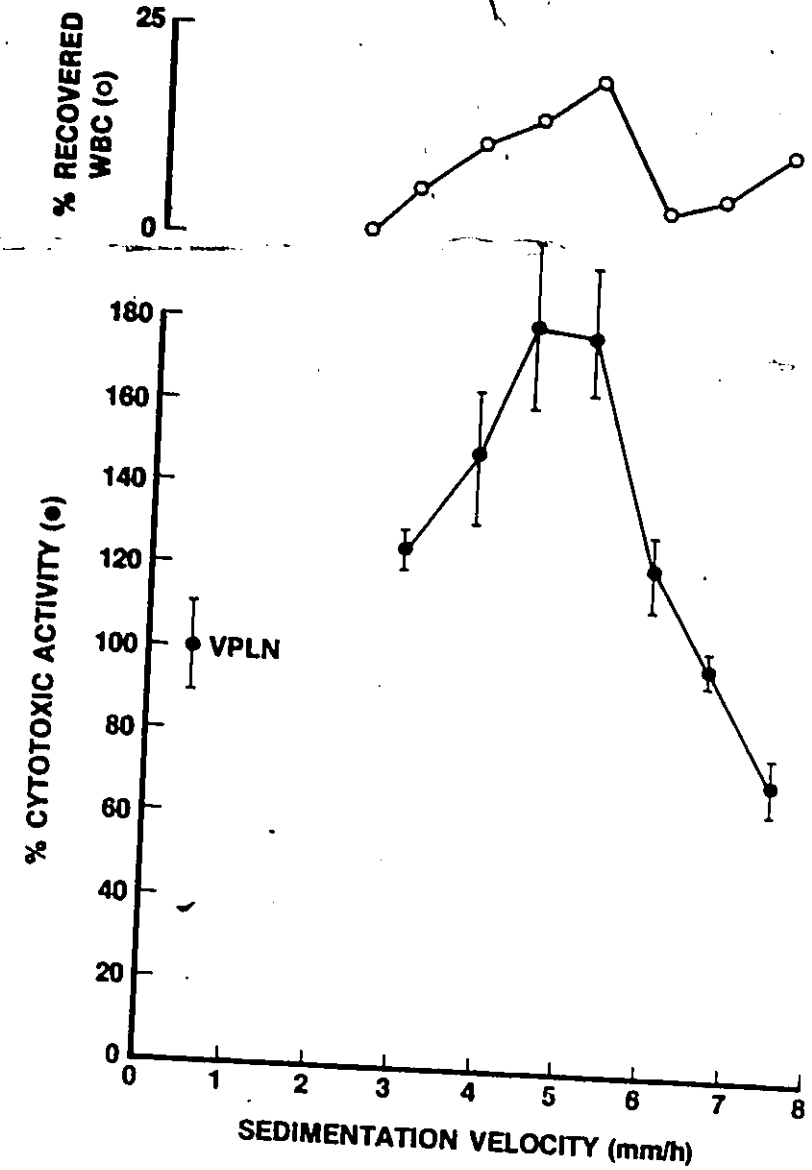
SEDIMENTATION VELOCITY (mm/h)

Figure 12

Velocity sedimentation and separation of cells from uterine decidua having received oil stimuli

Decidual cells obtained from pseudopregnant mice 6 days afer receiving the oil intrauterine stimuli and separated into fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 12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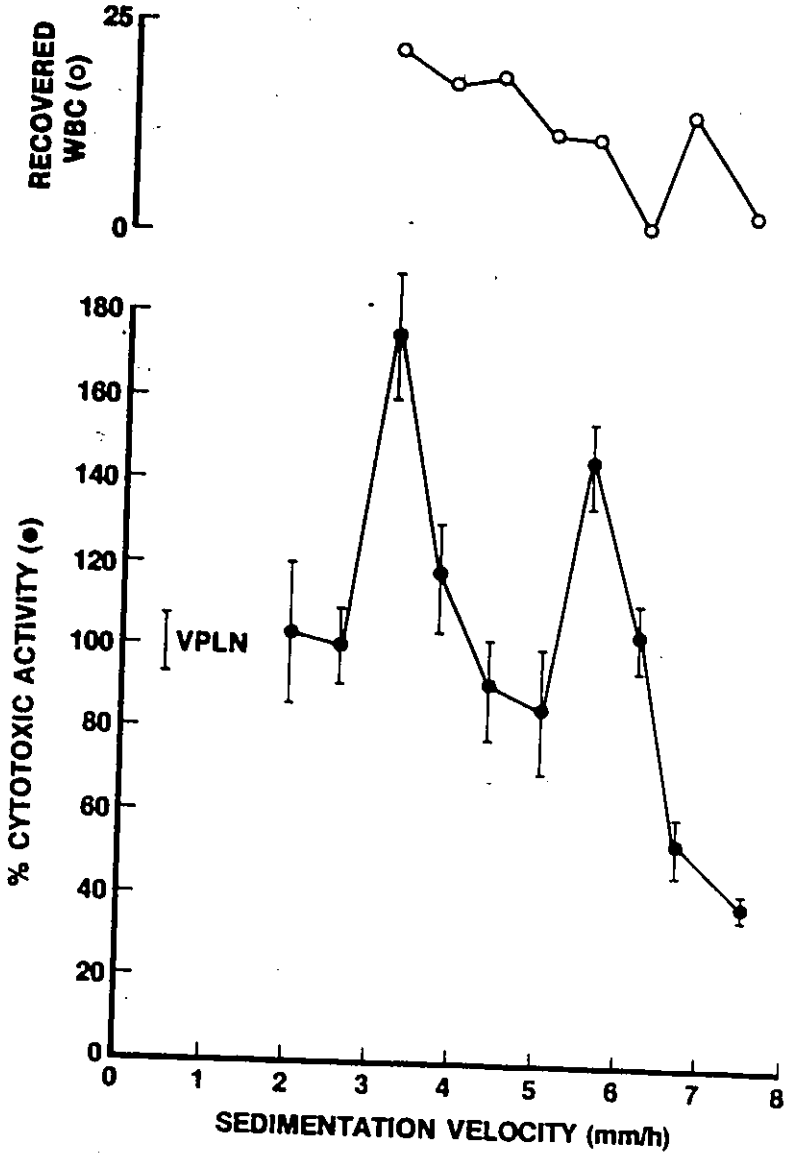
SEDIMENTATION VELOCITY (mm/h)

Figure 13

Velocity sedimentation and separation of cells from uterine decidua having received the P19 stimuli

Decidual cells obtained from pseudopregnant mice 6 days afer receiving the P19 cell line intrauterine stimuli and separated into fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 13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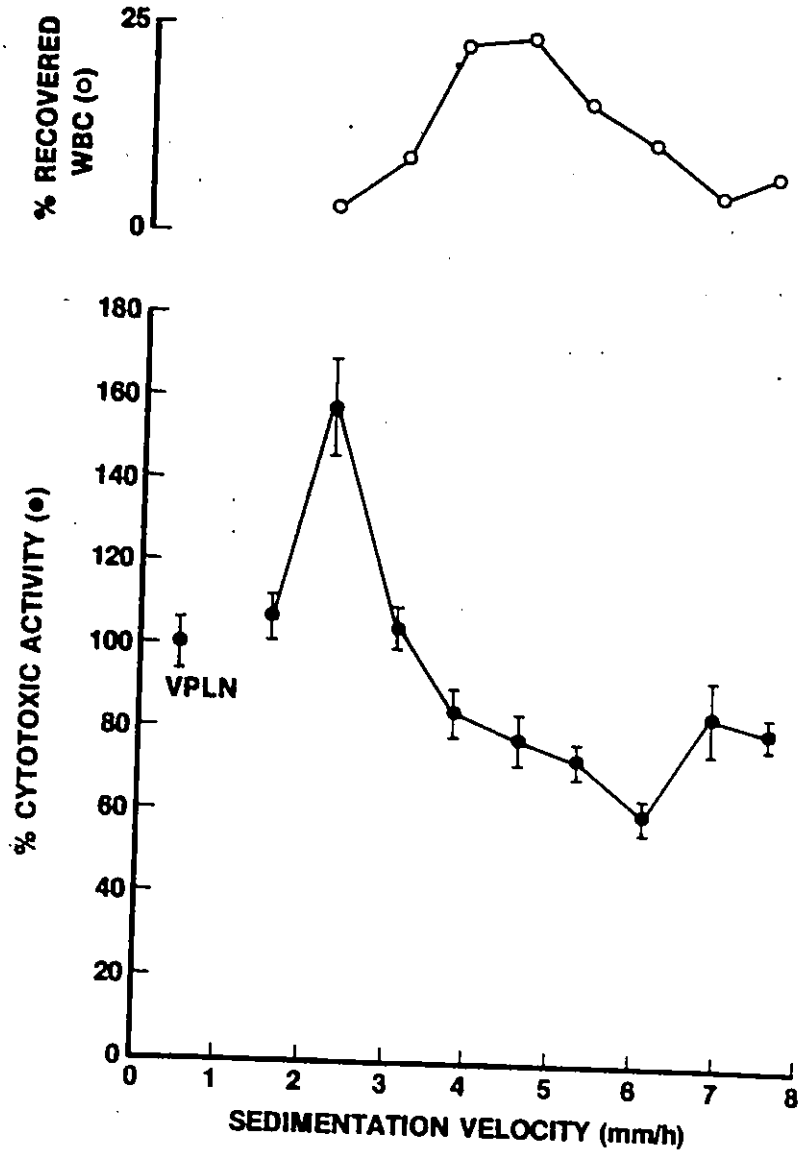
SEDIMENTATION VELOCITY (mm/h)

Figure 14

Velocity sedimentation and separation of cells from uterine decidua having received the P815 cell line stimuli

Decidual cells obtained from pseudopregnant mice 6 days afer receiving the P815 cell line intrauterine stimuli and separated into fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 14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SEDIMENTATION VELOCITY (mm/h)

appearance of granulated small lymphocytes with suppressor activity. The latter stimuli did lead to the appearance of a suppressor cell distinctly larger than the previously described decidua-associated suppressor cell, sedimenting at >6 mm/h. Preliminary characterization of the larger suppressor cell recovered from pseudopregnant animals, showed it to be resistant to treatment with anti-thy 1.2 + C' and anti-Mac 1 + C' (Table 15). In addition, when percent granulated small lymphocytes was enumerated it was found that the trophoblastic Be6 line recruited significantly more small granulated cells compared to the non-trophoblastic stimuli (Figure 15).

In the Mus caroli system it can be seen from Figure 15 that when Mus caroli females were mated to Mus caroli males the resulting allopregnant decidua yielded 30 ± 1% (mean ± SEM) small granulated lymphocytes similar to the percent recovered from allopregnant decidua of Mus musculus. When the suppressor activity of the Mus caroli decidua was tested, significant suppression was recovered as shown in Table 14. However, when the Mus musculus Be6 choriocarcinoma line was injected into the uterus of pseudopregnant Mus caroli, it failed to recruit small granulated cells, possibly demonstrating a species restriction in the trophoblast-decidua interaction. This could not be tested in the reciprocal direction due to lack of availability of a Mus caroli placental-trophoblast line.

3.7.3 The effect of injecting Be6 cell line supernatants into uterine lumen

Supernatants were prepared from the Be6 choriocarcinoma cell line, P815 mastocytoma line and day 9.5 ectoplacental cone as des-

Table 15

"Large" suppressor cell surface markers - anti-thy 1.2 and anti-Mac 1 plus complement treatment

Test cells	CTL Activity per culture Nαt x 10^3 ± SEM
Expt. 1.	
3 x 10^6 VPLN	4656 ± 477
" + 10^5 decidua-associated cells[a] (untreated)	414 ± 19[b]
" + 10^5 decidua-associated cells (anti-thy 1.2 + C′)	686 ± 25[b]
Expt. 2.	
3 x 10^6 VPLN	1564 ± 267
" + 10^5 decidua-associated cells (untreated)	582 ± 24[b]
" + 10^5 decidua-associated cells (anti-Mac 1 + C′)	318 ± 16[b]

a Decidual cells were obtained from pseudopregnant animals receiving oil intrauterine stimuli and were untreated or treated with anti-thy 1.2 + C′ or anti-Mac 1 + C′ as described in Materials and Methods. Anti thy 1 + C′ killed approximately 10% of the cells in the decidua and anti-Mac 1 + C′ killed approximately 22% of the cells. No correction was made for altered viability.

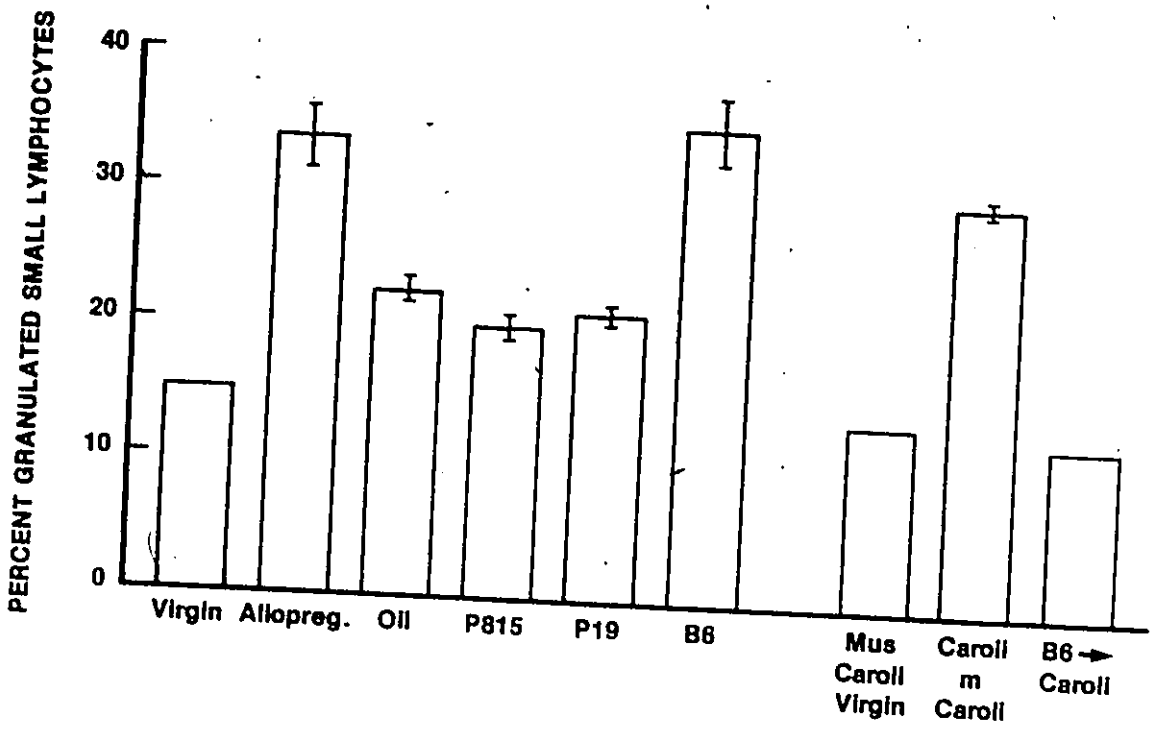
b Significant suppression by Student's t test $P < 0.05$.

Figure 15

Percent small granulated cells recovered from pseudopregnant animals receiving various intrauterine stimuli

Percent granulated cells enumerated by Diff-Quick staining of cytocentrifuge smears of decidual suspensions recovered from pseudopregnant animals.

Figure 15


PERCENT GRANULATED SMALL LYMPHOCYTES
40
30
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10
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Virgin
Allopreg.
Oil
P815
P19
B6
Mus Caroli Virgin
Caroli m Caroli
B6 → Caroli

cribed in Materials and Methods, sections 2.16.2 and 2.16.4. On the third day of the hCG (human chorionic gonadotropin) hormone regime, 20 μl of supernatant was injected into the uterine lumen of allopregnant mice along with 10 μl of peanut oil to ensure decidualization. On the eighth day of the hormone schedule the animals were boosted with 20 μl of supernatant in the same uterine horn as injected previously. Decidual cells were harvested after 6 days and examined for suppressor cell activity and percent granulated small lymphocytes. As shown in Figure 16, the Be6 trophoblastic line supernatant and the day 9.5 ectoplacental cone supernatant recruited significantly more small lymphocytes to the uterus compared to P815 supernatant.

Figure 17 shows that a suppressor cell sedimenting at 3 mm/h is recovered from the decidua of animals receiving Be6 trophoblastic supernatant. In addition, there also appears to be suppressor activity associated with a larger cell sedimenting at >5mm/h. This may represent the same uncharacterized cell discussed in the previous section which is apparent in pseudopregnant animals receiving the non-trophoblastic stimuli. Figure 18 demonstrates the ability of day 9.5 ectoplacental-cone supernatant in recruiting a small suppressor cell sedimenting at 3 mm/h to the uteri of pseudopregnant animals.

3.7.3.1 Characterization of the cell recovered from the decidua of animals stimulated with Be6 supernatant

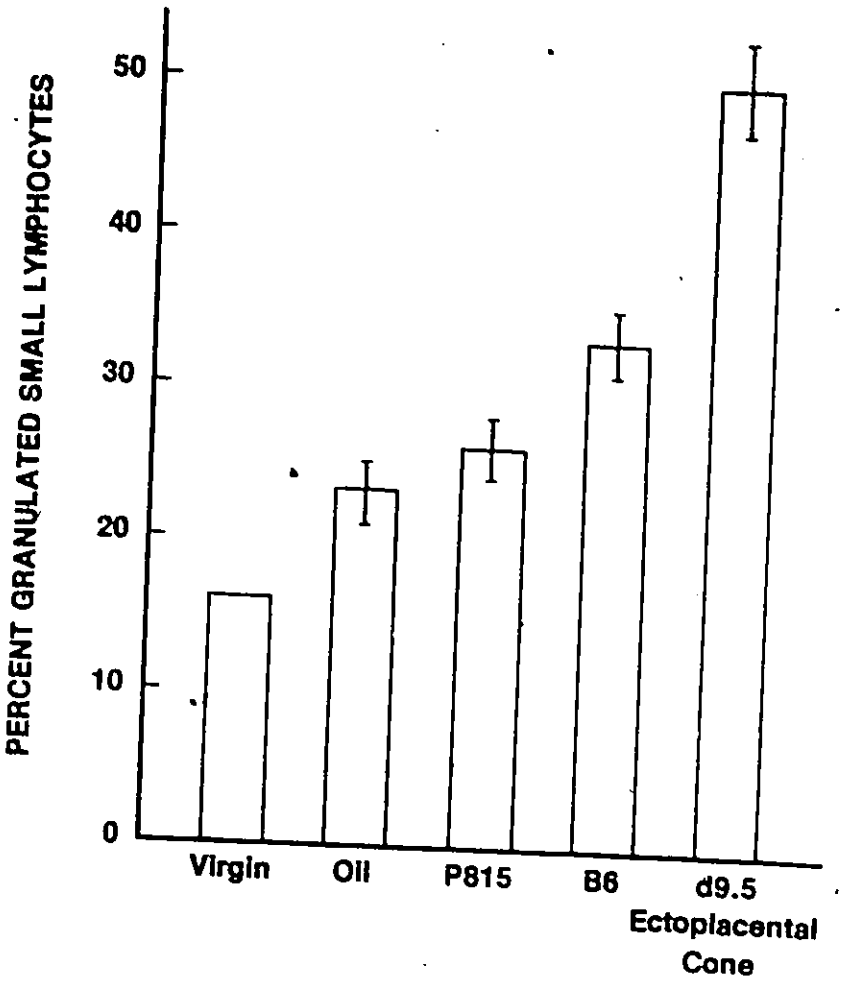
Since it has been previously shown that the decidua-associated suppressor cell recovered from allopregnant mice is resistant to anti-T cell antisera plus C′ treatment (Slapsys and Clark, 1983), we examined the sensitivity of the suppressor activity recovered from the

Figure 16.

Percent small granulated lymphocytes recovered from pseudopregnant animals receiving supernatants of various intrauterine stimuli

Percent granulated cells enumerated by Diff-Quick staining of cytocentrifuge smears of decidual suspensions recovered from pseudopregnant animals.

Figure 16


PERCENT GRANULATED SMALL LYMPHOCYTES
50
40
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Virgin
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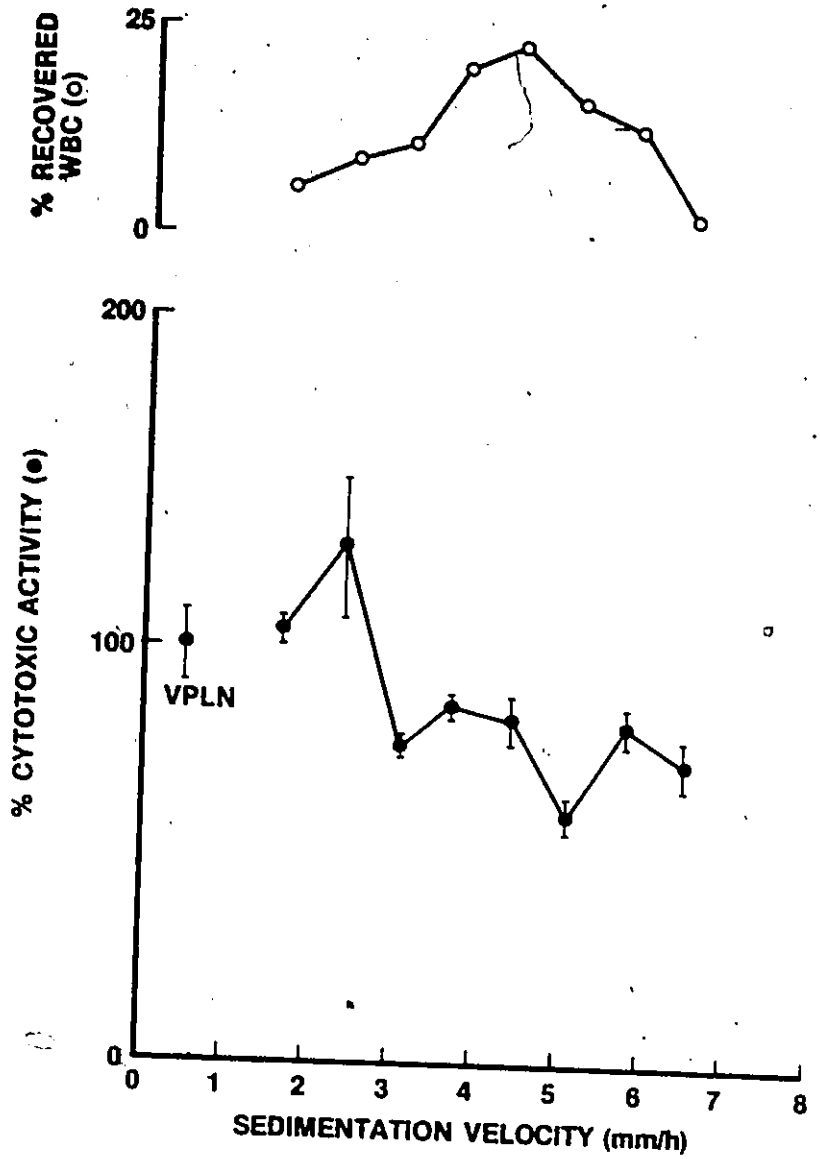
P815
B6
d9.5
Ectoplacental
Cone

Figure 17

Velocity sedimentation and separation of cells recovered from nu/nu uterine decidua having received Be6 supernatant

Decidua-associated cells obtained from athymic pseudopregnant mice 6 days after receiving Be6 supernatant intrauterine stimuli and separated in fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 17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SEDIMENTATION VELOCITY (mm/h)

pseudopregnant uterus stimulated with the Be6 cell line supernatant. Table 16 is a representative experiment demonstrating the resistance of the suppressor-activity from pseudopregnant animals which received Be6 supernatant to anti-thy 1.2 + C′ treatment, suggesting that the trophoblastic cell line is capable of recruiting a similar cell to that described during successful allopregnancy.

In addition, to substantiate the suppressor cells non-T cell nature Table 17 demonstrated that decidua-associated suppressor activity may be recovered from T cell deficient or nu/nu pseudopregnant females when stimulated with Be6 supernatant. Upon examination of the % granulated small lymphocytes in prepared decidual cell suspensions it was found that the pseudopregnant nu/nu females which received Be6 supernatants contained 28 ± 2% granulated small lymphocytes compared to 6 ± 1% from pseudopregnant nu/nu females not receiving the Be6 stimulation. Also, in confirmation, with previous reports, size characterization of the decidua-suppressor activity isolated from nu/nu, Be6 stimulated mice was associated with a small lymphocyte sedimenting at 3 mm/h. as seen in Figure 19. Therefore, it appears that a factor(s) elaborated by the trophoblast is important for the recruitment of a population of suppressor cells which are small, non-T, granulated lymphocytes to the decidua of pseudopregnant females similar to the decidua-associated suppressor cells described in successful allopregnancy.

To recapitulate, the studies reported in this chapter have shown that during successful pregnancy there is an accumulation of a novel type(s) of suppressor cell(s) in the uterus of allopregnant

Table 16

Suppressor cell surface marker – anti-Thy1.2 + C′ treatment

Test cells	CTL activity per culture Nαt x 10^3 ± SEM
3 x 10^6 VPLN	256 ± 7
" + 10^5 decidua-associated cells[a] (untreated)	56 ± 5[b]
" + 10^5 decidua-associated cells (C′ only)	N.D.
" + 10^5 decidua-associated cells (anti-Thy1.2 + C′)	53 ± 8[b]

a Decidua-associated cells were obtained from pseudopregnant C3H/HeJ females stimulated with Be6 supernatant and were untreated or exposed to anti-Thy 1.2 + C′ treatment as described in Materials and Methods. No correction was made for altered viability caused by treatment.

b Significant suppression by Student's t-test $P < 0.05$.

Table 17

Decidua-associated suppressor activity of nu/nu mice

Test cells	CTL activity per culture Not x 10^3 ± SEM
3×10^6 VPLN	805 ± 30
" + 10^5 nu/nu virgin uterine cells	1399 ± 36
" + 3×10^5 nu/nu mouse decidua-associated cells[a]	78 ± 8[b]
" + 1×10^5 nu/nu mouse decidua-associated cells	124 ± 9[b]

a Decidua-associated cells were isolated from nu/nu pseudopregnant mice having received Be6 supernatant.

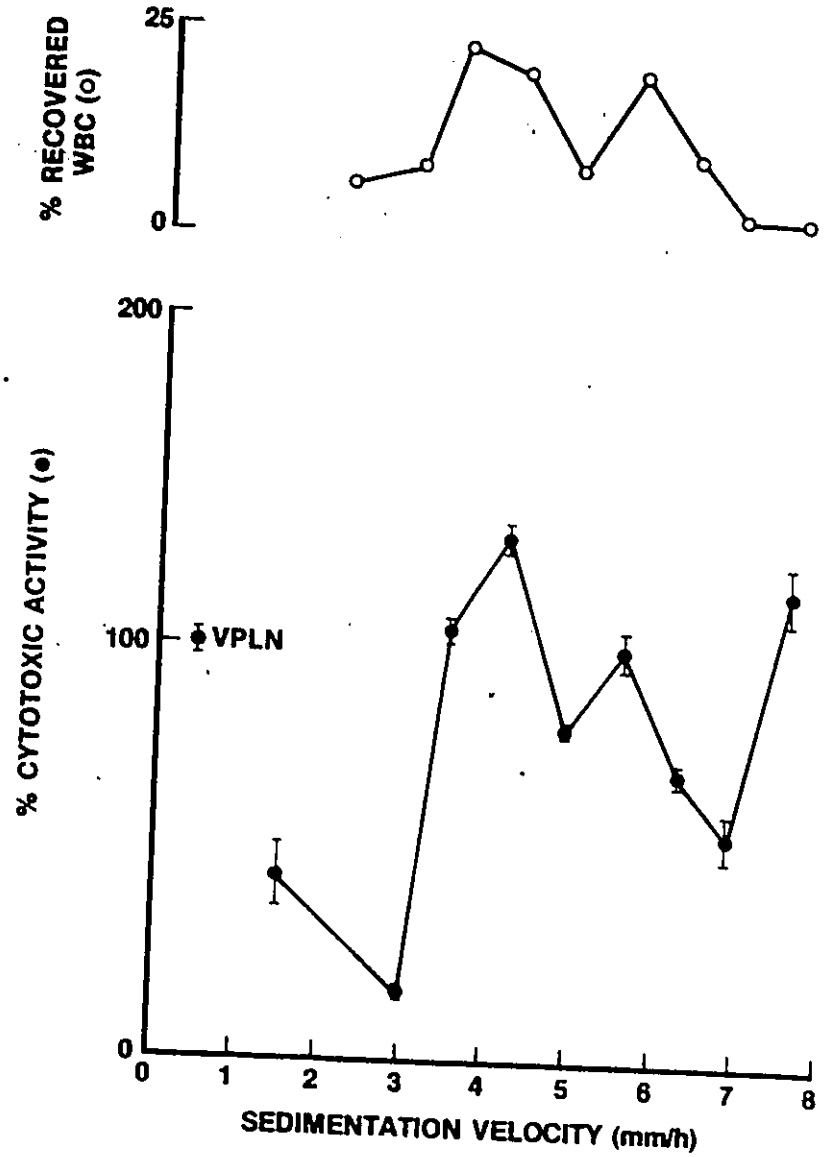
b Significant suppression by Student's t-test $P < 0.05$.

Figure 18

Velocity sedimentation and separation of cells recovered from uterine decidua having received day 9.5 ectoplacetal cone supernatant

Decidua-associated cells obtained from pseudopregnant mice 6 days after receiving day 9.5 ectoplacental cone supernatant intrauterine stimuli and separated in fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 18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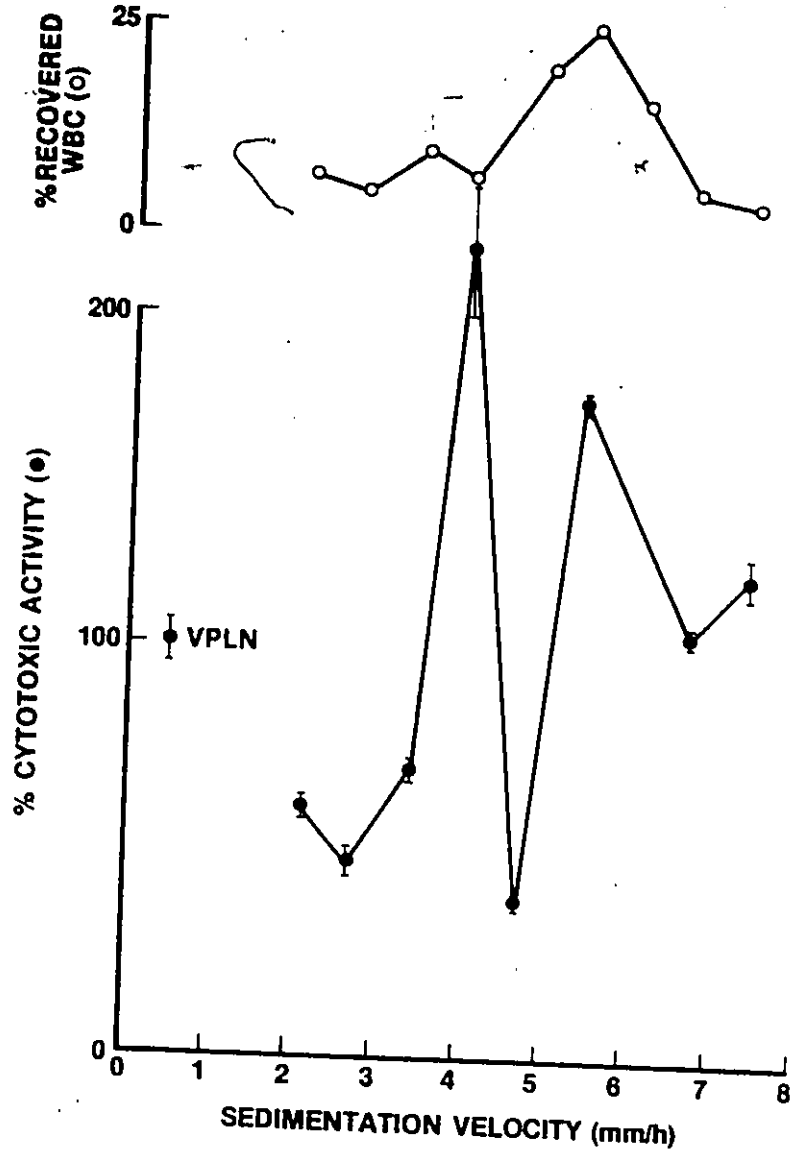
SEDIMENTATION VELOCITY (mm/h)

Figure 19

Velocity sedimentation and separation of cells recovered from uterine decidua of nu/nu mice that received Be6 supernatant

Decidua-associated cells obtained from nu/nu pseudopregnant mice 6 days after receiving Be6 supernatant intrauterine stimuli and separated in fractions of different sizes by velocity sedimentation. 10^5 cells of each fraction was then tested for its ability to suppress CTL generation (●). Percent viable cell numbers recovered per fraction (o). Percent CTL activity of each fraction against ^{51}Cr-labelled P815.

Figure 19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SEDIMENTATION VELOCITY (mm/h)

mice. Characterization of the decidua-associated suppressor cell has shown the cell to bear no positive surface markers tested (i.e. T-cell, macrophage, gramulocyte) except for the FcR. The mechanisms responsible in the recruitment or triggering of the decidua-associated suppressor activity remain unknown, although trophoblast factors have been shown to play a critical role in the accumulation of the described suppressor activity.

Chapter 4

Discussion

Discussion

The murine pregnancy provided a convenient model for studying the immunology of the feto-maternal interface. Mechanical isolation of the maternal uterine decidual cells provided a reliable technical tool by which we could examine their immunoregulatory capacity of cytotoxic events. There were two major findings. Firstly, aborting pregnancies fail to accumulate decidua-associated suppressor cells in the uterine decidua. Secondly, the appearance of decidua-associated suppressor cells is dependent upon a decidua-trophoblast interaction.

To recapitulate, successful murine pregnancy is associated with the presence of non T, small lymphocyte-like cells (sedimenting at 3.0 ± 0.5 mm/h) which appear to contain eosinophilic granules in the scant cytoplasm. Further characterization studies outlined in this thesis reveal the decidua-associated suppressor population to be devoid of conventional cell markers for macrophages (Table 4) and granulocytes (Tables 3 and 5) in addition to T cell markers (Slapsys and Clark, 1983). In contrast, the decidua-associated suppressor population did appear to bear FcR for immunoglobulin IgG (Tables 6 and 7). The significance of FcR on the suppressor population remains to be established. One may only speculate that immunoglobulin molecules of the IgG class may be involved in triggering the suppressor population. Fc mediated immunoregulation has been described for T cell and B cell mediated immunosuppression (Panoskaltsis and Sinclair, 1986). Further, blocking antibodies of the IgG class have been eluted from the maternal-placental interface by a number of independent labora-

tories (Voisin and Chaouat, 1974; Wegmann et al., 1979). There is evidence that the abortion rate can be decreased in particular stain matings (ie. CBA x DBA/2) when an appropriate IgG immunoglobulin is generated by vaccination (Chaouat et al, 1985) suggesting immunological significance of immunoglobulins in association with the decidua-associated suppressor cell.

Hopt et al. (1980) have shown that sensitized lymphocytes were preferentially recruited to allografts bearing the sensitizing alloantigen in mice. They were also able to show that unsensitized lymphocytes were also recruited to the graft site. This observation has been confirmed by Hanto et al. (1982) who have reported lymphocyte recruitment to a graft site, which may be mediated by the interaction of sensitized cells and alloantigen, resulting in the elaboration of lymphokines and increasing the accumulation of other sensitized and nonsensitized cells. However circulating sensitized CTL generated by immunizing females against paternal MHC allo-antigens does not threaten normal fetal development (Wegmann et al. 1979). In addition, NK cells have also been demonstrated in the decidua during pregnancy (Table 9; Croy et al., 1986). The demonstration of cytotoxic cells during pregnancy raises the question of whether cytotoxic cells possess a potential threat to the developing embryo. Since NK cells have been shown to selectively lyse target cells bearing embryonic differentiation antigens (Gidlund et al., 1981; Stern et al., 1980) such cells may in fact be capable of exerting harmful effects on the implanting embryo. Chang and Tanaka (1982) have reported that splenic NK cells can kill trophoblast cell lines in vitro. This observation is similar to that of Georgina Smith (1983) who showed that fresh

trypsinized trophoblast cells could be lysed by MHC-sensitized lymphocytes. Kreck et al. (1982) have reported that small lymphocytes infiltrate the trophoblast giant cell layer of the mouse spongiotrophoblast on day 10.5 of pregnancy and that greater infiltration was seen in allopregnant mice compared to syngeneic matings. It is of interest that we find maximum suppressor cell activity in the decidua at this time (Slapsys and Clark, 1983), at a developmental point when paternal H-2 antigens are expressed by the placenta as detected by the binding of circulating antibody (Raghupathy et al., 1981). Croy et al. (1982) have shown that xenogeneic Mus caroli embryos that implant in the uterus of Mus musculus females survive until day 9.5 of pregnancy where upon they are infiltrated by CTL and undergo necrosis and resorption by 12.5 days of pregnancy. The sponge graft data in Table 8 suggests that a small, non-T granulated suppressor population in the decidua may prevent the infiltration of the fetal allograft by sensitized CTL in vivo. Therefore the recruitment of non-T, granulated suppressor cells to the implant site may be an important immuno-regulatory mechanism to protect the fetal allograft from cell mediated immune rejection.

The mechanism of cytotoxic inhibition has been shown to be via a soluble factor isolated form the non-T suppressor population which blocks the IL-2 response of both CTL and an IL-2 dependent cloned NK cell line in vitro. (Clark et al., 1985), possibly by inhibiting the induction and function of the IL-2 receptor. (This issue is currently being investigated). Bulmer and Johnson (1986) reported that although T lymphocytes were abundant in first trimester human decidua, none expressed the IL-2 receptor. Thus, localization of non-T suppressor

cells in the decidua should be able to protect the fetal graft by blocking NK cell activation and generation of lymphokine activated killer cells (Grimm et al., 1982; Grimm et al., 1983) by IL-2. Indeed, NK cell activity in decidua appears to decline progressively between days 6.5 and 9.5 of pregnancy (Gambel et al., 1985) as suppressor cell activity is increasing (Slapsys and Clark, 1983).

The significance of local uterine suppressor cells in protecting the fetal allograft from rejection is supported by studies using failing pregnancy models. Firstly, it has been reported that suppressor activity is absent from the DLN of allopregnant CBA mice that resorb their litters (Clark et al., 1980; Chaouat et al., 1983; Chaouat et al., 1985). Similar findings in mice resorbing their litters have also been reported by Smith (1981). It is interesting that the decidua of CBA strain mice appears to be deficient in the small Fc-receptor positive cell population (Kirkwood, 1981) that has been associated with a soluble suppressor activity and in decidua-associated suppressor cell activity (Clark et al 1986b).

Secondly, xenopregnancy in sheep-goat, horse-donkey and Mus musculus - Mus caroli systems show a high rate of embryo failure associated with infiltration of the fetus by maternal cytotoxic lymphoid cells (Clark, 1984; Hancock et al, 1968; Croy et al, 1982). Immunization of the Mus musculus female against Mus caroli lymphoid cells accelerated the rate of "rejection" suggesting an immunological mechanism. It was of tremendous interest in that, in the Mus caroli - Mus musculus model system, viable Mus caroli fetuses could be obtained by enveloping the inner mass of the Mus caroli in a trophoblast of the Mus musculus type (Rossant et al., 1983). In contrast Mus musculus

inner cell mass that was successful when left within Mus musculus trophoblast underwent necrosis and resorption when enveloped in Mus caroli trophoblast (Rossant et al., 1983). This observation could be explained by firstly, incorrect xeno-matching between trophoblast and decidua that might lead to abnormal growth and development of the trophoblast/placenta. And secondly, species-restricted trophoblast-decidua interactions that may lead to failure to recruit local non-T suppressor cells. In support of the latter hypothesis we found that at day 9.5 of pregnancy the xenopregnancy embryo is viable and appears completely normal despite the dramatic deficiency of uterine suppressor cells (Table 13). However by 10.5 days of pregnancy, the Mus caroli embryo begin to die and the conceptuses became infiltrated by maternal cytotoxic cells.

Acquisition of a trophoblast cell line (Be6) has provided some direct evidence for a recruiting role of trophoblast in a species restricted fashion. Padykula et al. (1978) and Bulmer and Johnson (1985) have shown a lymphoid infiltrate some of which are granulated cells in association with maternal endometrial glands which have acquired fetal trophoblast antigens in early human pregnancy. It is tempting to postulate that maternal cells (ie. endometrial gland cells) modify their cellular antigens, possibly via hormonal control (Natalli et al., 1981; Natalli et al., 1984) to encourage recruitment of the non-specific, non-T granulated decidua-associated suppressor cell. It now appears that trophoblast soluble factors, elaborated in vitro by the trophoblast cell line and ectoplacental cone possess the ability to signal the decidua for the development of suppressor cells at the implantation site.

It has been demonstrated that suppressive activity can be measured in human decidua using soluble products elaborated by this tissue in culture (Golander et al., 1981), and using lymphocytes isolated from first trimester decidua (Daya et al., 1985). Further experiments are necessary to determine to what extent local activity in human pregnancy reflects the findings in murine pregnancy.

Conclusions

In conclusion, the presence or absence of a novel population of non-specific non-T granulated suppressor lymphocytes in the uterine decidua correlates closely with susceptibility to maternal immunity and the outcome of pregnancy. The fetal trophoblast appears to play a key role in the generation of this popultion of suppressor cells in the decidua. The precise cellular and molecular events underlying suppressor cell recruitment remain to be determined. The understanding of the mechanisms by which decidua-associated suppressor cells are regulated could have practical applications in dealing with problems of human pregnancy failure.

Bibliography

Abruzzo, L.V., and Rowley, D.A. Homeostasis of the antibody response. Immunoregulation by NK cells. Science 222:581, 1983.

Allen, W.R. Maternal recognition of pregnancy and immunological implications of trophoblast - endometrium interactions in equids. In: Maternal Recognition of Pregnancy (ed. Whelan, J.) Ciba Foundation Symposium 64, Excerpta Medica, New York p. 323-353, 1979.

Alter, B.J., Bach, F.H. Speculations on alternative pathways of T lymphocyte response. Scand. J. Immunol. 10:87-92, 1979.

Andersen, R.H., and Monroe, C.W. Experimental study of behaviour of adult skin homografts during pregnancy. Am. J. Obstet. Gynecol. 84:1096-1101, 1962.

Ansell, J.D., McDougall, C.M., Speedy, G., and Inchley, C.J. Changes in lymphocyte accumulation and proliferation in the lymph nodes draining the pregnant uterus. Clin. Exp. Immunol. 31:397-407, 1978.

Antczak, D.F. Antibodies to lymphocyte alloantigens produced during equine pregnancy. 4th International Congress of Immunol., Paris, Abstr. No. 16501, 1980.

Ascher, N.L., Chen, S., Hoffman, R., and Simmons, R.L. Maturation of cytotoxic effector cells at the site of allograft rejection. Transplant Proc. 13:1105-1107, 1981.

Badet, M.T., Bell, S.C., and Billington, W.B. Immunoregulatory activity of supernatants from short term cultures of mouse decidual tissue. J. Reprod. Fert. 68:351-358, 1983.

Bailey, K., Herrod, H.G., Younger, R. and Shaver, D. Functional

aspects of T-lymphocyte subsets in pregnancy. Obset Gynecol. 66:211-215, 1985.

Baines, M.G., Pross, H.F., Millar, K.G. Effects of pregnancy in the maternal lymphoid system in mice. Obst. Gynecol. 50:457-461, 1977.

Baines, M.G., Speers, E.A., Pross, H., and Millar, K.G. Characteristics of the maternal lymphoid response of mice to paternal strain antigens induced by homologous pregnancy. Immunol. 31:363-369, 1976.

Barg, M., Burton, R.C., Smith, J.A., Luckenbach, G.A., Decker, J., and Mitchell, G.F. Effects of placental tissue on immunological responses. Clin. Exp. Immunol. 34:441-448, 1978.

Barnes, R.D., and Holliday, J. The morphologic identity of maternal cells in newborn mice. Blood 36:480-490, 1970.

Beer, A.E., and Billingham, R.E. Host responses to intrauterine tissue, cellular and fetal allografts. J. Reprod. Fertil. (Suppl.) 21:59-88, 1974.

Beer, A.E., and Billingham, R.E. Maternal immunological recognition mechanisms during pregnancy. In Maternal Recognition of Pregnancy, (ed. Whelan J), CIBA Foundation, Symposium 64, Excerpta Medica, Amsterdam, 293-323, 1979.

Beer, A.E., Billingham, R.E. and Hoerr, R.A. Elicitation and expression of transplantation immunity in the uterus. Transplant. Proc. 3:609-611, 1971.

Beeson, J., Daynes, R., Wernet, A., Gahring, L. Tumors arising from injection of cultured murine placental cells into normal

animals: Direct evidence for placental origin of tumors of two histological types. J. Natn. Cancer Inst. 72:705–711, 1984.

Bell, S.C. Decidualization and associated cell types: Implications for the role of the placental bed in the materno-fetal immunological relationship. J. Reprod. Immunol. 5: 185–194, 1983.

Bell, S.C. Humoral immune responses in murine pregnancy. IV. Strain dependency and alloantibody specificity. J. Immunogenetics 11:21–31, 1984.

Bell, S.C., and Billington, W.D. Humoral immune responses in murine pregnancy. I. Anti-paternal alloantibody levels in maternal serum. J. Reprod. Immunol. 3:3, 1981.

Bell, S.C., and Billington, W.D. Major anti-paternal alloantibody induced by murine pregnancy is non-complement fixing IgG. Nature 288:387, 1980.

Benacerraf, B. In Regulatory T lymphocytes (ed. Pernis, B., and Vogel, H.J.), Academic Press, New York, p.3, 1980.

Bennett, D. The T-locus of the mouse. Cell 6:441, 1975.

Bernard, I., Scheid, M.P., Ripoche, M, and Bennett, D. Immunological studies of mouse decidual cells. I. Membrane markers of decidual cells in the days after implantation. J. Exp. Med. 148:580–591, 1978.

Billington, W.D. Organization, ultrastructure and histochemistry of the placenta: Immunological considerations. In Immunology of the trophoblast (ed. Edwards, R.G., Howe, C.W.S., and Johnson,

M.H.) Cambridge University Press, Cambridge, p. 67-78, 1975.

Billington, W.D. Immunology of trophoblast. In: Immunology of Human Reproduction (ed. by Scott, J.S., and Jones, W.R.), Grune & Stratton Inc., New York, p. 81, 1976.

Billington, W.D., Jenkinson, E.J., Searle, R.F., and Sellens, M.H. Alloantigen expression during early embryogenesis and placental ontogeny in the mouse: Immunoperoxidase and mixed hemadsorption studies. Transplant Proc. 9:1371-1377, 1977.

Birkeland, S.A. and Kristoffersen, K. Lymphocyte transformation with mitogens and antigens during normal human pregnancy : A longitudinal study. Scand. J. Immunol. 11:321-325, 1980.

Boue, J., Boue, A., and Lazar, P. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratology 12:11, 1973.

Brieva, J., Targan, S., and Stevens, R. NK and T cell subsets regulate antibody production by human in vivo antigen-induced lymphoblastoid B cells. J. Immunol. 132:611, 1984.

Brunner, K.T., Manel, J., Cerottini, J.C., and Chapuis, B. Quantitative assay of the lytic action of immune lymphoid cells in ^{51}Cr-labelled allogeneic target cells in vitro : inhibition by isoantibody and drugs. Immunology 4:181-196, 1968.

Bulmer, J.N., and Johnson, P.M. Immunohistological characterization of the decidual leucocytic infiltrate related to endometrial gland epithelium in early human pregnancy. Immunol. 55:35-44, 1985.

Bulmer, J.N., and Johnson, P.M. The T-lymphocyte population in first trimester of human decidua does not express interleukin 2 receptor. J. Reprod. Immunol. Suppl. 3rd. ICRI p. 127, 1986.

Burton, R.C., and Russell, P.S. T-cell responses to allo-antigens. II. In vitro induction of specific Ts by heat-inactivated allogeneic lymphoid cells. Transplantation 31 : 445-448, 1981.

Burwell, R.G. Studies of the primary and the secondary immune responses of lymph nodes draining homografts of fresh cancellous bone (with particular reference to mechanisms of lymph node reactivity). Ann. N.Y. Acad. Sci. 99:821, 1962.

Byrd, J.R., Askew, D.E., and McDonough, P.G. Cytogenetic findings in fifty-five couples with recurrent fetal wastage. Fertil. Steril. 28:246, 1977.

Carr, M.C., Stites, D.P., and Fudenberg, H.H. Cellular immune aspects of the human fetal-maternal relationship. iii) Mixed lymphocyte reactivity between related maternal and cord blood lymphocytes. Cell. Immunol. 11:332-341, 1974.

Cerottini, J.C., and Brunner, K.T. Cell mediated cytotoxicity, allograft rejection and tumor immunity. Adv. Immunol. 18:67, 1974.

Chambers, S.P., and Clarke, A.G. Measurement of thymus weight, lumbar node weight and progesterone levels in syngeneically pregnant, allogeneically pregnant and pseudopregnant mice. J. Reprod. Fert. 55:309-315, 1979.

Chang, A.E., and Sugarbaker, P.H. Abrogation of T cell homing by alloantibody. Transplantation 29:127-132, 1980.

Chang, K.S.S., and Tanaka, K. Effects of IFN and tumor promoter, 12-0-tetracarbonyl-13-acetate, on the sensitivity of trophoblast cells to NK cell activity. In "NK cells and other natural effector cells" ed. R.B. Herberman, Academic Press, New York, p. 751-756, 1982.

Chaouat, G., Kiger, N., and Wegmann, T.G. Vaccination against spontaneous abortion in mice. J. Reprod. Immunol. 5:389-392, 1983.

Chaouat, G., Kolb, J., Kiger, N., Stanislawski, M., and Wegmann, T.G. Immunological consequences of vaccination against abortion. J. Immunol. 134:1594-1599, 1985.

Chaouat, G., Kolb, J.P., and Wegmann, T.G. Immunological concommitants of the CBA/J vaccination against abortion. 6th ICI Abst. 6.21.47, 1986.

Chaouat, G., Monnot, P., Hoffman, M., and Voisin, G.A. Regulatory T cells in pregnancy. VI. Evidence of T cell mediated suppression of CTL generation toward paternal alloantigens. Cell. Immunol. 68:322-331, 1982.

Chaouat, G., Voisin, G.A. Regulatory T cell subpopulations in pregnancy. I. Evidence for suppressive activity of the early phase MLR. J. Immunol. 122:1383-1388, 1979.

Chaouat, G., and Voisin, G.A. Regulatory T-cell subpopulations in pregnancy. II. Evidence for suppressive actiity of the late phase MLR. Immunol. 39:239-248, 1980.

Chaouat, G., Voisin, G.A., Escalier, D., and Robert, P. Facilitation Reaction (enhancing antibodies and suppressor cells) and

rejection reaction (sensitized cells from the mother to the paternal antigens of the conceptus). Clin. Exp. Immunol. 35:13-24, 1979.

Chardonnens, X., and Jeannet, M. Lymphocyte mediated cytotoxicity and humoral antibodies in human pregnancy. Int. Arch. Allergy Appl. Immunol. 61:467-471, 1980.

Chateaureynaud, P., Badet, M.T., and Voisin, G.A. Antagonistic maternal immune reactions (rejection and facilitation) to the embryo in the urodele amphibian Salamandra salamandra Lin. J. Reprod. Immunol. 1:47-60, 1979.

Chatterjee-Hasrouni, S., and Lala, P.K. MHC antigens on the mouse trophoblast cells: Paucity of Ia antigens despite the presence of H-2K and D. J. Immunol. 127:2070-2073, 1981.

Chatterjee-Hasrouni, S., Santer, V., and Lala, P.K. Characterization of material small lymphocyte subsets during allogeneic pregnancy in the mouse. Cell. Immunol. 50:290-304, 1980.

Chew, N.J., Sherman, M.I. Biochemistry of differentiation of mouse trophoblast: Δ^5, 3β-hydroxy-steroid dehydrogenase. Biol. Reprod. 12:357-359, 1975.

Clark, D. Local suppressor cells and the success or failure of the "fetal-allograft"? Ann. Immunol., Paris 135D:321-324, 1984.

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

Clark, D.A., Chaput, A., Walker, C., and Rosenthal, K.L. Active suppression of host-vs-host graft reaction in pregnant mice. VI. Soluble suppressor activity obtained from decidua blocks the response to IL-2. J. Immunol. 134:1659-1664, 1985.

Clark, D.A., Damji, N., Chaput, A., Daya, S., Rosenthal, K.L., and Brierly, J. Decidua-associated suppressor cells and suppressor factors regulating interlenkin 2: their role in the survival of the "fetal allograft". In: Progress in Immunology VI (Eds. B. Cinader, R.G. Miller), Academic Press Inc. (in press), 1986a.

Clark, D.A., and McDermott, M.R. Impairment of host vs graft reaction in pregnant mice. I. Suppression of cytotoxic T cell generation in lymph nodes draining the uterus. J. Immunol. 121:1389-1393, 1978.

Clark, D.A., McDermott, M.R., and Szewczuk, M.R. Impairment of host vs graft reaction in pregnant mice. II. Selective suppression of cytotoxic T cell generation correlates with soluble suppressor activity and with successful allogeneic pregnancy. Cell Immunol. 52:106-118, 1980.

Clark, D.A., and McDermott, M.R. Active suppression of host vs graft reaction in pregnant mice. III. Developmental kinetics, properties, and mechanism of induction of suppressor cells during first pregnancy. J. Immunol. 127:1267-1273, 1981.

Croy, B.A., De Rusha, L., and Rossant, J. Localization of NK cells in murine decidua. 6th ICI Abst. 6.21.18, 1986.

Croy, A., Rossant, J., and Clark, D.A. Histological and immunological

studies of post implantation death of Mus caroli embryos in the Mus musculus uterus. J. Reprod. Immunol. 4:277-293, 1982.

Cummings, A.M., Yochim, J.M. Differentiation of the uterus in preparation for gestation: A model for the action of progesterone. J. Theor. Biol. 106:353-374, 1984.

Currie, G.A., and Bagshawe, K.D. The masking of antigens on trophoblast and cancer cells. Lancet i:708-710, 1967.

Currie, G.A., Van Doorninck, W., and Bagshawe, K.D. Effect of neuraminidase on the immunogenecity of early mouse trophoblast. Nature 219:191-192, 1968.

Dattwyler, R.J., and Tomasi, T.B. Inhibition of sensitization of T cells by alpha-fetoprotein. Int. J. Cancer 16:942-945, 1975.

Davies, M. The formation of immune complexes in primiparous and multiparous human pregnancies. Immunol. Letters 10:199-205, 1985.

Davies, M., and Browne, G.M. Anti-trophoblast antibody responses during normal human pregnancy. J. Reprod. Immunol. 7:285-297, 1985.

Daya, S., Clark, D.A., Devlin, C., Jarrell, J., Chaput, A. Suppressor cells in human decidua. Am. J. Obstet. Gynecol. 151:267-270, 1985.

Degenne, D., Canepa, S., Horowitz, R., Khalfoun, B., Gutman, N., and Bardos, P. Effect of human syncytiotrophoblast extract on in vitro proliferative responses. Am. J. Reprod. Immunol. 8:20-26, 1985.

Dodd, M., Andrew, T.A. and Coles, J.S. Functional behavior of skin allografts transplanted to rabbit deciduomata. J. Anat.

130:381-390, 1980.

Drake, B.L., and Rodger, J.C. The in vitro immunoregulatory properties of cultured murine trophoblast are not unique to this tissue. Immunol. 55:325-329, 1985.

Edmonds, D.K., Lindsay, K.S., Miller, J.F., Williamson, E., and Wood, P.V. Early embryonic mortality in women. Fertil. Steril. 38:447-453, 1982.

Duc, H.T., Masse, A., Bobe, P., Kinsley, R.G., and Voisin, G.A. Deviation of humoral and cellular alloimmune reactions by placental extracts. J. Reprod. Immunol. 7:27-39, 1985.

Faanes, R.B., Choi, Y.S., and Good, R.A. Escape from isoantiserum inhibition of lymphocyte-mediated cytotoxicity. J. Exp. Med. 137:171-182, 1973.

Fabris, N. Immunological reactivity during pregnancy in the mouse. Experientia 29:610-612, 1973.

Fabris, N., Piantanelli, L., and Muzzioli M. Differential effect or pregnancy of gestagus on humoral and cell-mediated immunity. Clin. Exp. Immunol, 28:306-314, 1977.

Faulk, W.P., and Temple, A. Distribution of β2 microglobulin and HLA in chorionic villi of human placenta. Nature 262:799-802, 1976.

Faulk, W.P., Sanderson, A.R., and Temple, A. Distribution of MHC antigens in human placenta chorion villi. Transpl. Proc. ix:1379-1384, 1977.

Faulk, W.P., Yeager, C., McIntyre, J.A., and Meda, M. Oncofetal antigens of human trophoblast. Proc. Royal Soc. London, B

206:163-182, 1979.

Fernandez-Cruz, E., Woda, B.A., and Feldman, J.D. Elimination of syngeneic sarcomas in rats by a subset of T-lymphocytes. J. Exp. Med. 152:823, 1980.

Foster, P.M., McLean, J.M., and Gibbs, A.C.C. Lymphoid response to pregnant and pseudopregnancy in the rat. J. Anat. 128:837-845, 1979.

Fowlis, D.J., and Ansell, J.D. Evidence that decidual cells are not derived from bone marrow. Transpl. 39:445-446, 1985.

Gambel, P., Croy, B.A., Moore, W.D., Hunziker, R.D., Wegmann, T.G. and Rossant, J. Characterization of immune effector cells present in early murine decidua. Cell. Immunol. 93:303-314, 1985b.

Gambel, P., Rossant, J., and Hunziker, R.D. Origin of decidual cells in murine pregnancy and pseudopregnancy. Transplantation, 39:443-445, 1985a.

Gauldie, J., Richards, C., and Lamontagne, L. Fc Receptors for IgA and other immunoglobulins on resident and activated alveolor macrophages. Mol. Immunol. 20:1029, 1983.

Genetet, N., Genetet, B., Amice, V., and Fauchet, R. Allogeneic responses in vitro induced by fetomaternal alloimmunization. Am. J. Reprod. Immunol. 2:90-96, 1982.

Ghani, A., Gill, T.J. III, Misra, D.N., and Kunz, H.W. The antibody response to the fetus and its genetic control: Monoclonal antibodies to a unique fetal antigen. Transpl. Proceed. XVII:1842-1843, 1985.

Gidlund, M., Orn, A., Pattengale, P.K., Jansson, M., Wigzell, H.K.,

and Nilsson, K. Natural killer cells kill tumor cells at a given stage of differentiation. Nature 292:848–850, 1981.

Gill, T.G. Chimerism in humans. Transpl. Proc. IX:1423, 1977.

Gill, T.J. III, and Kunz, H.W. Gene complex controlling growth and fertility linked to the MHC in the rat. Am. J. Pathol. 96:185, 1979.

Gill, T.J., and Repetti, C.F. Immunologic and genetic factors influencing reproduction – A review. Amer. J. Path. 95:465–570, 1979.

Golander, A., Zakuth, V., Shechter, Y., Spirer, Z. Suppression of lymphocyte reactivity in vitro by soluble factor secreted by explants of human decidua. Eur. J. Immunol. 11:849–851, 1981.

Goodfellow, P.N., Barnstable, C.J., Bodmer, W.F., Snary, D., and Crumpton, M.J. Expression of HLA system antigens on placenta. Transpl. 22:595–603, 1976.

Goodlin, R.C., and Herzenberg, L.A. Pregnancy induced hemagglutinins to paternal H-2 antigens in multiparous mice. Transplantation 2:57, 1964.

Grimm, E.A., Mazunder, A., Zhang, A., and Rosenberg, S.A. Lysis of NK-resistant fresh solid tumor cells by IL-2 activated autologous human peripheral blood lymphocytes. J. Exp. Med. 155:1823–1841, 1982.

Grimm, E.A., Ramsey, K.M., Mazumder, A., Wilson, D., Djeu, J.Y., and Rosenberg, S. II. Precursor phenotype is seriologically distinct from peripheral T lymphocytes, memory cytotoxic thymus-derived lymphocytes and Natural Killer cells. J. Exp.

Med. 157:884-897, 1983.

Gustafsson, D.C., and Kottmeier, H.L. Carcinoma of the cervix associated with pregnancy. Acta. Obstet. Gynecol. Scand. 41:1, 1962.

Hamilton, M.S., Beer, A.E., May, R.D., Vitetta, E.S. The influence of immunization of female mice with F9 tetracarcinoma cells on their reproductive performance. Transpl. Proc. XI:1069-1072, 1979.

Hamilton, M.S., and Helltrom, I. Altered immune responses in pregnant mice. Transplantation. 23:423-430, 1977.

Hancock, J., McGovern, P., and Stamp, J. Failure of gestation of goat and sheep hybrids in goats and sheep. J. Reprod. Fert. 3:Suppl 29-36, 1968.

Hanto, D.W., Hopt, U.T., Hoffman, R., and Simmons, R.L. Recruitment of unsensitized circulating lymphocytes to sites of allogeneic cellular interactions. Transplantation 33:541-546, 1982.

Halle-Pannenko, O., Pritchard, L.L., and Motta, R., and Mathe, G. Non-H-2 antigens can induce high GvH mortality in adult recipients of normal cells. Biomedicine 29:253-255, 1978.

Harrison, M.R. Maternal Immunocompotence. II. Proliferative responses of maternal lymphocytes in vitro and inhibition by serum from pregnant rats. Scand. J. Immunol. 5:582-589, 1976.

Hatanaka, K., Kitamura, Y., Maeyama, K., Watanabe, T., and Matsumoto, K. Deciduoma formation in uterus of genetically mast cell-deficient W/W^V mice. Biol. of Reprod. 27:25-28, 1982.

Hay, M.F., and Moore, R.M. Distribution of $^3\beta$-hydroxysteroid dehydro-

genase activity in the Graafian follicle of the sheep. J. Reprod. Fert. 43:313-322, 1975.

Hayry, P., Von Willebrand, E.V., and Soots, A. In situ effector mechanism in rat kidney allograft rejection. III. Kinetics of the inflammatory response and generation of donor-killer cells directed killer cells. Scand. J. Immunol. 10:95-108, 1979.

Head, J.R., and Seeling, L.L. Jr. Lymphatic vessels in the uterine endometrium of virgin rats. J. Reprod. Immunol. 6:157-166, 1984.

Hellstrom, K.E., and Hellstrom, I. Immunologic enhancement as studied by cell culture technique. Ann. Rev. Microbiol. 24:373-398, 1970.

Hellstrom, K.E., Hellstrom, J., and Brown, J. Abrogation of cellular immunity to antigenically foreign mouse embryonic cells by a serum factor. Nature 224:914-915, 1960.

Herberman, R.B., Djeu, J.Y., Kay, D., Ortaldo, J.R., Riccardi, C., Bonnard, G.D., Holden, H.T., Fagnai, R., Santoni, A., and Puccetti, P. Natural killer cells: characteristics and regulation activity. Immunol. Rev. 44:43-70, 1979.

Herzenberg, L.A., Bianchi, D.W., Schroeder, J., Cann, H.M., and Iverson, G.M. Fetal cells in the blood of pregnant women. Detection and enrichment by fluorescence-activated cell sorting. Proc. Natl. Acad. Sci. 76:1453-1455, 1979.

Holland, N.H., and Holland, P. Immunological maturation in an infant of an aggammaglobulinaemic mother. Lancet II:1152-1155, 1966.

Hooper, D.L., and Murgita, R.A. Regulaton of murine T-cell responses

to autologous antiens by α-fetoprotein. Cell. Immunol. 63:417-425, 1981.

Hopt, U.T., Sullivan, W., Hoffman, R., Simmons, R.L. Migration and cell recruiting activity of specifically sensitized lymphocytes in sponge matrix allografts. Transplantation 30:411-416, 1980.

Hunziker, R.D., Gambel, P., and Wegmann, T.G. Placenta as a selective barrier to cellular traffic. J. Immunol. 133:667-671, 1984.

Jenkinson, E.J., and Owen, V. Ontogeny and distribution of major histocompatibility (MHC) antigens on mouse placental trophoblast. J. Reprod. Immunol. 2:173-181, 1980.

Jenkinson, E.J., and Searle, R.F. Ia antigen expression on the developing mouse entryo and placentae. J. Reprod. Immunol. 1:3-10, 1979.

Johnson, P.M., Barnes, R.M.R., Hart, C.A., and Francis, W.J.A. Determinants of immunological responsiveness in recurrent spontaneous abortion. Transplantation, 38:280-284, 1984.

Kabawat, S., Mostoufi-Zadeh, M., Driscoll, S., and Bhan, A.K. Implantation site in normal pregnancy. A study with monoclonal antibodies. Am. J. Pathol. 118:76-84, 1985.

Kaneko, Y., and Nishmura, T. Factors affecting trophoblast growth in extrauterine sites. Transplantation 25:309-314, 1978.

Kastner, D., Rich, R., Chu, L., and Rich S. Regulatory mechanisms in cell-mediated immune responses. V. H-2 homology requirements for the production of a minor locus induced suppressor factor. J. Expt. Med. 146:1152-1157, 1977.

Kearns, M., and Lala, P.K. Bone marrow origin of decidual cell precursors in the pseudopregnant mouse uterus. J. Exp. Med. 155:1537-1154, 1982.

Keith, A.B. Effect of pregnancy on experimental allergic encephalomyelitis in guinea pigs and rats. J. Neurol. Sci. 38:317-326, 1978.

Kenny, J., and Diamond, M. Immunological responsiveness to Escherichia coli during pregnancy. Infect. and Immunol. 16:174-180, 1977.

Khudr, G. Cytogenetics of habitual abortion. Obstet. and Gynecol. 29:299, 1974.

Kirby, D.R.S. The immunological consequences of extrauterine development of allogeneic mouse blastocysts. Transplantation 6:1005-1009, 1968.

Kirby, D.R.S., Billington, W.D., Bradbury, S., and Goldstein, D.S. Antigen barrier of the mouse placenta. Nature 204:548-549, 1964.

Kirby, D.R.S., Potts, D.M., and Wilson, I.B. On the orientation of the implanting mouse blastocyst. J. Emb. Exp. Morph. 17:527-532, 1967.

Kirkwood, K.J. Immunoglobulin and complement receptor-bearing cells in cultures of mouse decidual tissue. J. Reprod. Fert. 62:345-352, 1981.

Kirkwood, K.J., and Bell, S.C. Inhibitory activity of supernatants from murine decidual cell cultures on mixed lymphocyte reaction. J. Reprod. Immunol. 3:243, 1981.

Klein, J., Hammerberg, C. The control of differentiation by the T

complex. Immunol. Rev. 33:70, 1977.

Kobayashi, R.H., Hyman, C.J., and Stiehm, E.R. Immunological maturation in an infant born to a mother with agammaglobulinemia. Am. J. Dis. Child. 134:942-944, 1980.

Krcek, J.P., Dickson, A.D., Biddle, F.G. Numbers of mononuclear cells in the mouse trophoblast giant cell circulation on the 9th and 11th days of gestation. Proc. Can. Fed. Biol. Soc. Abst. #92, 1982.

Krehbiel, R.H. Cytochemical studies of the decidual reaction in the rat during early pregnancy in the production of deciduomata. Physiol. Zool. 10:212, 1937.

Kunz, H.W., Gill, T.J. III, and Dixon, B.D. et al. Genes linked to the MHC that affect development. J. Exp. Med. 152:1506, 1980.

Loke, Y.W. Antigen bearing trophoblast in human placental implantation site. 3rd International Congress of Reproductive Immunology. J. Reprod. Immunol. Suppl. 1986, p 5, 1986.

Loveland, B., Cereding, R.H., Hogarth, M., and McKenzie, I. The key role of Lyt-1$^+$ cells in skin graft rejection in the mouse. Transplant. Proceed. XIII:1079-1081, 1981.

Lucivero, G., Selvaggi, L., Dell'Osso, A., Antonaci, S., Iannone, A., Bettocchi, S., and Bonomo, L. Mononuclear cell subpopulations during normal pregnancy: I. Analysis of cell surface markers using conventinal techniques and monoclonal antibodies. Am. J. Reprod. Immunol. 4:142-145, 1983.

Luini, W., Boraschi, D., Alberts, S., Aleotti, A., and Tagliabue, A. Morphological characterization of a cell popultion responsible

for natural killer activity. Immunol. 43:663, 1981.

Maraz, A., and Petri, I. Intrinsic alterations of lymphocyte reactivity in women with normal pregnancy or tumor of placental origin. Cell. Immunol. 10:496-499, 1974.

Maroni, E.S., and DeSousa, M. The lymphoid organs during pregnancy in the mouse. A comparison between syngeneic and an allogeneic mating. Clin. Exp. Immunol. 31:107-124, 1973.

Maroni, E.S., and Parrot, D.M.V. Progressive increase in cell-mediated immunity against paternal transplantation antigens in mice after multiple pregnancies. Clin. Exp. Immunol. 13:253-262, 1973.

Masson, P.L., Delire, M., and Cambiaso, C.L. Circulating immune complexes in normal human pregnancy. Nature 286:542-543, 1977.

Medawar, P.B. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp. Soc. Exp. Biol. 7:320, 1953.

Merritt, K., and Galton, M. Antibody formation during pregnancy. Transplantation 7:562-566, 1969.

Milgrom, F., Comini-Andrada, E., and Chaudhry, A.P. Fetal and neonatal fatality in rat hybrids from mothers stimulated with paternal skin. Transplant. Proc. IX:1409-1415, 1977.

Miller, G.B., and Emmens, C.W. The effects of oestradiol with progesterone on the incorporation of tritiated uridine into the genital tract of the mouse. J. Endocrinol. 43:427-436, 1969.

Miller, J.E., Williamson, E. and Glue, J. Fetal loss after implantation: a prospective study. Lancet, 2:554-556, 1980.

Mishell, R.I., Herzenberg, L.A., Herzenberg, L.A. Leukocyte agglutination in mice. Detection of H-2 and non H-2 isoantigens. J. Immunol. 90:628-633, 1963.

Moore, K.L. The Developing Human (ed. Moore, K.L.), W.B. Saunders Comp., Philadelphia, p. 96-118, 1977.

Moore, M.P., Sargent, I.L., Ting, A., and Redman, C.W.G. Maternal cell-mediated immunity in pregnancy lymphocyte responses of mothers and their non-pregnant HLA identical sisters to paternal HLA. Clin. Exp. Immunol. 54:91-94, 1983.

Murray, D.L., Imagara, D.T., Okada, D.M., and St. Comp, J.W. Antibody response to monovalent A/New Jersey/8/76 influenza vaccine in pregnant women. J. Clin. Microbiol. 10:184, 1979.

McDermott, M.R., Clark, D.A., and Bienenstock, J. Evidence for a common mucosal immunological system. II. Influence of the esterous cycle of B immunoblast migration into genital and intestinal tissues. J. Immunol. 124:2536-2539, 1980.

McDevitt, H.O. The role of H-2I region genes in regulation of the immune response. In: Progress in Immunology, Vol. IV, (ed. Fongerian, M., and Dausset, J.), Academic Press, London, p. 503, 1980.

McIntyre, J.A., Faulk, W.P., Verhulst, S.J., and Colliver, J.A. Human trophoblast-lymphocyte cross-reactive (TLX) antigens defined a new alloantigen system. Science 22:1135-1137, 1983.

McLean, J.M., Shaya, E.I., and Gibbs, A.C.C. Immune response to first mating in the female rat. J. Reprod. Immunol. 1:285, 1980.

McMichael, A.J., and Sasazuki, T. A suppressor cell in the human

mixed lymphocyte reaction. J. Exp. Med. 146:368–380, 1977.

Nagarkatti, P., and Clark, D.A. In vitro activity and in vivo correlates of alloantigen-specific murine suppressor T cells induced by allogeneic pregnancy. J. Immunol. 131:638–643, 1983.

Nakayama, E., Asano, S., Kodo, H., and Miwa, S. Suppression of mixed lymphocyte reaction by cells of human first trimester pregnancy endothelium. J. Reprod. Immunol. 8:25–31, 1985.

Natalli, P.G., De Martino, C., Quaranta, V., Nicotra, M.R., Frezza, F., Pellegrino, M.A., and Ferrone, S. Expression of Ia-like antigens in normal human non-lymphoid tissues. Transplantation 31:75, 1981.

Natalli, P.G., Segatto, O., et al. Differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens. Immunogenetics 19:109, 1984.

Nelson, J.H., Hall, J.E., Manuel-Limson, G., Freidberg, H., and O'Brien, F.J. Effect of pregnancy on the thymolymphatic system. Am. J. Obstet. Gynecol. 98:895–903, 1967.

Nelson, J.H., Lu, T., Hall, J.E., Krown, S., Nelson, J.M., and Wright Fox, C. The effect of trophoblast on immune state of women. Am. J. Obstet. Gynecol. 117:689, 1973.

Nicklin, S., and Billington, W.D. Impairment of graft versus host reactivity in pregnant mice. Clin. Exp. Immunol. 49:135–141, 1982.

Ninneman, J.L. Melanoma-associated immunosuppression through B cell activation of suppressor T cells. J. Immunol. 120:1573–1579, 1978.

Noonan, F.P., Halliday, W.J., Morron, H., and Clunie, G. Early pregnancy factor is immunosuppressive. Nature 278:649-650, 1979.

Nymand, G., Heron, I., Jensen, K., and Landsgaard, A. Cytotoxic antibodies in serum of pregnant women at delivery. Acta Pathol. Microbiol. Scand. (13) 79:595-598, 1971.

Ohta, Y. Decidua formation in persistent estrous rats produced by neonatal androgenization. Biol. of Reprod. 29:93-98, 1983.

Oppenheim, J.J., Whang, J., and Frei, E. The effect of skin homograft rejection on the recipient and donor mixed leukocyte cultures. J. Exp. Med. 122:651-654, 1965.

Padykula, H.A., Driscoll, S.G. and Cardasis, C.A. Decidual differentiation in the normal early gestational human uterus includes lymphoid infiltration. Anat. Record. 190:500-501, 1978.

Panoskaltsis, A., and Sinclair, N.R. Fc-mediated immunoregulation. 6th ICI Abst. 2.63.26, 1986.

Parrmiani, G., and Della Porta, G. Effects of antitumor immunity on pregnancy in the mouse. Nature (New Biol.) 241:26-28, 1973.

Parrott, D.M.V. The response of draining lymph nodes to immunological stimulation in intact and thymectomized animals. J. Clin. Path. 20:456, 1967.

Pearson, T.W., Roolants, G.E., Pinder, M., Ludin, L.B., and Mayor-Withey, K.S. Immune depression in trypanosome-infected mice. III. Suppressor cells. Eur. J. Immunol. 9:200-204, 1979.

Peck, A.B., Murgita, R.A., and Wigzell, H. Cellular and genetic

restrictions in the immunoregulatory activity of α-fetoprotein. III. Role of the MLC-stimulating cell population in α-fetoprotein-induced suppression of T cell mediated cytotoxicity. J. Immunol. 128:1134-1140, 1982.

Pence, H., Petty, W.M., and Rocklin, R.E. Suppression of maternal responsiveness to paternal antigens by maternal plasma. J. Immunol. 114:525-528, 1975.

Pepper, F.J. The effect of age, pregnancy and lactation on the thymus gland and lymph nodes of the mouse. J. Endocrinol. 22:335, 1961.

Persellin, R.H. The effect of pregnancy on rheumatoid arthritis. Bull. Rheum. Dis. 9(27):922, 1977.

Persike, E.L. Involution of thymus during pregnancy in young mice. Proc. Soc. Exp. Biol. 45:315, 1940.

Phuc, L.H., Papiernik, M., and Dardenne, M. Thymic involution in pregnant mice. II. Functional aspects of the remaining lymphocytes. Clin. Exp. Immunol. 44:253-261, 1981.

Power, D.A., Mason, R.J., Stewart, K.N. et al. B-lymphocyte antibodies associated with successful pregnancy: Evidence for MHC linkage. Transplant. Proc. 15:890-892, 1983.

Power, D.A., Mason, R.A., Stewart, G.H., Catto, G.R., MacLeod, A.M., and Stewart, K.N. The fetus as an allograft: Evidence for protective antibodies to HLA-linked paternal antigens. Lancet 701-704, 1983.

Raghupathy, R., Singh, B., Leigh, J.B., and Wegmann, T.G. The ontogeny and turnover kinetics of paternal H-2K antigenic determi-

nants on the allogeneic murine placenta. J. Immunol. 127:2074-2079, 1981.

Rao, V.S., Bennet, J.A., Shen, F.W., Gershon, R.K., and Mitchell, M.S. Antigen-antibody complexes generate Lyt 1 inducers of suppressor cells. J. Immunol. 125:63-67, 1980.

Remacle-Bonnet, M., Culouscou, J.H., Pamnier, G., Rance, R., and Depieds, R. Immunoregulatory activities of human trophoblasts mediated by polyamine complexes. Am. J. Reprod. Immunol. 8:55-61, 1985.

Rich, S.S., David, C.S., and Rich, R.R. Regulatory mechanisms in cell-mediated immune responses. VII. Presence of I-C subregion determinants on mixed leukocyte reaction suppressor factor. J. Exp. Med. 149:114-126, 1979.

Rich, R.R., and Pierce, C.W. Biological expressions of lymphocyte activation. III. Suppression of plaque-forming cell responses in vitro by supernatant fluids from concanavalin A - activated spleen cell cultures. J. Immunol. 112:1360-1368, 1974.

Roberts, P.J., and Hayry, D. Sponge matrix allografts: A model for analysis of killer cells infiltrating mouse allografts. Transplantation 21:437-445, 1976.

Rocklin, R.E., Kitzmiller, J.L., Carpenter, C.B., Garovoy, M.R., David, J.R. Maternal-fetal relation. Absence of an immunological blocking factors from the serum of women with chronic abortions. New Engl. J. Med. 295:1209-1213, 1976.

Rocklin, R., Zuckerman, J.E., Alpert, E., and David, J.R. Effect of

multiparity on human maternal hypersensitivity to foetal antigen. Nature 241:130-131, 1973.

Roder, J.C., Bell, D.A., and Singhal, S.K. Regulation of the immune response in autoimmune NVZB/NZW F_1 mice. Cell. Immunol. 29:272-284, 1977.

Rodriguez, G., Andersson, G., Wigzell, H., and Peck, A.B. Non-T cell nature of the naturally occurring, spleen-associated suppressor cells present in the newborn mouse. Eur. J. Immunol. 9:737-746, 1979.

Rollinghoff, M., Starzinski-Powitz, A., Pfizenmaier, K., and Wagner, H. Cyclophosphamide-sensitive T-lymphocytes suppress the in vivo generation of antigen-specific cytotoxic T-lymphocytes. J. Exp. Med. 145:455-459, 1977.

Rossant, J., and Croy, B.A. Genetic identification of tissue of origin of cellular populations within mouse placenta. J. Embryol. exp. Morph. 86:177-189, 1985.

Rossant, J., Croy, B.A., Clark, D.A., and Chapman, V.M. Interspecific hybrids and chimeras in mice. J. Exp. Zool. 228:223-233, 1983.

Rossant, J., and Frels, W.I. Interspecific chimeras in mammals: Successful production of live chimeras between Mus musculus and Mus caroli. Science 208:419, 1980.

Roth, D.B. The frequency of spontaneous abortion. Int. J. Fertil. 8:431-434, 1963.

Russell, A.S., Liburd, E.M., and Diener, E. In vitro suppression of cell-mediated autoimmunity in NZB mice. Nature 249:43-45,

1974.

Sargent, I.L., Redman, C.W.G., Stirrat, G.M. Maternal cell-mediated immunity in normal and preeclamptic pregnancy. Clin. Exp. Immunol. 50:601-609, 1982.

Sasaki, K., and Ishida, N. Diminished immune response in pregnant mice. Tohoku J. Exp. Med. 116:391, 1975.

Scothorne, R.J., and McGregor, I.A. Cellular changes in lymph nodes and spleen following skin homografting in the rabbit. J. Anat. 89:283, 1955.

Schuster, D., Bunstein, S., and Cooke, B.A. Biosynthesis of steroid hormones. In: Molecular Endocrinology of Steroid Hormones. John Wiley & Sons, London, pgs. 44-57, 1976.

Searle, R.F., Sellens, M.H., Elson, J., Jenkinson, E.J., and Billington, W.D. Detection of alloantigens during preimplantation development and early trophoblast differentiation in the mouse by immunoperoxidase labelling. J. Exp. Med. 143:348-359, 1976.

Segal, S., Siegal, T., Altaraz, H., Lev-El, A., Nevo, Z., Nebel, L., Katzenelsin, H., Feldman, M. Fetal bone grafts do not elicit allograft rejection because of protecting anti-Ia alloantibodies. Transplantation 28:88-95, 1979.

Sellens, M.H., Jenkinson, E.T., and Billington, W.D. Major histocompatibility complex and non-major histocompatibility complex antigens on mouse ectoplacental cone and placental trophoblast. Transplantation 25:173-179, 1978.

Shiu, H.M., Scholtenfeld, D., Maclean, B., and Fortner, J.G. Adverse

effect of pregnancy in melanoma. Cancer 37:181-187, 1976.

Siiteri, P., Febres, F., Clemens, L.E., Chang, R.J., Gondos, B., and Stites, D. Progesterone and maintenance of pregnancy: Is progesterone nature's immunosuppressant? Ann. N.Y. Acad. Sci. 286:384-397, 1977.

Silverstein, A.M. Ontogeny of the immune response: A perspective. In: Development of Host Defences (ed. by Cooper, M.D., Dayton, D.H.), Raven Press, New York, p. 1, 1977.

Simmons, R.L., and Russell, P.S. The antigenicity of mouse trophoblast. Ann. N.Y. Acad. Sci. 99:717-732, 1962.

Singal, D.P., Butler, L., Liao, S-K., and Joseph, J. The fetus as an allograft: Evidence for antiidiotypic antibodies induced by pregnancy. Am. J. Reprod. Immunol. 6:145-151, 1984.

Slapsys, R.M., and Clark, D.A. Active suppression of host-vs-graft in pregnant mice: IV. Local suppressor cells in decidua and uterine blood. J. Reprod. Immunol. 4:355-364, 1982.

Slapsys, R.M., and Clark, D.A. Active suppression of host vs graft reaction in pregnant mice. V. Kinetics, specificity and in vivo activity of non-T suppressor cells localized to the genital tract of mice during first pregnancy. Am. J. Reprod. Immunol. 3:65-71, 1983.

Smith, G. Maternal regulatory cells during murine pregnancy. Clin. Exp. Immunol. 44:90-99, 1981.

Smith, G. In vitro susceptibility of mouse placental trophoblast to cytotoxic effector cells. J. Reprod. Immunol. 5:39, 1983.

Smith, N.C., and Brush, M.G. Preparation and characterization of

human synchtiotrophoblast plasma membranes. Med. Biol. 56:272, 1978.

Smith, R.N., and Sternlicht, M. The alloantibody response in the allogenecity pregnant rat. III. The location of alloantibody forming cells. J. Immunol 129:783-786, 1982.

Stern, P., Gidlund, M., Orr, A., Wigzell, H. Natural killer cells mediate lysis of embryonal carcinoma cells lacking MHC. Nature 285:341-342, 1980.

Stimson, W.H., Strachan, A.F., and Shepherd, A. Studies in the maternal response to placental antigens: Absence of blocking factor from the blood of abortion-prone women. Brit. J. Obs. Gyn. 86:41-45, 1979.

Storm, T.B., Tilney, N.L., Paradysz, J.M., Bancewicz, J., and Carpenter, C.B. Cellular components of allograft rejection: Identity, specificity and cytotoxic function of cells infiltrating acutely rejecting allografts. J. Immunol. 118:2020-2026, 1977.

Strelkauskas, A.J., Davies, I.J., and Dray, S. Logitudinal studies showing alterations in the levels and funtional response of T and B lymphocytes in human pregnancy. Clin. Exp. Immunol. 32:531-539, 1978.

Strober, W., Hague, N.E., and Henkart, P.A. IgA-Fc receptors on mouse lympoid cells. J. Immunol. 121:2440-2445, 1978.

Sutton, W.D., and McCallum, M.J. Related satellite DNA's in the Genus Mus. J. Mol. Biol. 71:633-656, 1972.

Sunderland, C.A., Naiem, M.,D.Y., Redman, C.W., and Stirrat, G.M. The

expression of major histocompatibility antigens by human chorionic villi. J. Reprod. Immunol. 3:323-331, 1981.

Tagliabue, A., Befus, A.D., Clark, D.A., and Bienenstock, J. Characterization of natural killer cells in the murine intestinal epithelium and lamina propia. J. Exp. Med. 155:1785-1796, 1982.

Taina, E., Hanninen, P., and Gronroose, M. Viral infections in pregnancy. Acta Obstet. Gynecol. Scand. 64:167-173, 1985.

Tallon, D.F., Darach Corcoran, D.J., O.Dwyer, E.M. and Greally, J.F. Circulating lymphocyte subpopulations in pregnancy: A longitudinal study. J. Immunol. 132:1784-1787, 1984.

Taylor, P.V., and Hancock, K.W. Antigenicity of trophoblast and possible antigen masking effects during pregnancy. Immunol. 28:973, 1975.

Terasakai, P.I., Mickey, M.R., Yamazaki, J.N., and Vredevoe, D. Maternal-fetal incompatibility. I. Incidence of HLA antibodies and possible association with congenital abnormalities. Transplantation 9:538, 1970.

Theiler, K. In: . The House Mouse. Springer-Verlag, New York, p. 1, 1972.

Truitt, G.A., Rich, R.R., and Rich, S.S. Suppression of cytotoxic lymphocyte responses in vitro by soluble products of alloantigen activated spleen cells. J. Immunol. 121:1045-1051, 1978.

Tung, K.S.K. Immune complex in one renal glomerulus during normal pregnancy: A study in the guinea pig and mouse. J. Immunol.

112:186-200, 1974.

Vanderbeeken, Y., Vlieghe, M.P., Duchateau, J., and Delespesse, G. Suppressor T-lymphocytes in pregnancy. Am. J. Reprod. Immunol. 5:20-24, 1984.

Van Zon, A.A.J.C., and Eling, W.M. Depressed malarial immunity in pregnant mice. Infect. Immunity 28:630-632, 1980.

Voisin, G.A., and Chaouat, G. Demonstration, nature and properties of maternal antibodies fixed on placenta and direted against paternal antigens. J. Reprod. Fert. Suppl. 21:89-103, 1974.

VonWillebrand, E., Soots, A., and Hayry, P. In situ effector mechanisms in rat kidney allograft rejection. II. Heterogeneity of the effector cells in the inflammatory infiltrate vs that in the spleen of the recipient rat. Cell. Immunol. 46:327-336, 1979.

Ward, F.E., Mendell, N.R., Seigler, H.F., MacQueen, J.M., and Amos, D.B. Factors which have a significant effect on the survival of human skin grafts. Transplantation 26:194-198, 1978.

Wattenberg, L.W. Microscopic histochemical demonstration of steroid-3β-hydroxy steroid dehydrogenase in tissue sections. J. Histochem. Cytochem. 6:225-232, 1958.

Webb, C.G. Decreased fertility in mice immmunized with tetracarcinoma OTT6050. Biol. Reprod. 22:695-704, 1980.

Webb, C.G., Gall, W.E., and Edelman, G.M. Synthesis and distribution of H-2 antigens in preimplantation mouse embryo. J. Exp. Med. 146:923-932, 1977.

Wegmann, T.G. The presence of class I MHC antigens at the maternal-

fetal interface and hypothesis concerning the survival of the murine fetal allograft. J. Reprod. Immunol. 3:267-270, 1981.

Wegmann, T.G., Waters, C.a., Drell, D.W., and Carlson, G.A. Pregnant mice are not primed but can be primed to fetal alloantigens. Immunology 76:2410-2414, 1979.

Wira, C.R., Hyde, E., Sandoe, C.P., Sullivan, D., and Spencer, S. Cellular aspects of the rat uterine IgA response to estradiol and progesterone. J. Steroid. Biochem. 12:451-459, 1980.

Woodruff, M.F.A. Transplantation immunity and the immunological problem of pregnancy. Proc. Royal Soc. London, Ser. B. 148:68-75, 1958.

Wordinger, R.J., Orr, E.L., Pace, K., Oakford, L., and Morrill, A. An assessment of mast-cell deficient mice (W/W^V) as a model system to study the role of histamine in implantation and decidua formation. J. Reprod. Fert. 73:451-456, 1985.

Yochim, J.M. Modulation of uterine sensitivity to decidual induction in the rat by nicotinamide; challenge and extension of a model of progestational differentiation. Biol. of Reprod. 30:637-645, 1984.

Young, E.J., and Gomez, C.I. Enhancement of Herpes Virus type 2 infection in pregnant mice. Proc. Soc. Exp. Biol. Med. 160:416-420, 1979.

Youtananukorn, V., Matangkasombut, P., and Osathanondh, V. On set of human maternal cell-mediated immune reactions to placental antigens during the first pregnancy. Clin. Exp. Immunol. 16:593-598, 1974.