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AN INVESTIGATION OF THE EFFECTS OF GENETIC CONSTITUTION OF THE FETAL ALLOGRAFT AND OF ITS MOTHER ON PLACENTAL MONONUCLEAR AND METRIAL GLAND CELLS

by

JERRY P. KRCEK, M.Sc.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

(MEDICAL SCIENCE)

DEPARTMENT OF ANATOMY

FACULTY OF MEDICINE

CALGARY, ALBERTA

September, 1982

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "AN INVESTIGATION OF THE EFFECTS OF GENETIC CONSTITUTION OF THE FETAL ALLOGRAFT AND OF ITS MOTHER ON PLACENTAL MONONUCLEAR AND METRIAL GLAND METRIAL GLAND CELLS" submitted by Jerry P. Krcek, M.Sc., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

A. D. Dickson

Supervisor: Dr. A. D. Dickson
Department of Anatomy

Fred G. Biddle

Dr. F. G. Biddle
Departments of Pediatrics and
Medical Biochemistry

Michael J. Cavey

Dr. M. J. Cavey
Department of Biology

Dr. R. S. Hannah
Department of Anatomy

J. Klassen

Dr. J. Klassen
Departments of Pathology and
Internal Medicine

J. H. Stimpfling

External Examiner:
Dr. J. H. Stimpfling
McLaughlin Research Institute,
Columbus Hospital, Great Falls,
Montana

September 20, 1982
DATE

ABSTRACT

A series of investigations was carried out on the effects of genetic constitution of the fetal allograft (conceptus) and its mother on two cell types that have recently been implicated in the immunology of pregnancy; mononuclear cells (MNC) in the trophoblastic giant cell (TGC) layer of the conceptus and metrial gland cells (MGC) in the maternal vessels of the placental labyrinth. Throughout the study, only histocompatibility resistant congenic (C57BL/10Sn and B10.A/SgSn) and inbred (SWR/J and DBA/2J) strains were employed in order to allow genetic control of the experiments.

In the first series of experiments it was found that high numbers of MNC (MNC accumulation) in the TGC layer are found only at approximately 9 a.m. on the 10th day of gestation and are associated with maternal-conceptual H-2 disparity. No effects of strain genotype on low numbers of MNC in isogenic matings were seen.

The results of experiments involving backcrossing of the congenic resistant strains appear to support the hypothesis that H-2 haplotype and numbers of MNC are associated. Furthermore, it was found that the maternal system appears to respond to each conceptus individually.

In a preliminary study in which the numbers of MNC at 9 a.m. on the 10th day were examined in H-2 disparate conceptuses of 1st and 2nd pregnancies, it was found that lower numbers of MNC were present in 2nd pregnancy than in 1st pregnancy, consistent with the hypothesis that the MNC accumulation is an immune-type response.

The final series of experiments revealed that, unlike the situation with the numbers of MNC, a factor other than H-2, perhaps a strain specific trait under genetic control, is responsible for differences in numbers of labyrinthine MGC.

ACKNOWLEDGEMENTS

I would like to thank Dr. A. D. Dickson for introducing me to a fascinating new area of research, constantly sharing his time and knowledge with me and for his patience in supervising my graduate work. During our acquaintance I have come to know a man who is truly unequaled.

I would like to thank Dr. F. G. Biddle who also gave freely of his time and expertise and whose support was always forthcoming, especially when most needed. I thank Drs. M. J. Cavey and J. Klassen, the other members of my supervisory committee, for their help and guidance throughout this project.

To Dr. J. H. Stimpfling I extend a word of special thanks for acting as my external examiner and for donating B10.A/SgSn male mice from his laboratory so that I could proceed with my research.

The countless hours of technical assistance and friendship, throughout this project, of Ms. Maureen Sinclair are greatly appreciated and will never be forgotten.

I also wish to thank the people of MAVU for their cooperation with the artwork and photography, especially Mr. Will Symmes whose diagrams and graphs appear throughout this thesis.

This thesis was typed by Ms. Lynda Gourlie. I would like to thank her for the hard work, time and patience that she unselfishly provided.

All the members of the Faculty of Medicine, especially Drs. D. S. Matheson and A. W. Rademaker, were always willing to give freely of their knowledge and discuss ideas and problems with me. This contact with one's colleagues, I feel, is an essential part of education. All told, my stay at the University of Calgary, Health Sciences Centre, and participation in its graduate program in Medical Science has opened wide the pupil of my mind's eye and has indeed been a unique and true learning experience.

I acknowledge gratefully the financial support received from the University of Calgary, Graduate Awards Program, and from a Studentship awarded by the Alberta Heritage Foundation for Medical Research.

TO JAROSLAV AND BOZENA

MY PARENTS

Stoutness of heart, humility of soul and open-mindedness are the keys to human understanding and happiness; no one endowed with these virtues can be but honest, just and tolerant to his neighbor and himself.

....Melville Sahyun

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CHAPTER I

The conceptus: Nature's successful allograft

Review of the Literature

a) Introduction

Grafts taken from and placed on the same individual are called autogenic (autografts). Grafts transplanted between genetically identical individuals (for example, between mice of the same inbred strain) are called isogenic (isografts) whereas grafts transplanted between genetically different individuals of the same species (for example, between two different inbred strains of mice) are called allogenic (allografts).

The fate of a graft is determined by the genetic relationship between the donor and the host (Klein, 1975). Each species has a set of genes coding for antigens (histocompatibility antigens) that determine compatibility or incompatibility of tissue transplants (Klein, 1975). Generally, grafts exchanged between animals that do not differ in the histocompatibility genes are accepted, whereas those transplanted between individuals differing in the histocompatibility genes are

rejected. The rejection is caused by a specific immune response against the histocompatibility antigens (Klein, 1975).

In the mouse, histocompatibility loci, identified as distinct from other known loci, are designated by the prefix H, followed by a serial number in the order of their discovery (i.e. H-1, H-2, H-3 etc.).

It was demonstrated (Counce et al., 1956) that skin grafts exchanged between mice differing in the H-2 system survived an average of 8.5 days, whereas grafts exchanged between mice differing at the H-3 (or H-1) locus survived an average of 24 days or more. On the basis of these results Counce et al. distinguished between a strong H locus and a weak H locus, the former causing rapid rejection of skin allografts, the latter causing delayed rejection.

The H-2 complex remains the sole representative of the strong category; all other subsequently discovered histocompatibility loci are weak. The distinction between major and minor histocompatibility systems seems to be largely operational (Stimpfling, 1971). The antigens of a major system evoke a more vigorous immune response than do antigens of minor systems.

The major histocompatibility complex of the mouse, H-2, is a cluster of 10 loci, grouped into regions and subregions and located on

chromosome 17 (Klein et al., 1978). The five main regions are: K, I, S, G and D (Caldwell, 1980). The K and D regions form the boundaries of the H-2 gene complex and code for the serologically defined classic transplantation antigens present on almost all mouse cells. The I region is subdivided into five subregions (IR-1A, -1B, -1J, -1E, -1C) and is responsible for the control of immune responses to a variety of synthetic and natural antigens. The S region controls the quantitative and qualitative expression of a serum β-globulin which is demonstrated to be one of the precursor molecules of the complement system, a system which is the primary humoral mediator of antigen-antibody reactions. Most of the genes in the H-2 complex control membrane proteins but the mechanisms by which these surface proteins control the response of cells to antigen exposure still remains largely unknown (Caldwell, 1980).

The immunological basis of rejection of normal tissue transplants by allogeneic recipient (of the same species but unrelated to the donors) was demonstrated using skin tissues as grafts (Medawar, 1944). The results of this allografting were compared with those of autografting. In the first week after transplantation, both types of grafts healed and established a normal blood supply. In the second week, while the autografts gradually returned to the condition of normal skin, the allografts began to show signs of degeneration, becoming inflamed and edematous and their dermis being infiltrated by mononuclear cells. This was followed by obliteration of the graft's vascular

system and disintegration of the epidermis and dermis. The rejection of the graft was completed usually two or three weeks after transplantation.

A second allograft from the same donor placed on the same recipient is rejected much more rapidly than the first one, usually within the first week after transplantation. Apparently, the first graft specifically sensitizes the host and second-set grafts from the same donor as the first-set grafts are rejected by an accelerated reaction. The sensitization is systemic, with the second graft provoking a second-set reaction in almost any part of the body, regardless of the location of the first graft. Second grafts from unrelated allogeneic donors are rejected by a typical first-set reaction (Klein, 1975).

It was initially assumed that the allograft reaction, like many other immunological phenomena, was mediated by antibodies. However, evidence was gathered to indicate that the humoral antibody response was not the full explanation of graft rejection. For example, the sensitized state of the recipient that is rejecting a tumor graft can be transferred to a new recipient with lymphoid cells but not with serum (Mitchison, 1954). Therefore, the allograft reaction, although it is frequently accompanied by humoral antibody formation, is basically a cell-mediated type of immunity.

The mammalian fetoplacental unit which results from allogeneic

mating is potentially alien to the mother, with half of its genome (including histocompatibility factors) having been derived from the father. If the conceptus were an ordinary transplant, the trophoblast cells invading the endometrium and tapping its blood vessels would suffer cytolysis by maternal lymphocytes in response to antigenic stimulation. However, the conceptus is not rejected by the mother. Its survival is not consistent with the known laws of transplantation immunology. The immunobiology of the fetomaternal relationship has been the subject of extensive investigation but the precise mechanism that allows allogeneic pregnancies to proceed to term has as yet not been elucidated. Several factors of importance to this problem have, however, been the subject of an increasing volume of work in recent years.

b) Antigenicity of the conceptus

Initially it was thought that the embryo possessed no definite physiologic characteristics which were individual enough to be recognized as foreign to the mother (Little, 1924). Apparently, investigation of this area was sparse save for studies that tended to refute Little's hypothesis by furnishing evidence favouring alloantigenicity of embryonic and fetal tissue (Billingham & Head, 1981). Finally, however, Medawar (1953) articulated the immunologic problem of pregnancy by posing the question "How does the pregnant mother continue to nourish within itself, for many weeks or months, a fetus that is an

antigenically foreign body?" Subsequently, the antigenic nature of the conceptus has been the subject of arduous debate.

During its early development the embryo is enclosed within the zona pellucida which should provide an effective barrier to immunologically competent maternal cells (Searle et al., 1976) although it may be permeable to antibody (Sellens & Jenkinson, 1975). At implantation, which, in the mouse, occurs on the 5th day (Snell & Stevens, 1966), this acellular coat is shed and the blastocyst attaches to the uterine epithelium, which soon degenerates, allowing the trophoblast to come into close contract with the uterine stroma and maternal blood elements (Searle et al., 1976).

This close apposition of the trophoblast to the maternal tissues, especially in haemochorial placentation, demands a consideration of the nature of potentially antigenic substances on the surface of trophoblast cells. These substances may be histocompatibility antigens or other types of antigens.

(i) Histocompatibility antigens

The expression of histocompatibility antigens on trophoblast cells of pre-implantation embryos has been the subject of considerable investigation. Initially, it was considered that histocompatibility antigens (including H-2) were expressed on pre-implantation embryos

(Simmons & Russell, 1965; Olds, 1968; Kirby, 1969). Since then, some researchers (Palm et al., 1971; Gardner et al., 1973; Searle et al., 1974) questioned these findings and reported instead that only minor histocompatibility antigens were present on pre-implantation blastocysts (Palm et al., 1971; Searle et al., 1974). More recent studies (Searle et al., 1976; Billington et al., 1977; Webb et al., 1977) have verified the presence of H-2 antigens on blastocysts.

It was also believed that H-2 antigens were present on the cells of the inner cell mass (Billington, 1973; Gardner et al., 1973; Webb et al., 1977) and that the lack of H-2 antigen on the trophoblast layer may allow implantation of the embryo, while its impermeability may permit expression of H-2 antigens by the inner cell mass without risk of exposure to immunological rejection (Webb et al., 1977). However, the presence of H-2 antigens has been demonstrated on trophectoderm of early blastocyst stages (Billington et al., 1977).

Of greater consequence to the experiments that will be presented in this thesis is the matter of histocompatibility antigens on trophoblast at the time of and after implantation. This topic has also been the subject of much investigation. Many reports in the literature support the hypothesis that trophoblast does not possess histocompatibility antigens, neither in the mouse (Simmons & Russell, 1962; Vandeputte & Sobis, 1972; Jenkinson & Billington, 1974; Searle et al., 1975) nor in the human (van der Werf, 1971; Goodfellow et al., 1976;

Faulk & Temple, 1976; Faulk et al., 1977). Although evidence in favour of the presence of trophoblastic histocompatibility antigens in the human (for example, Currie, 1967; Loke et al., 1971) was sparse and not generally accepted, there now appears to be mounting evidence of its existence (Montgomery & Lala, 1982; Sutton et al., 1982). In the mouse, initially only a few studies supported the existence of trophoblastic antigenicity (for example, Kirby, 1968, 1969; Heyner, 1973) but the development of increasing sensitivity of tests for detection of antigens has permitted the presence of H-2 and non-H-2 antigens on trophoblast in the mouse to be demonstrated (Carter, 1976, 1978; Sellens et al., 1978; Searle & Jenkinson, 1978). Most recently, studies involving immunolabelling followed by quantitative radiography revealed the presence of H-2 antigens of both parental haplotypes on 11-13 day F_1 trophoblast cells (Chatterjee-Hasrouni & Lala, 1979). By 14-16 days of gestation the antigen density was equivalent to that on adult thymocytes and was further increased on day 18.

Other histocompatibility factors, although weak, that exist in mice are H-X (Bailey, 1963), associated with the X chromosome, and H-Y (Eichwald & Silmser, 1955; Snell & Stimpfling, 1966), associated with the Y chromosome. H-Y antibodies were detected in the serum of postpartum female rats (Shalev, 1980) and the presence of H-Y antigen has now been demonstrated on pre-implantation mouse embryos (Krco & Goldberg, 1976; Epstein et al., 1980).

(ii) Other antigens

Other antigens that have been demonstrated on murine embryonic or trophoblast cells are: methylcholanthrene-induced type (Parmiani & Della Porta, 1973); F9, a surface antigen common to embryonic carcinoma cells (Buc-Caron et al., 1978) and Forssman antigen (Williamson & Stern, 1978). Maternal antibodies (IgA, M, G_1 & G_2) directed against paternal antigens were identified in the mouse placenta (Voisin & Chaouat, 1974). Fetal or tissue-specific antigens are apparently associated with trophoblast (Beer et al., 1976; Artzt et al., 1976; Chang & Angellis, 1976). A developmental stage-specific antigen was detected on the plasma membrane of rat embryo cells (Baldwin & Vose, 1974).

In the human, two categories of membrane antigens, TA_1 and TA_2, have been identified on the trophoblast (Faulk et al., 1978).

The situation concerning conceptual antigenicity may be summarized as follows:

1. Evidence exists in favour of the presence of some products of the major histocompatibility complex on trophectoderm of the preimplantation blastocyst, on cells of post-implantation egg cylinder and on trophoblast populations at implantation and of the definitive placenta.

2. Evidence exists in favour of the presence of minor (non-H-2) histocompatibility antigens, including H-X and H-Y, on embryonic and extraembryonic tissues.

3. Several antigens of other types appear to be present on embryonic and extraembryonic tissues.

4. It may be possible that other, as yet unidentified, histocompatibility or other surface antigens will be demonstrated on cells of the developing conceptus.

(c) Maternal response to conceptual antigenicity

A humoral immune response has been shown to occur in normal pregnancy, cytotoxic antibodies in the human (Nymand et al., 1971) and anti-paternal antibodies in the mouse (Herzenberg & Gonzales, 1962; Goodlin & Herzenberg, 1964; Kaliss and Dagg, 1964; Rubenstein & Kaliss, 1964) having been detected.

Pregnancy-associated immune complexes have been shown to accumulate in the renal glomeruli of guinea pigs and mice (Tung, 1974). They were found (a) to increase with successive pregnancies, (b) to appear in the second half of pregnancy, (c) not to decrease following pregnancy and (d) to be present in pregnancy of syngeneic mating. No

deposition of immune complexes was found in pseudopregnant mice. The antibodies in the immune complexes may have been produced by the pregnant animals against antigens of paternal, placental or fetal origin.

A cellular immune response, in addition to the humoral response discussed above, also takes place normally during first pregnancy in man (Youtananukorn et al., 1974) and the mouse (Hellstrom et al., 1969; Baines et al., 1976; Smith et al., 1978). This is evidenced by an increase in weight of the lymph nodes draining the uterus, but not in lymph nodes elsewhere, of the pregnant mouse (Maroni & de Sousa, 1973). The increase started by the 6th day of gestation and, up to the 11th day, the gain was similar in syngeneic, BALB/C ($\underline{H\text{-}2}^d$), and allogeneic, BALB/C ($\underline{H\text{-}2}^d$) X C3H/B ($\underline{H\text{-}2}^k$) matings but thereafter became highly significantly greater in the case of the latter. A similar situation has been described in A ($\underline{H\text{-}2}^a$) and C57B1/6 ($\underline{H\text{-}2}^b$) matings but when C3H ($\underline{H\text{-}2}^k$) females were mated with CBA ($\underline{H\text{-}2}^k$) males there was no significant enlargement of the regional nodes (Beer et al., 1975). The reverse mating, however, did produce regional node enlargement. Syngeneic C3H ($\underline{H\text{-}2}^k$) and CBA ($\underline{H\text{-}2}^k$) matings both produced regional node enlargement. Although this picture is a little confused, it would appear that a mating between strains which are incompatible at the $\underline{H\text{-}2}$ locus usually produces greater lymph node enlargement than a mating between strains which are compatible. Since a mating between strains compatible at the $\underline{H\text{-}2}$ locus is sometimes associated with regional node enlargement, there also appears to be involvement of other loci.

Maroni & de Sousa (1973), examining the regional lymph nodes histologically, found greater numbers of blast cells in the thymus-dependent areas following allogeneic mating. There is also an increase in the number of plasma cells in the medullary cords (Tofoski & Gill, 1977). Baines et al. (1977), although they found that cell numbers increased in regional nodes, did not observe significant changes in the proportions of thymus-derived (Theta allo-antigen positive) and bone marrow-derived (Fc receptor positive) lymphocytes.

The spleen was found to increase in weight in both syngeneic and allogeneic matings (Maroni & de Sousa, 1973), although mesenteric, inguinal and axillary lymph nodes did not increase in either. This strengthened the existing evidence that immunization of females to fetal transplantation antigens of paternal origin occurs during pregnancy. However, it might also be due to increased haemopoiesis in pregnancy (Maroni & de Sousa, 1973). Greater enlargement of the spleen was noted by Beer et al. (1975) in CBA ($\underline{H\text{-}2}^k$) females pregnant by C3H ($\underline{H\text{-}2}^k$) males than when the male was CBA. The same was true of the reverse mating of these strains. Their findings with A ($\underline{H\text{-}2}^a$) and C57BL/6 ($\underline{H\text{-}2}^b$) strains were similar to those of Maroni & de Sousa (1973). Unfortunately, Beer et al. (1975) didn't look at the spleens histologically.

Changes in the maternal small lymphocyte subsets within bone marrow, spleen, paraortic lymph nodes and blood during allogeneic (CBA X

C57BL) and syngeneic (CBA X CBA) pregnancy were investigated by radioautographic examination of surface markers on these cells (Chatterjee-Hasrouni et al., 1980). Temporal changes in absolute numbers of these small lymphocyte subsets in different lymphoid organs were qualitatively similar for both types of pregnancies, but these changes were more marked in the allogeneic type. They suggested that, while these changes common to both kinds of pregnancy may reflect a maternal response to fetal antigens, the more pronounced alterations seen during allogeneic pregnancy may result from an additional response to paternal type alloantigens, including H-2.

(d) Factors modifying maternal response to conceptual antigenicity

(i) Barrier hypotheses

Trophoblastic antigenicity was postulated to be masked in mice by a layer of mucoprotein (Kirby et al., 1964) or inert fibrinoid material (Bradbury et al., 1965; Billington, 1971) and in humans by deposits of sulphated mucoprotein or fibrinoid (Bradbury et al., 1969). It has been suggested that the coats of fibrinoid or sialomucin overlying the trophoblast neutralize or mask antigens on these cells (Bradbury et al., 1965; Currie & Bagshawe, 1967; Jones & Kemp, 1969; Douthwaite & Urbach, 1971) or impart an electronegative charge to them, thus

repelling maternal lymphocytes which are similarly charged (Currie & Bagshawe, 1967). Furthermore, the thickness of these layers increased with wider genetic differences between mother and fetus (Bradbury et al., 1965) while their enzymatic removal from the cell surface was claimed to expose transplantation antigens on the underlying ectoplacental cone, evidenced by the observation that the trophoblast could then sensitize recipients to allogeneic grafts (Currie et al., 1968). However, much of the aforementioned evidence is considered tenuous and many of the conclusions have been strongly questioned, by, for example, Edwards & Coombs (1975).

Doubts have been raised about the presence and significance of a barrier layer as it could not be detected by some workers (e.g. Wynn, 1967; 1969), who consequently rejected the concept of a protective layer on the trophoblast in favour of antigenic neutrality of these cells after implantation (e.g. Simmons & Russell, 1967). The fibrinoid layer could not be identified in rodent embryos during the early days of pregnancy or in embryos of other species throughout the whole of pregnancy (Potts, 1968).

Barker & Billingham (1977) proposed that decidual tissue impairs the afferent pathway of the immunologic reflex and suggested that the uterus itself provides an immunologically-privileged site on the anatomical basis of the lack of lymphatics pervading the endometrium (Hoggan & Hoggan, 1881; McLean & Scothorne, 1970). However, it was

found that a dye injected into the wall of the non-pregnant mouse uterus travelled to the lumbar and renal lymph nodes (Maroni & de Sousa, 1973). It could be that this injection was made into the uterine wall far enough out for the material to enter lymphatics in the vicinity of the internal muscle layer where they are acknowledged to be present in the rabbit (McLean & Scothorne, 1970). An important question, in the mouse, is whether the decidua capsularis, which contains the conceptus during implantation and for some time thereafter, is completely or relatively impermeable to antigens or sensitized lymphocytes. Although it does not appear to have been addressed directly in this species, it does not appear likely since blood vessels have been observed passing through it (Dickson, personal communication).

In the stage following implantation in the mouse and rat, the maternal blood circulates through the trophoblastic giant cell layer, apparently bathing the surfaces of the cells directly (Snell & Stevens, 1966; Everett, 1935; Dickson, 1977). The mucoprotein that Kirby et al. (1964) described in the mouse may constitute a barrier in this situation but their description related to a different place and time, viz. the labyrinth on the 16th day.

Recently, an interesting observation, that murine trophoblast cells contain maternal immunoglobulins either as granules or a more diffuse staining substance in the cytoplasm, was reported (Bernard, 1977). This pattern was observed in a few trophoblast cells

immediately after implantation and increased as the cells became more numerous. By the 9th day, the embryo was surrounded by layers of immunoglobulin-containing trophoblastic giant cells. Furthermore, it was determined that, at the time of implantation, the embryo is surrounded by maternal immunoglobulins, which are taken up by trophoblastic giant cells, suggesting that these cells may constitute a barrier around the embryo (Bernard et al., 1977).

The increased weight, referred to above, of lymph nodes draining the pregnant murine uterus (Maroni & de Sousa, 1973) appears to indicate that there is at least not an absolute barrier to the transmission of antigenic information from the conceptus via the uterine lymphatics.

(ii) Hormones

Evidence presently exists in favour of steroid synthesis and catabolism by trophoblast cells, which occurs before implantation in some species, including the rat, mouse, hamster, rabbit, pig, sheep and cow (Heap et al., 1979). Progesterone is commonly described as the hormone of pregnancy and an immunosuppressive role has been claimed for it (Siiteri et al., 1977; Beer & Billingham, 1979). The same role appears to exist for estrogens and glucocorticoids, of which there is a steady increase in production and excretion during pregnancy in many

mammals (Amoroso, 1981). The production of glycoprotein hormones (e.g. hCG, CG, PMSG) attributable to the placenta is now generally accepted (Amoroso, 1981). It may be possible that the glycoprotein hormones may be signals directed by the conceptus at the mother, perhaps acting in concert with steroids (progesterone) to reduce the maternal immune response by suppressing lymphocyte transformation (Contractor & Davis, 1973). It has also been reported that chorionic gonadotropins will inhibit an immunological system dependent on antigen recognition and will synergize with steroids (Amoroso, 1981). Furthermore, the implanting mammalian blastocyst has the ability to synthesize both estrogens and gonadotropins in sufficient quantities to be a factor in the survival of the trophoblast (Amoroso & Perry, 1975).

(iii) Mechanisms akin to tolerance or enhancement

Inert barriers or modification of the maternal immune response by hormones has been questioned as being alone sufficient to prevent the rejection of the conceptus. The current trend of research in this area has therefore turned increasingly to the study of additional forms of protection, notably tolerance and enhancement.

Immunological tolerance is defined as an immunological response consisting of the development of specific non-reactivity of the lymphoid tissues to an antigen which is capable of inducing, in other circumstances, cell-mediated or humoral immunity (Herbert & Wilkinson,

1971). It may follow contact with antigen in fetal or early post-natal life or, in adults, administration of very high or very low doses of certain antigens. In the latter case (i.e. acquired tolerance) the tolerance persists only as long as the antigen remains in the body. Immunological reactions to unrelated antigens are not affected by the induction of tolerance to any given antigen.

Immunological enhancement is said (Herbert & Wilkinson, 1971) to occur following transplantation of an experimental tumor to an animal which has antibody to the tumour and in which a greater than expected rate of tumor growth takes place. It may be attributed to masking of tumor antigens. Whether the mechanisms of tolerance and enhancement are different, in principle, is not known (Klein, 1975).

Herbert & Wilkinson (1971) define a third condition, immunological inertia, which they state is a depression of immunity, other than immunological tolerance, towards the histocompatibility antigens of a partner in viviparity (i.e. of mother or of fetus). The term immunological inertia is not commonly employed.

The possibility exists that mechanisms similar to tolerance or enhancement may be operative during pregnancy. It has been reported that successive pregnancies result in an impairment of the ability of female mice to reject grafts of a genotype similar to the fetuses (Breyere & Barrett, 1960; Prehn, 1960; Breyere & Burhoe, 1963). This

led to attempts to find blocking antibodies in maternal and fetal sera. The presence of sensitized maternal lymphocytes and blocking antibodies was shown in mice by Hellstrom et al. (1969). Similar studies in humans revealed that maternal lymphocytes from parous women exhibited decreased reactivity against fetal lymphocytes in mixed leucocyte culture (MLC) tests. This reaction could apparently be blocked by serum from the same mother (Ceppellini et al., 1971). According to Edwards & Coombs (1975), the mode of action of such sera may involve soluble antigen, antigen-antibody complexes or free antibody capable of inhibiting lymphocyte recognition.

Concentrations of IgG, relative to the other immunoglobulins, in the mother rise sharply during pregnancy (Ralph et al., 1972). It was suggested that IgG_3, which is preferentially transported to the fetus, may serve as a factor (perhaps a blocking antibody) protecting it from possible destructive responses of cytotoxic IgM antibodies and lymphoid cells of the mother (Ralph et al., 1972). In the human, IgG_3 is also transmitted preferentially (Adinolfi & Kohn, 1971).

Tolerance to C57BL/An male antigens, demonstrated by successful skin grafts from the male donors, has been induced in C57BL/An female mice by repeated matings with the same males (Prehn, 1960). The degree of tolerance was proportional to the number of prior pregnancies. It has been postulated that tolerance could also be induced in the mother during parturition by contact with histocompatibility antigens

present in amniotic fluid (Goodlin et al., 1964). However, later investigations favoured events during pregnancy as being more important in the induction of maternal tolerance.

Reservations about accepting enhancement as a major protective mechanism against immunological damage to the fetus have been expressed (Edwards & Coombs, 1975) because the methods of measuring blocking factors in pregnancy have not proved to be readily repeatable and blocking antibody has not been characterized. Furthermore, although some form of blocking antibody has been demonstrated in in vitro tests such as MLC, it has been shown that non-specific agents will also interfere with this reaction.

Another possible mechanism is that mouse paternal strain antigens may be presented in a manner capable of neutralizing the maternal immune response. Allogeneically pregnant mice rapidly absorbed passively administered antibody (IgG) against paternal strain H-2 antigens and there was no deleterious effect on the conceptus but it was uncertain whether the absorption took place in the blood, by the trophoblast in the placenta or by transmission to the fetus (Wegmann & Carlson, 1977). In a later paper (Wegmann et al., 1979), written after the antibody was partially purified and labelled with ^{125}I, it was reported that the placenta differentially absorbs the antigen. Nevertheless, blocking factors may be present in the blood (Pence et al., 1975) and have been reported lacking in abortion-prone women (Rocklin

et al., 1976; Stimson et al., 1979).

(iv) Cellular mechanisms

It is difficult to appreciate why maternal immunoglobulins are present rather abundantly in particular places in the pregnant uterus. They have been shown to have enhancing (i.e. immunosuppressive) properties after having been eluted from late placentae of the mouse (Voisin & Chaouat, 1974) and human (Faulk et al., 1974). They are found in the mouse (Bernard 1977; Bernard et al. 1977) at implantation in the uterine glands and lumen as well as in the trophoectoderm, blastocele and early endoderm of the embryo. These antibodies were found, later in gestation, in the trophoblastic giant cells, both as granules and diffusely dispersed in the cytoplasm. Goetze & Franke (1967) described trophoblastic giant cells in the rat as binding humoral antibodies. Morisada et al. (1976) were of the opinion that the embryo may be protected from maternal antibodies against its antigens by resolution of antigen-antibody complexes inside the trophoblast.

The presence of immunoglobulin G has been reported in the metrial gland cells of the rat as well as in trophoblast, yolk sac, fibrinoid and uterine connective tissue and some decidual cells (Bulmer & Peel, 1977). The occurrence in metrial gland cells is interesting because of the suggestion that these cells are derived from lymphocytes

(Smith, 1966; Peel & Bulmer, 1977). Bernard et al. (1978) found, shortly after implantation in the mouse, decidual cells bearing receptors for the Fc portion of IgG which they considered might protect the embryo by interacting with maternal blocking antibodies and trophoblasts.

Lack of the cellular reaction in the regional lymph nodes during pregnancy may have a detrimental effect on fertility. In rats, the removal before mating of the regional lymph nodes draining the uterus reduced mean litter size and increased the incidence of stillbirths (Tofoski & Gill, 1977). It was ascertained, also in rats, that excision of the regional nodes prior to mating was followed by a decrease in weight of both the uterus and the placenta (Beer & Billingham, 1977). Clark & McDermott (1978) suggested that the cellular activity in the regional lymph nodes may benefit fertility by immunostimulation or by providing a mechanism for preventing harm to the conceptus, such as activation of suppressor mechanisms (e.g. suppressor T cells, Diener, 1974; Kilshaw et al., 1975). They found a reduction in mortality of newborn C3H X DBA/2 mice injected with draining lymph node cells from C3H females pregnant by DBA/2 males in comparison with injection of the corresponding cells from virgin C3H females. The reduction in cytotoxic T lymphocyte (CTL) generation from regional lymph nodes during gestation could, they proposed, be explained by a reduced frequency of CTL precursors, by a reduction in the frequency or activity of helper T cells (Miller et al., 1977; Cantor & Boyse, 1975b) or

by suppressor T cells (Cantor & Boyse, 1975a; Rollinghoff et al., 1977). They felt that their demonstration of suppression of CTL generation in vitro by regional lymph nodes from pregnant mice suggests that cellular suppressor mechanisms may be in part responsible, which is supported by the work of Hamilton & Hellstrom (1977). The latter authors demonstrated suppression of CTL generation to alloantigens after adoptive transfer of mixtures of lymphoid cells from pregnant and non-pregnant mice to irradiated syngeneic hosts. Clark & McDermott (1978) found evidence of suppression on the eighth day of gestation and postulated that it may be due to a maternal cell to which a factor (e.g. α-fetoprotein or antigen-antibody complexes) has become firmly bound or which had become activated by antigen-antibody complexes (Gershon et al., 1974), a non-specific immunoregulatory factor such as α -fetoprotein (Murgita & Tomasi, 1975; Murgita et al., 1977) or by a specific antigen derived from the embryo.

The presence of significant aggregations of mononuclear cells, at midgestation, in the maternal vascular channels of the trophoblastic giant cell layer of random bred Swiss-Webster mice has recently been reported (Dickson, 1979). The high number and location of these cells was suggestive of their possible local involvement in the maintenance of the fetal allograft, whatever the mechanism may be.

Metrial gland cells of the rat and mouse may also be involved in the maintenance of the fetal allograft. These cells, first seen in

the decidua basalis early in pregnancy, later form an organized structure, the metrial gland, within the meso-metrial triangle. It has been suggested, as mentioned previously, that they may be derived from a cell of the lymphocytic series (Smith, 1966). They contain, and have been suggested to synthesize, immunoglobulin (Sharma & Peel, 1979). Moreover, comparison of the numbers of metrial gland cells in maternal labyrinthine vessels in an inbred (C57BL/HPB) and an outbred (Swiss Webster) mating demonstrate that there is a greater accumulation of these cells in the maternal vessels in the labyrinth of the outbred strain (Dickson, 1980). This finding is, at least, consistent with the postulate that these cells are involved in some, as yet unknown, immunosuppressive mechanism during pregnancy.

Therefore, the studies presented in this thesis will be concerned with possible cellular mechanisms, operative during pregnancy and involving mononuclear and metrial gland cells, that may play a role in the survival of the fetal allograft.

CHAPTER 2

Mononuclear cells in the murine trophoblastic giant cell layer: effects of the major histocompatibility complex and of strain genotype

INTRODUCTION

The mammalian conceptus is akin to a foreign transplant by virtue of its paternal complement of genes. If this conceptus is identified by the maternal immune system, it is crucial to the viviparous form of mammalian reproduction that the conceptus not be rejected and sloughed off like a foreign tissue transplant.

While the mechanism that prevents rejection of the mammalian conceptus is not understood, the hypotheses that have been postulated can be grouped into three categories. First, the trophoblast may not be antigenic (Faulk & Temple, 1976; Faulk et al., 1977; Chatterjee-Hasrouni & Lala, 1979). Second, a barrier may prevent the antigens from being detected by the mother's immune system (Kirby et al., 1964; Kirby, 1968). However, neither of these hypotheses is in accord with the observation that the lymph nodes draining the uterus become enlarged during pregnancy in the mouse (Beer et al., 1975; Maroni & de Sousa, 1973) and the rat (McLean & Shaya, 1978), apparently due to

proliferation of lymphocytes, presumably in response to conceptual antigenicity (McLean et al., 1980). Nevertheless, this observation does fit with the third hypothesis that the maternal immune system recognizes the foreign nature of the conceptus but its response is somehow modified (Wegmann & Carlson, 1977; Clark & McDermott, 1978; Clark et al., 1980).

A very marked accumulation of mononuclear cells (MNC), which appeared to be maximal on the morning of the 10th day of gestation, was noted in the trophoblastic giant cell (TGC) layer of random-bred Swiss Webster mice (Dickson, 1979). The conceptuses of isogenically mated inbred C57BL/HPB females contained relatively low numbers (unpublished results). A random-bred mouse, such as the Swiss-Webster, contains a heterogeneous complement of genes derived from the general gene pool of the breeding population. If any two such mice are compared, a large number of genetic differences between them will be apparent. In a highly inbred strain, however, such as the C57BL/HPB, all mice of that strain are genetically identical, except for differences in the sex chromosomes. It therefore appeared possible that the high numbers of MNC present in the random-bred Swiss-Webster conceptuses might represent a maternal immune response, that may be involved in the maintainance of the fetal allograft, to the maternal-conceptual genetic disparity. Also, the possibility existed that these cells might be present at some other time(s) in the isogenic conceptuses or in the allogeneic conceptuses.

The present study was undertaken in an attempt to explore possible genetic factors that may be associated with the high numbers of MNC and to investigate whether the MNC accumulation is found at a time other than 9 a.m. on the 10th day. Since it is generally accepted that a difference in the major histocompatibility complex (H-2 in the mouse) between two individuals is associated with the greatest response of a host to an allograft, its role in the MNC accumulation was studied. The timing and duration of maximum accumulation were investigated simultaneously. The effects of strain genotype were then studied since the possibility also existed that differences in MNC numbers may be purely a strain-related phenomenon.

The effect of differences at the H-2 locus were investigated using the C57BL/10Sn (abbreviated to B10) strain and the histocompatibility congenic resistant strain, B10.A/SgSn (abbreviated to B10.A). The B10.A mouse differs from its background strain (B10) only at H-2, allowing the study of the effects of a single genetic difference, on an otherwise identical background, on MNC numbers. The effect of strain genotype on the accumulation was investigated by comparing conceptuses from isogenic matings of the B10 (and B10.A), SWR/J and DBA/2J strains, which are genetically distinct at a number of polymorphic loci.

MATERIALS AND METHODS

The congenic strains C57BL/10Sn and B10.A/SgSn, which differ at the major histocompatibility locus, were obtained from the Jackson Laboratory (Bar Harbor, Maine). SWR/J and DBA/2J mice were obtained from the colony of Dr. F. G. Biddle at the University of Calgary. The mice were maintained on a light-dark cycle of 14 and 10 hours, the lights being switched on at 6 a.m. In this and all other experiments in this thesis, the first day of gestation is that during which a copulation plug was found at the 9 a.m. examination.

Female B10, B10.A, SWR/J and DBA/2J mice were mated isogenically (i.e. with B10, B10.A, SWR/J and DBA/2J male mice, respectively). Of these, three B10 females were killed during the first hour of each three hour period from 12:01 a.m. on the 9th day to 11:59 p.m. on the 11th day of gestation and three B10.A, SWR/J and DBA/2J females every 3rd hour from 6 a.m. to 1 p.m. on the 10th day only. Subsequently, seven additional isogenically-mated B10 females were killed between 9 and 10 a.m. on the 10th day, bringing the total for that mating and time to 10.

Three B10 females, mated with B10.A males, were killed every third hour from 12:01 a.m. on the 9th day to 11:59 p.m. on the 11th day of gestation and three B10.A females, mated with B10 males, every third hour from 6 a.m. to 1 p.m. on the 10th day only. Later, seven

additional B10 females, mated with B10.A males, were killed between 9 and 10 a.m. on the 10th day, making a total of 10. In the remainder of this thesis, reference to 6 a.m. (or 9 a.m., etc.) specimens is to be construed as referring to 6-7 a.m. (or 9-10 a.m., etc.) specimens.

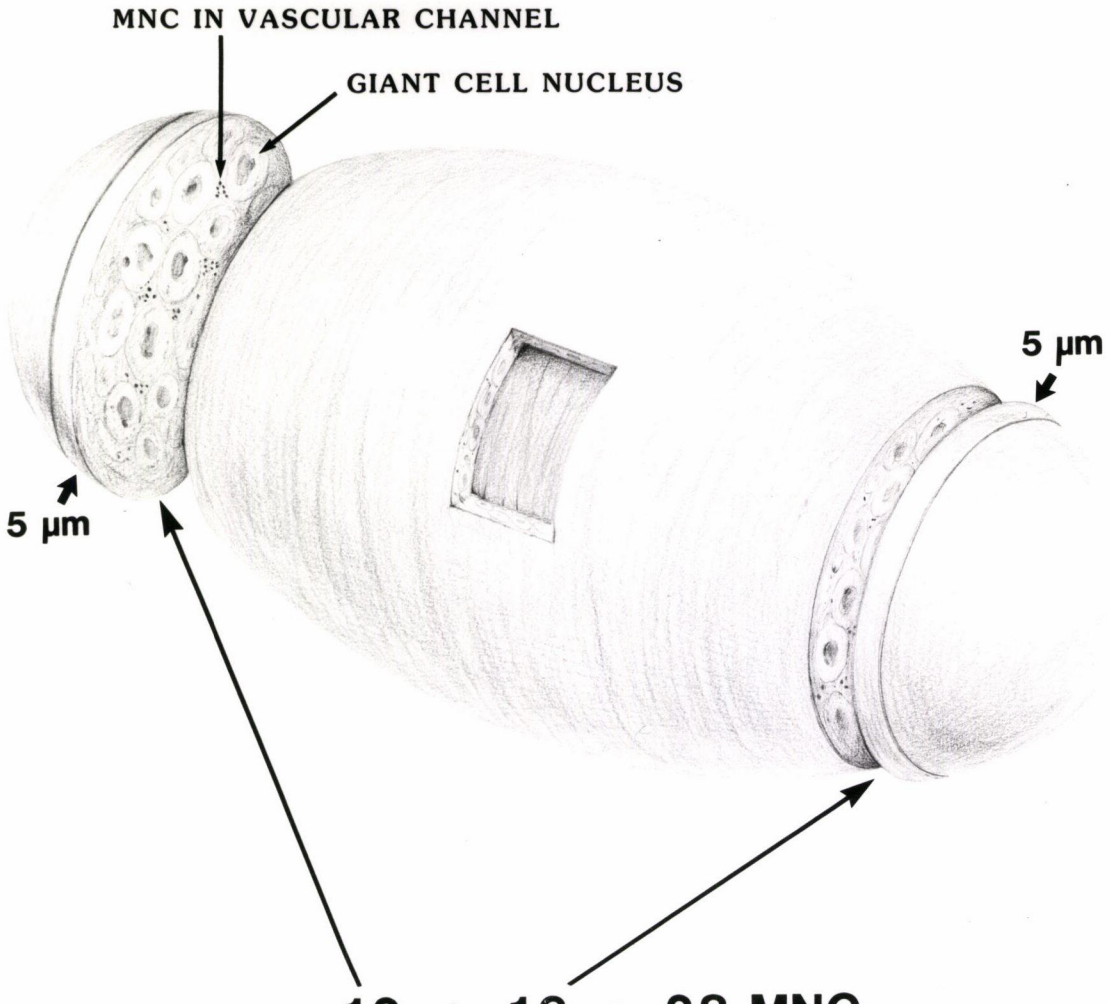
The uterine horns were excised and fixed in a phosphate-buffered 2% glutaraldehyde-10% formalin mixture modified from Karnovsky (1965). One conceptus per mouse was dehydrated in a graded series of ethyl alcohol solutions, embedded in paraffin wax and sectioned at 5 μm perpendicular to the long axis of the uterus. One section in every 20 (9th day specimens) and one in every 50 (10th and 11th day specimens) was mounted and stained with PAS/diastase and haematoxylin.

The number of mononuclear cells in the maternal blood channels in the TGC layer was counted in two tangential sections of each conceptus, one section at each end (see Figure 2.1). The number of giant cell nuclei in the same sections was used to standardize for variations in the area of the sections. The non-parametric Mann-Whitney U-test (MW) and Kruskal-Wallis (KW) one-way analysis of variance were used to compare the different groups (Siegel, 1956).

In describing matings (e.g. B10 X B10.A), the female will precede the male throughout this thesis.

Figure 2.1

Diagram of a 10th day conceptus removed from the uterus. The thickness of the giant cell layer in the middle of the conceptus is indicated by the window. Tangential sections through the ends of the layer are indicated. An example of the method of standardization is also included.



12 + 16 = 28 MNC

40 + 74 = 114 GCN

STANDARDIZATION

BASED ON 50 GCN

= 12 MNC

RESULTS

Tangential histological sections showing MNC in the maternal blood channels in the trophoblastic giant cell layer of conceptuses from B10 X B10 and B10 X B10.A matings are exemplified by Figures 2.2 and 2.3, respectively. The numbers of MNC counted in each conceptus are given in Table 2.1. Five conceptuses indicated by single asterisks in the table have been omitted because the development of both the embryo and the placenta appeared to be retarded. The numbers of MNC that were counted in the TGC layer on the 9th and 11th days in both the B10 X B10 and B10 X B10.A conceptuses are, in general, lower than the numbers on the 10th day in both types of conceptuses. However, since the area occupied by the TGC layer varies from one conceptus to another, it is difficult to make meaningful comparisons using these figures. Therefore, the number of trophoblastic giant cell nuclei, in the areas in which MNC were counted, were also counted in each specimen as a basis for standardization. The area throughout which 50 giant cell nuclei were dispersed in the two sections per conceptus was taken as the standard area.

The result of this standardization for each conceptus is given in Table 2.2 and the mean values for each three-hour interval are shown in Figure 2.4. Inspection of the table and figure suggests that, within each mating, the numbers of MNC on the 9th day are similar to those on the 11th day and that, moreover, the numbers on both the 9th

Figure 2.2

A tangential section of a 9 a.m., 10th day B10 X B10 conceptus illustrating the number of mononuclear cells found in the maternal channels in the trophoblastic giant cell layer. X400.

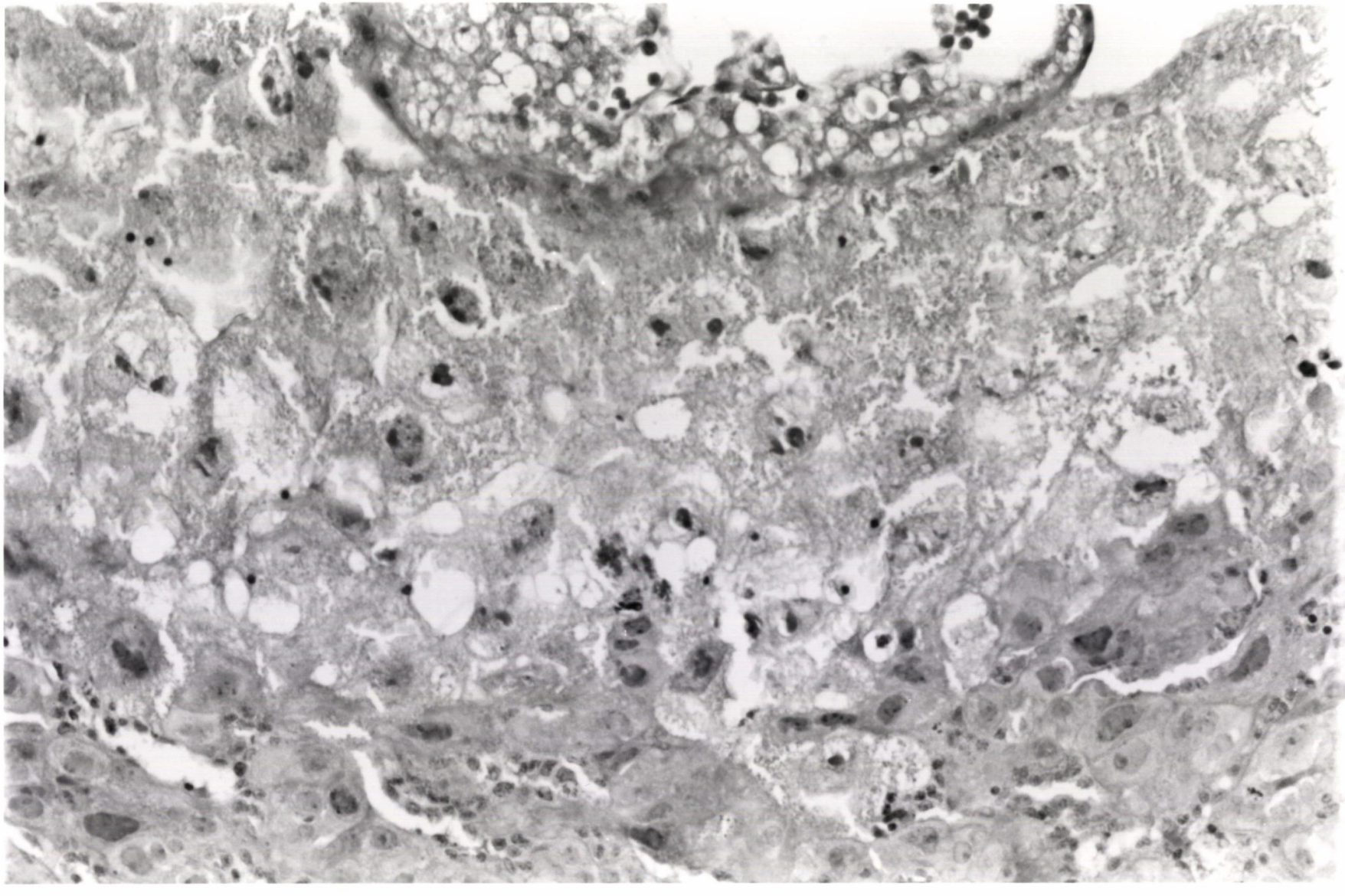


Figure 2.3

A tangential section of a 9 a.m., 10th day B10 X B10.A conceptus showing the abundance of mononuclear cells observed in the maternal channels in the trophoblastic giant cell layer. X400.

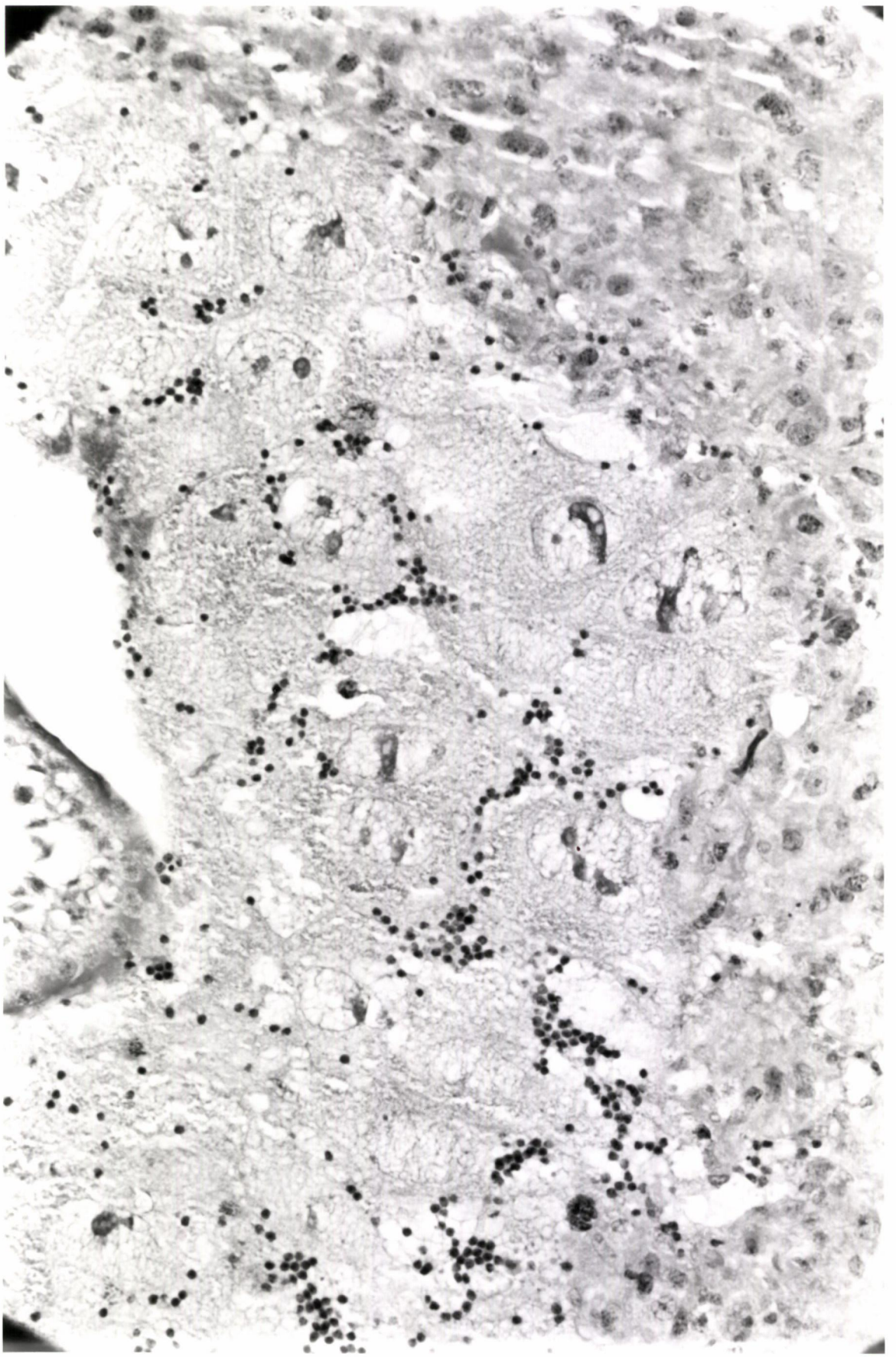


TABLE 2.1

Numbers of mononuclear cells counted in the maternal blood channels in the trophoblastic giant cell layer of conceptuses of B10 female mice mated isogenically and with B10.A males. Each entry represents one conceptus.

MATING	MIDNIGHT	3 a.m.	6 a.m.	9 a.m.	NOON	3 p.m.	6 p.m.	9 p.m.
				9TH DAY				
B10 X B10	32	28	46	234	17	17	41	53
	30	34	24	47	21	78	27	100
	*	46	23	58	30	*	258	20
B10 X B10.A	32	26	63	58	35	34	34	6
	57	61	30	55	36	43	22	77
	28	54	22	35	44	20	*	27
				10TH DAY				
B10 X B10	41	74	87	126	620	295	295	127
	36	209	149	204	45	175	199	98
	73	209	439	184	463	72	204	121
B10 X B10.A	75	129	598	977	77	820	228	188
	529	213	103	935	241	509	232	410
	38	232	373	2165	**3222	234	440	161
				11TH DAY				
B10 X B10	40	53	64	115	62	158	53	146
	40	116	156	82	85	51	59	119
	126	67	32	50	87	36	90	54
B10 X B10.A	239	137	43	55	33	54	60	33
	135	55	36	167	48	55	27	23
	28	*	70	*	65	82	83	36

* specimen omitted because of delayed development
** possible slight delayed development

TABLE 2.2

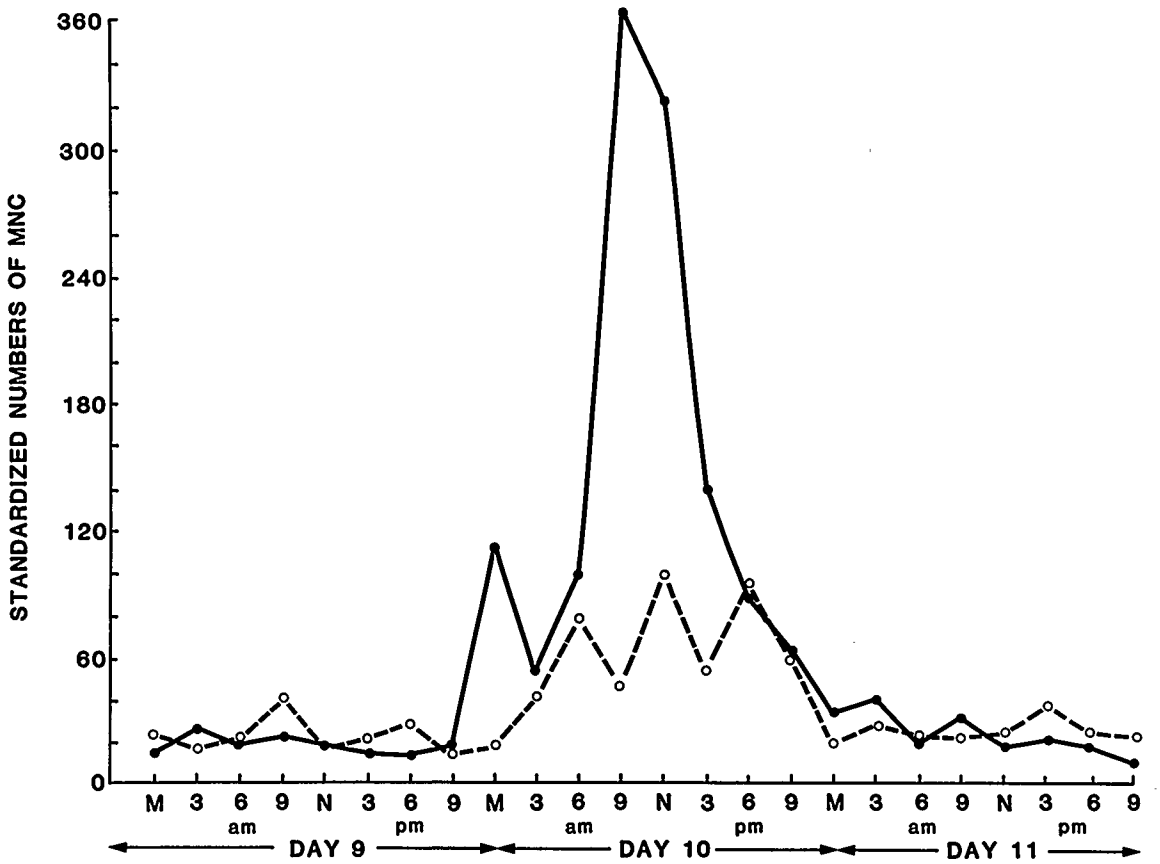
Standardized numbers of mononuclear cells (per 50 giant cell nuclei) in the trophoblastic giant cell layer of conceptuses of B10 female mice mated isogenically and with B10.A males. Each entry represents one conceptus.

MATING	MIDNIGHT	3 a.m.	6 a.m.	9 a.m.	NOON	3 p.m.	6 p.m.	9 p.m.
				9TH DAY				
	14	9	29	62	12	27	24	6
B10 X B10	23	18	17	37	17	16	10	27
	*	22	19	28	25	*	50	7
	9	22	29	22	14	12	14	4
B10 X B10.A	22	36	12	21	12	17	12	37
	12	24	16	26	35	17	*	12
				10TH DAY				
	9	31	55	34	111	87	83	110
B10 X B10	10	51	46	49	17	51	101	37
	34	43	136	56	174	18	58	28
	39	34	154	411	32	229	113	42
B10 X B10.A	288	56	33	205	107	113	64	106
	17	71	108	485	**834	83	84	41
				11TH DAY				
	14	21	23	17	24	67	18	25
B10 X B10	8	31	29	28	25	27	23	23
	32	29	7	18	23	18	32	17
	68	67	28	28	14	23	21	8
B10 X B10.A	17	13	9	33	18	21	17	8
	14	*	19	*	20	20	17	15

* specimen omitted because of delayed development
** possible slight delayed development

Figure 2.4

Standardized numbers of mononuclear cells in the trophoblastic giant cell layer throughout the 9th, 10th and 11th days of gestation. Each point represents the mean for three conceptuses. o--o B10 X B10 ●—● B10 X B10.A


STANDARDIZED NUMBERS OF MNC
360
300
240
180
120
60
0
M 3 6 9 N 3 6 9 M 3 6 9 N 3 6 9 M 3 6 9 N 3 6 9
am pm am pm am pm
DAY 9
DAY 10
DAY 11

and 11th days in the B10 X B10 conceptuses are similar to those in the B10 X B10.A conceptuses (KW, $p > 0.2$). However, the number of MNC are significantly greater on the 10th day in both the B10 X B10 (KW, $p < 0.001$) and B10 X B10.A conceptuses (KW, $p < 0.001$) than on either the 9th or 11th day.

While it can be seen from Table 2.2 and Figure 2.4 that increased numbers of MNC in both the B10 X B10 and B10 X B10.A conceptuses are found throughout the 10th day, there appears to be a particularly large increase in numbers of MNC at 9 a.m. on that day only in the B10 X B10.A conceptuses. The numbers of MNC counted at 9 a.m. in B10 X B10.A conceptuses were greater than those in conceptuses collected at 6 a.m. (MW, $p = 0.05$) and greater than those in two of the three conceptuses collected at noon, the number in the third of the noon conceptuses being exceptionally high. Furthermore, it can be seen from Table 2.2 that, of the conceptuses collected at 6 a.m., 9 a.m. and 12 noon on the 10th day from both the B10 X B10 and B10 X B10.A matings, it is only in those from the B10 X B10.A matings and collected at 9 a.m. that the standardized numbers of MNC in all three conceptuses are greater than 200. Also, it is only at 9 a.m. on the 10th day that the numbers of MNC in all three B10 X B10.A conceptuses are greater than in all three B10 X B10 conceptuses (MW, $p = 0.05$). When the B10 X B10 conceptuses, collected at 6 a.m. and noon, are compared with those collected at 9 a.m., there is no significant difference in numbers of MNC (MW, $p = 0.35$ in each case).

TABLE 2.3

Standardized numbers of mononuclear cells at 9 a.m. on the 10th day of gestation in conceptuses of seven additional B10 female mice mated isogenically and with B10.A males. The number in brackets is the actual number of mononuclear cells counted. Each entry represents one conceptus.

B10 X B10	B10 X B10.A
23 (92)	101 (336)
103 (133)	117 (150)
24 (39)	354 (771)
19 (64)	205 (455)
48 (138)	265 (509)
32 (90)	119 (299)
99 (291)	125 (435)

In order to confirm the finding of greater numbers of MNC at 9 a.m. on the 10th day in B10 X B10.A than in B10 X B10 conceptuses, the numbers of MNC were counted in additional conceptuses collected at 9 a.m. on the 10th day from 14 further B10 females, seven mated with B10 males and seven with B10.A males (Table 2.3). The cells were indeed found to be present in greater numbers at this time in the B10 X B10A conceptuses than in the B10 X B10 conceptuses (MW, $p = 0.001$).

Mononuclear cells were also counted in B10.A X B10.A and B10.A X B10 conceptuses. These figures, presented in Table 2.4, suggest that greater numbers of MNC may be found at 6 a.m. (MW, $p = 0.05$) and 9 a.m. (MW, $p = 0.05$) but not at 12 noon (MW, $p = 0.5$) in B10.A X B10 than in B10.A X B10.A conceptuses.

The numbers of MNC at 6 a.m., 9 a.m. and 12 noon on the 10th day in the TGC layer of conceptuses of isogenically mated SWR/J and DBA/2J female mice are shown in Table 2.5, the data for B10 and B10.A females from Tables 2.1 and 2.2 being included to facilitate comparison. The numbers found in conceptuses of isogenically mated B10.A, SWR/J and DBA/2J females do not differ significantly from those found in isogenically mated B10 females (KW, $p > 0.025$).

TABLE 2.4

Standardized numbers of mononuclear cells on the 10th day of gestation in conceptuses of B10.A females mated isogenically and with B10 males. The number in brackets is the actual number of mononuclear cells counted. Each entry represents one conceptus.

B10.A X B10.A		B10.A X B10
24 (74)	6 a.m.	109 (249)
37 (139)		151 (304)
58 (166)		97 (189)
6 (40)	9 a.m.	45 (135)
13 (46)		44 (84)
39 (123)		224 (403)
	12 noon	
226 (767)		39 (37)
22 (62)		175 (660)
78 (313)		206 (583)

TABLE 2.5

Standardized numbers of mononuclear cells on the 10th day of gestation in conceptuses of isogenically mated female mice. The number in brackets is the actual number of mononuclear cells counted. Each entry represents one conceptus.

BIO	B10.A	SWR/J	DBA/2J
	6 a.m.		
55 (87)	24 (74)	22 (52)	16 (18)
46 (149)	37 (139)	33 (68)	12 (26)
136 (439)	58 (166)	41 (79)	19 (46)
	9 a.m.		
34 (126)	6 (40)	54 (109)	24 (40)
49 (204)	13 (46)	16 (32)	8 (10)
56 (184)	39 (123)	55 (246)	7 (25)
	12 noon		
111 (620)	226 (767)	30 (34)	27 (52)
17 (45)	22 (62)	12 (14)	78 (80)
174 (463)	78 (313)	37 (81)	63 (107)

DISCUSSION

In the initial experiments, specimens were collected from B10 females, mated isogenically and with B10.A males, from the beginning of the 9th day to the end of the 11th day. This was done to ascertain whether an accumulation of MNC, as was seen in Swiss Webster mice (Dickson, 1979), occurred in these mice and, if so, to study its timing. The time of 9 a.m. (which, as mentioned above, in this study implies a one hour period beginning at 9 a.m.) on the 10th day was confirmed to be the approximate time of greatest accumulation of MNC; all further studies were restricted to specimens collected at 6 a.m., 9 a.m. and 12 noon on the 10th day only. It would be necessary to collect more samples at closer time intervals in any attempt to localize more precisely the time of maximal accumulation.

Careful attention was paid to comparability of counts of MNC numbers, since this study depends on it. The reliability of the counts and thus the probability of detecting a difference, if present, between groups of conceptuses was maximized by choosing those sections containing the largest giant cell area and, therefore, if the distribution of MNC is homogeneous throughout the TGC layer, the greatest number of MNC. Such sections are found at the proximal and distal ends of the conceptus. One from each end of each conceptus was used. The number of giant cell nuclei in these sections was also counted and employed as a means of expressing the area and thus, indirectly, the

volume of the giant cell layer in these sections. The assumption was made that, in general, the ratio of giant cell volume to maternal blood channel volume did not vary significantly from specimen to specimen. Shrinkage following fixation could not affect the count since counting encompassed the whole giant cell layer in the selected sections.

It is interesting to note that the mean numbers of MNC found in the TGC layer (a) in both B10 X B10 and B10 X B10.A conceptuses and (b) on both the 9th and 11th days were in all four cases very similar to one another, being approximately 20 MNC per 50 giant cell nuclei. This suggests that, at least in the B10 X B10 and B10 X B10.A conceptuses and on the 9th and 11th days, there is a basal number of MNC in the TGC layer. It is tempting to speculate that a basal number of MNC might also be present on these days in the TGC layer in other strains of mice.

The finding that, in the B10 X B10 conceptuses, the number of MNC on the 10th day (about 60 per 50 giant cell nuclei) is, on the average, higher than on the 9th and 11th days suggests that the maternal immune system recognizes the presence of a conceptus even though it is virtually genetically identical to the mother. Furthermore, in the B10 X B10.A conceptuses, a very pronounced increase in MNC numbers (to about 240 per 50 giant cell nuclei) is seen at approximately 9 a.m. on the 10th day. This increase appears to be quite rapid, occurring

between 6 and 9 a.m., and rather evanescent, for the numbers of MNC begin to diminish again between 9 a.m. and 12 noon and have fallen back to the basal level by 3 p.m.

The greater increase in MNC numbers in the B10 X B10.A than in the B10 X B10 conceptuses is consistent with observations in allogeneic and syngeneic matings reported by other researchers. For example, more pronounced changes in the numbers of maternal small lymphocytes in mouse bone marrow, spleen, para-aortic lymph nodes and blood occurred in allogeneic than in syngeneic pregnancies, even though the temporal patterns of changes in their absolute numbers were qualitatively similar for both types of pregnancies (Chatterjee-Hasrouni et al., 1980). Similarly, greater proliferations of lymphocytes were observed in allogeneic than in syngeneic matings in iliac and popliteal lymph nodes of rats (McLean et al., 1980) and in para-aortic lymph nodes of mice, hamsters and rats (Beer et al., 1975). The more pronounced alterations that occur as a result of allogeneic mating may result from a maternal response to paternal-type antigens (Chatterjee-Hasrouni et al., 1980). While it is difficult to propose a suitable hypothesis to explain the changes seen in the case of syngeneic matings, a plausible explanation is that they may reflect some common, time-dependent immunological response to auto-antigens associated with trophoblast (Beer et al., 1972) or phase-specific fetal or developmental antigens (Baldwin & Vose, 1974; Chatterjee-Hasrouni et al., 1980).

Even though the number of MNC at 9 a.m. on the 10th day in B10.A X B10 conceptuses is higher than in B10.A X B10.A conceptuses, it is not as high as in B10 X B10.A conceptuses. This may be due, for example, to some inherent strain characteristic or a difference in the timing of maximal accumulation that may be under the influence of the major histocompatibility complex (Verbanac & Warner, 1981). A difference in the H-2 haplotype may, at least in part, be responsible, since conceptuses from both B10 and B10.A females mated with males that differ virtually only at the major histocompatibility locus (H-2) contained higher numbers of MNC at 9 a.m. on the 10th day than those from the corresponding isogenically mated females. However, neither chromosomal segments (Boyse, 1977) that may be attached to either side of the H-2 locus nor a role for the H-Y antigen, with which the variability of MNC counts within groups is consistent, can be excluded.

The possibility that the very high numbers of MNC observed in conceptuses of random-bred Swiss Webster mice (Dickson, 1979) is a strain-specific phenomenon was investigated by looking for it in other strains of mice, namely SWR/J and DBA/2J. These are both inbred strains and are genetically different from one another as well as from B10 (Taylor, 1972). The failure to find any accumulation in conceptuses of isogenically mated SWR/J, DBA/2J and B10 females suggests that it is not a normal occurrence in intra-strain matings of inbred mice. Furthermore, its absence in the inbred SWR/J strain may be due to reduction of genetic heterogeneity at multiple loci (probably

including H-2) in the parent, random-bred, Swiss Webster stock (Rice & O'Brien, 1980).

Descriptions of mononuclear cell accumulations such as those found in this study have not, to my knowledge, appeared previously in the literature describing normal placentation. Minor accumulations in trophoblastic lacunae seem to be present in some illustrations in, for example, Boyd & Hamilton (1970) and Hamilton et al. (1972).

It may be concluded from the results of the present study that the MNC accumulations 1) occur only at approximately 9 a.m. on the 10th day of gestation; 2) are evanescent, that is, within a three hour period their numbers begin to fall toward a baseline number and 3) are associated with maternal-conceptual H-2 disparity. Furthermore, no major strain difference exists in the low number of MNC found in the isogenic conceptuses.

It would be appropriate to further test the association of maternal-conceptual H-2 disparity with high numbers of MNC by asking the question: if both heterozygous and homozygous conceptuses were present, simultaneously, in a homozygous female, would the MNC accumulation exhibit site-specificity? That is, would there be segregation of conceptuses exhibiting high and low numbers of MNC respectively? This problem will be addressed in the next chapter.

CHAPTER 3

Effects of maternal-conceptual H-2 disparity on segregation of conceptuses exhibiting high and low numbers of MNC

INTRODUCTION

In the previous chapter and a subsequent report (Krcek et al., 1982) it was concluded that a difference in H-2 haplotype between the conceptus and its mother may, at least in part, be responsible for the high numbers of MNC, present at 9 a.m. on the 10th day, in the TGC layer. The purpose of the present investigation was twofold: 1) to explore further the association of the MNC accumulations with maternal-conceptual H-2 disparity; 2) to investigate whether the MNC accumulation is a site-specific response. It would therefore be desirable to identify MNC and H-2 haplotype in the same sections of tissue.

H-2 antigens have been identified on various embryonic tissues (Kirkwood & Billington, 1981) and trophoblast (Chatterjee-Hasrouni & Lala, 1979) in tissue cultures and single-cell suspensions, respectively, but the precise identification of two different H-2 haplotypes (i.e. maternal and paternal) on specific cells in the same tissue

sections still remains extremely tenuous. Therefore, a backcross mating design, which has been employed to study the genetics of susceptibility and resistance to allografts (Snell & Stimpfling, 1966), was used to investigate further the association of MNC accumulation with H-2 disparity between mother and conceptus in females homozygous and heterozygous for H-2 and derived from the B10 and B10.A congenic strains.

In a backcross mating between the B10 strain and the F_1 derived from a cross between B10 and B10.A, each female would be expected to contain conceptuses that are homozygous ($H\text{-}2^b/H\text{-}2^b$) and heterozygous ($H\text{-}2^a/H\text{-}2^b$) for H-2 alleles. In the backcross mating of B10 females with F_1 males, it is predicted that, if maternal recognition of H-2 disparity is related to high numbers of MNC, the homozygous ($H\text{-}2^b/H\text{-}2^b$) females will contain both homozygous ($H\text{-}2^b/H\text{-}2^b$) and heterozygous ($H\text{-}2^a/H\text{-}2^b$) conceptuses with low and high numbers of MNC, respectively. It is also predicted that, in the reciprocal mating of F_1 females to B10 males, the heterozygous ($H\text{-}2^a/H\text{-}2^b$) females will contain the same two genotypes of conceptuses but only low numbers of MNC will be found. Furthermore, if a single factor (i.e. recognition of H-2 similarity and dissimilarity) which determines low and high numbers of MNC is segregating, it is predicted that, in the homozygous mothers, the conceptuses containing low and high numbers of MNC will be in a 1:1 ratio.

MATERIALS AND METHODS

The congenic strains B10 and B10.A, differing at the major histocompatibility locus, were obtained from the Jackson Laboratory (Bar Harbor, Maine). The mice were maintained as described previously (Chapter 2).

Female B10 and B10.A mice were mated with B10.A and B10 males, respectively. Regardless of the type of mating, subsequent F_1 progeny were divided into two groups according to their sex. Of these, the F_1 males were backcrossed to B10 females and the F_1 females to B10 males (see Figure 3.1). Seven B10 and nine F_1 females were killed between 9 and 10 a.m. on the 10th day.

The uterine horns were excised and fixed in a gluteraldehyde-formalin solution (see Chapter 2). All conceptual sites were employed when the total number of both uterine horns was less than six. When the total was more than six, half of the sites (rounded up to the nearest whole number when the total was odd) were selected at random. The sites were dehydrated in a graded series of ethyl alcohol solutions, embedded in paraffin wax and sectioned at 5 μm perpendicular to the long axis of the uterus. One section in every 50 was mounted and stained with PAS/diastase and hematoxylin.

Figure 3.1

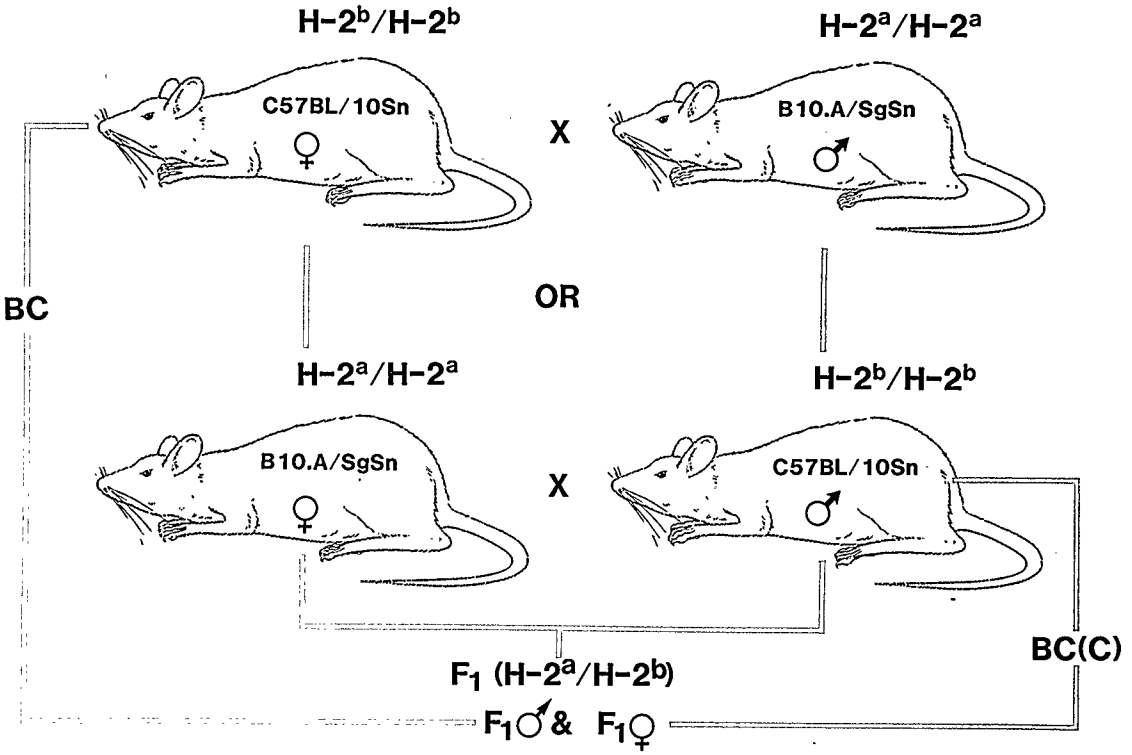
Diagram illustrating the method of backcrossing employed in this experiment

BC = backcross (B10 female X F_1 male)

BCC = backcross control (F_1 female X B10 male)

METHODS

A.


H-2b/H-2b
C57BL/10Sn
X
H-2a/H-2a
B10.A/SgSn
BC
OR
H-2a/H-2a
B10.A/SgSn
X
H-2b/H-2b
C57BL/10Sn
BC(C)
F1 (H-2a/H-2b)
F1♂ & F1♀

The number of mononuclear cells in the maternal blood channels in the TGC layer was counted in two tangential sections of each conceptus, one section at each end, and was standardized by the method described in Chapter 2. The Smirnov distribution free test (Conover, 1980) was used to test for segregation of single gene differences (Mode & Gasser, 1972) and to compare the numbers of MNC obtained from the two types of backcross matings. The data were also displayed by the graphic procedure of ranked normal deviates or rankits (Sokal & Rohlf, 1969).

Backross matings of B10 females and F_1 males are abbreviated to BC; backcross matings of F_1 females and B10 males are abbreviated to BCC.

RESULTS AND DISCUSSION

Tangential histological sections showing low and high numbers of MNC in the maternal vascular channels in the trophoblastic giant cell layer of BC conceptuses are similar to Figures 2.2 and 2.3, respectively, of Chapter 2. A similar appearance to Figure 2.2 was seen with the BCC conceptuses. The ranked standardized numbers of MNC, at 9 a.m. on the 10th day, in the BC and BCC conceptuses from individual litters are shown in Table 3.1.

TABLE 3.1

Rank-ordered standardized numbers of MNC (per 50 giant cell nuclei) in the trophoblastic giant cell layer of BC and BCC conceptuses in each litter examined. Each entry represents one conceptus.

BC (B10 X F_1) CONCEPTUSES FROM HOMOZYGOUS FEMALES			BC (F_1 X B10) CONCEPTUSES FROM HETEROZYGOUS FEMALES		
LITTER NUMBER	NUMBER OF CONCEPTUSES	STANDARDIZED NUMBER OF MNC	LITTER NUMBER	NUMBER OF CONCEPTUSES	STANDARDIZED NUMBER OF MNC
1	6	39, 72, 117, 199, 234, 257	1	5	9, 17, 18, 19, 21
2	5	22, 35, 51, 119, 156	2	5	29, 53, 60, 97, 102
3	6	10, 12, 15, 15, 17, 24	3	4	18, 25, 28, 32
4	5	50, 56, 94, 174, 477	4	6	7, 14, 20, 20, 27, 37
5	1	10	5	1	30
6	3	10, 32, 84	6	1	7
7	6	6, 10, 18, 22, 40, 47	7	5	18, 18, 18, 32, 48
			8	2	29, 30
			9	5	17, 47, 51, 61, 65

Overall, it appears that the numbers in the BC conceptuses (ranging from 6-477) are higher than in the BCC conceptuses (ranging from 7-102). Also, it appears possible that the BC conceptuses may be distributed in two populations, one with low (6-94) and the other with high (117-477) numbers of MNC, while the BCC conceptuses form one population comprised of only low numbers.

As a basis for interpretation of the present data, the numbers of MNC, at 9 a.m. on the 10th day, in 10 B10 (derived from B10 X B10 matings) and 10 F_1 (derived from B10 X B10.A matings) conceptuses from homozygous ($\underline{H\text{-}2^b}/\underline{H\text{-}2^b}$) B10 females from Chapter 2 have been incorporated into the statistical analysis. The homozygous ($\underline{H\text{-}2^b}/\underline{H\text{-}2^b}$) conceptuses (from the B10 X B10 matings) contained low numbers of MNC (ranging from 19 to 103) whereas the heterozygous ($\underline{H\text{-}2^a}/\underline{H\text{-}2^b}$) conceptuses (from the B10 X B10.A matings) contained high numbers (ranging from 101-485). The B10 and F_1 data were combined to create a sample of homozygous and heterozygous conceptuses with low and high numbers of MNC, respectively, in a 1:1 ratio. The observed distribution of the number of MNC in the 32 BC conceptuses (Table 3.1) did not differ significantly ($p > 0.05$) from the expected distribution derived from the combined B10 and F_1 data (Conover, 1980; Mode & Gasser, 1972). Therefore, segregation of allelic differences at the $\underline{H\text{-}2}$ locus in the BC conceptuses in the B10 mothers, which are homozygous $\underline{H\text{-}2^b}/\underline{H\text{-}2^b}$, appears to be responsible for the low and high numbers of MNC.

The observed distribution of the number of MNC in the 34 BCC conceptuses (Table 3.1) was also compared with the combined distribution from the B10 and F_1 data by a one-sided Smirnov test (Conover, 1980). A significant difference ($p < 0.01$) was found, which is consistent with the expectation that the BCC conceptuses would exhibit low numbers of MNC similar to those found in B10 conceptuses. Since the observed distribution of BCC conceptuses differed from the combined B10 and F_1 data but the BC distribution did not, the BC and BCC distributions would be expected to differ from one another. However, no difference was found ($p > 0.05$) (Conover, 1980).

To clarify this unexpected finding, the distribution of the numbers of MNC in the different matings were explored by a graphical analysis. When the ranked order of the 10 B10 and 10 F_1 conceptuses were plotted separately (Figure 3.2), by the method of rankits (Sokal & Rohlf, 1969), on a scale that was linear in terms of numbers of MNC, there was greater systematic deviation of the F_1 data from a fit to a normal distribution. This type of systematic deviation suggested that a logarithmic transformation of the numbers of MNC might improve the fit to a normal distribution. When the B10 and F_1 data are plotted on a log scale (Figure 3.3), they appear to fit separate normal distributions with the same variance (i.e. slope) and means of 40 and 200 MNC, respectively. No test is possible for fit to a normal distribution of the log values of the MNC from the B10 and F_1 conceptuses except the apparent fit by eye. From an exploration of the B10 and F_1 data by

Figure 3.2

Numbers of MNC in B10 and F_1 conceptuses plotted by rankits, using a linear scale.

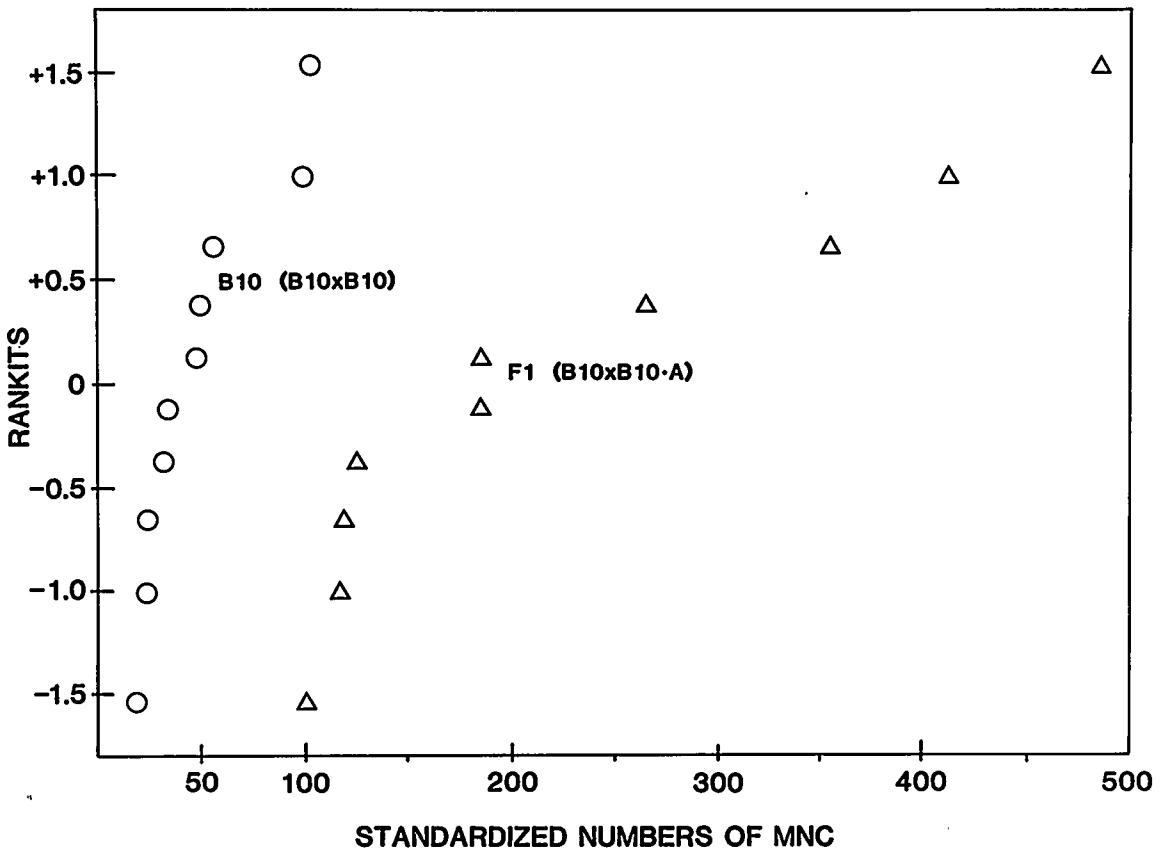
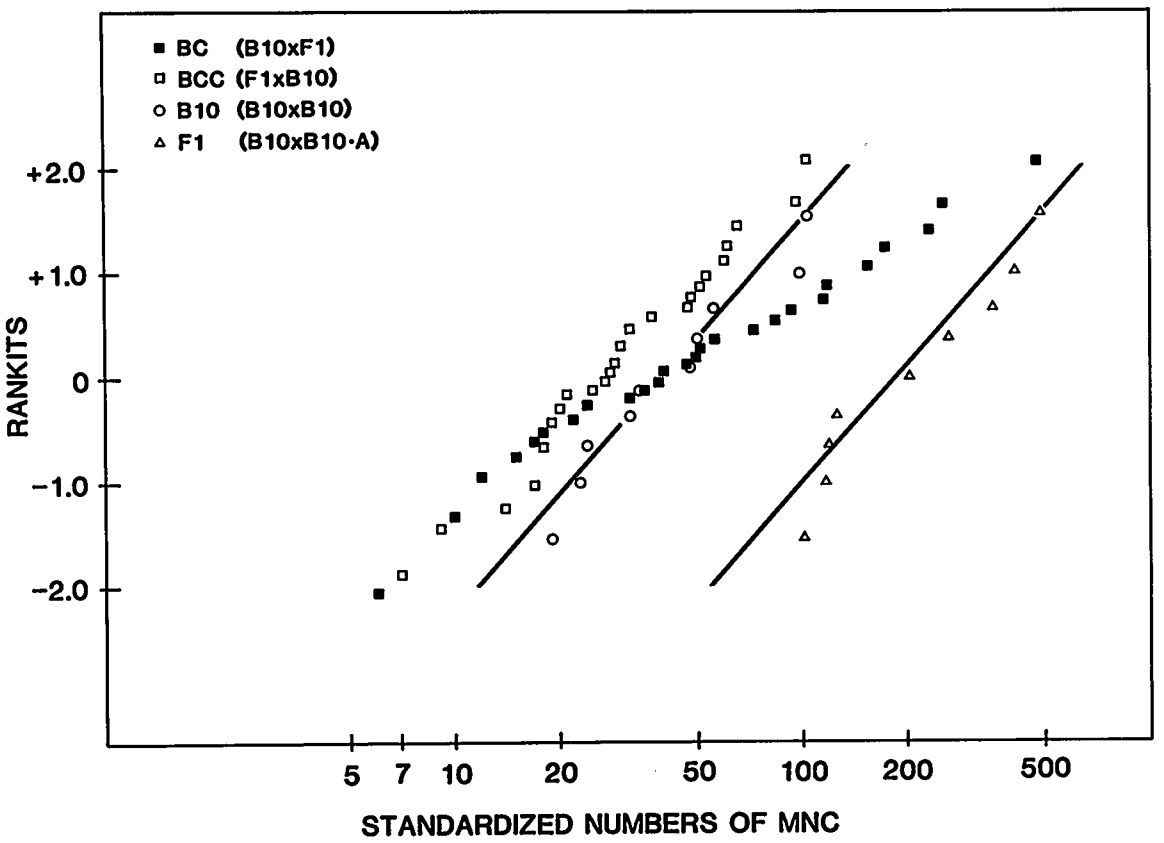

+1.5
+1.0
+0.5
0
−0.5
−1.0
−1.5
RANKITS
B10 (B10xB10)
F1 (B10xB10·A)
50
100
200
300
400
500
STANDARDIZED NUMBERS OF MNC

Figure 3.3

Numbers of MNC in B10, F_1, BC and BCC conceptuses plotted by rankits, using a logarithmic scale.


■ BC (B10xF1)
□ BCC (F1xB10)
○ B10 (B10xB10)
△ F1 (B10xB10·A)
+2.0
+1.0
0
-1.0
-2.0
RANKITS
5
7
10
20
50
100
200
500
STANDARDIZED NUMBERS OF MNC

the rankit procedure (Figure 3.3), the mean numbers of MNC are obtained at 0 rankits and their approximate 95% confidence limits are given by ± (plus and minus) 2 rankits (i.e. ± 2 standard deviation units). From Figure 3.3, the approximate 95% confidence limits of the numbers of MNC in the B10 and F_1 conceptuses are 12-150 and 58-700, respectively, with a region of overlap from approximately 58-150 MNC.

Two suggestions appear to arise from the rankit plots of the log numbers of MNC in the BC and BCC conceptuses (Figure 3.3). The distribution of BC conceptuses appears to depart from a linear graph in a direction suggestive of bimodality, whereas the distribution of BCC conceptuses appears to fit one normal distribution as expected. In addition, the distribution of the BCC conceptuses appears to be shifted toward a lower mean number of MNC than the B10 conceptuses. The reason for this may reside in the interval of 1.5 years between obtaining the B10 and F_1 conceptuses (employed in Chapter 2) and obtaining the BC and BCC conceptuses of this study. The lower end of the distribution of BC conceptuses also appears to be shifted toward a lower number of MNC.

A new basis may exist for comparing the distribution of BC and BCC conceptuses with the earlier data from the B10 and F_1 conceptuses. The mean numbers of MNC from B10 and BCC conceptuses are predicted to be equivalent and their difference in Figure 3.3 is suggested to be temporal. The 95% confidence range of BCC conceptuses (approximately

8-100 MNC) is suggested to be the expected distribution of B10 conceptuses rather than the previously observed range of 12-150 MNC. If there was a similar temporal effect on the F_1 conceptuses and this effect was additive, the expected 95% confidence range would be approximately 40-500 MNC rather than the previously observed range of 58-700 MNC. This would imply that there would be an area of overlap between approximately 40 and 150 MNC between the distribution of the B10 and F_1 conceptuses if they had been collected contemporaneously with the BC and BCC conceptuses in the present study.

From Table 3.1, 8 of the 32 BC conceptuses fall within this region of overlap, 16 lie below it in the predicted exclusive B10 range and 8 lie above it in the predicted exclusive F_1 range. If the BC conceptuses in this region of overlap are excluded, there appears to exist a 16:8 ratio of conceptuses containing low and high numbers of MNC, respectively, which does not differ from an expected 1:1 ratio ($p > 0.10$, Chi-squared test). The 8 BC conceptuses in the proposed region of overlap cannot be assigned exclusively to either the expected low or high group. Any further analysis with the present sample size should probably be resisted.

The key to an experiment of this nature, using the B10 and B10.A strains of mice congenic for H-2, lies in the ability to discriminate between the B10-like and the F_1-like conceptuses (i.e. $\underline{H\text{-}2^b}/\underline{H\text{-}2^b}$ and $\underline{H\text{-}2^a}/\underline{H\text{-}2^b}$, respectively). By the quantitative methods employed in

this study, these two groups are not mutually exclusive. What is required is a method to identify the specific H-2 haplotype (H-2b or H-2ab) of individual conceptuses in the same tissue sections in which the numbers of MNC are also ascertained. In addition, the proposed apparent shift in mean number of MNC with time (Figure 3.3) suggests that conceptuses of different strains and hybrids should be obtained contemporaneously.

The data, taken as a whole, appear to support the original hypothesis that H-2 haplotype and numbers of MNC are associated, although this cannot be stated unequivocally. Also, since BC conceptuses containing high and low numbers of MNC were found in the same homozygous female, the maternal system appears to respond to each conceptus individually.

It appears that high numbers of MNC in the TGC layer are associated with maternal-conceptual histoincompatibility and that the possibility exists that the MNC accumulation may be a manifestation of maternal immune response to the presence of a histoincompatible conceptus. If the MNC accumulation that is present in a first pregnancy is indeed a primary immune response resulting from first contact with conceptual antigenicity, a modification of the MNC accumulation should be observed following a second exposure to the same conceptual antigens (i.e. in a second pregnancy). The results of a preliminary experiment to test this hypothesis will be presented in the following chapter.

Chapter 4

Numbers of mononuclear cells in the trophoblastic giant cell layer in second pregnancy

INTRODUCTION

Thus far, it has been shown that MNC are present in higher numbers, at approximately 9 a.m. on the 10th day of gestation, in F_1 conceptuses, heterozygous for H-2, than in inbred homozygous conceptuses when either is present in a mother of homozygous H-2 haplotype. Furthermore, it was demonstrated that this association of high and low numbers of MNC with heterozygous and homozygous conceptuses, respectively, obtains when both were present simultaneously in a homozygous mother. Moreover, the accumulation of MNC in high numbers appears to be a site-specific response.

It is possible that the MNC, which may be lymphocytes, are involved in the maternal immune response to the conceptus. If their appearance at 9 a.m. on the 10th day in a first pregnancy represents a primary immune response, it is possible that this timing may be modified in a second pregnancy of identical genotype. If a second

pregnancy of identical genotype is analogous to a secondary immune response, the prediction is that the accumulation of MNC would not be found at 9 a.m. on the 10th day of gestation, having taken place earlier (possibly by as much as several days).

Rather than search for the exact time of MNC accumulation in second pregnancy, this preliminary study compared first and second pregnancies at 9 a.m. on the 10th day.

MATERIALS AND METHODS

The congenic strains B10 and B10.A, differing at the major histocompatibility (H-2) locus, were obtained from the Jackson Laboratory (Bar Harbor, Maine). They were maintained as described previously (Chapter 2).

Female B10 mice were mated with B10.A males, allowed to give birth to a first litter and then re-mated randomly with B10.A males. Five B10 females carrying a second litter were killed at 9 a.m. on the 10th day.

The uterine horns were excised and fixed in a gluteraldehyde-formalin solution (see Chapter 2). One conceptus per mouse was

dehydrated in a graded series of ethyl alcohol solutions, embedded in paraffin wax and sectioned at 5 μm perpendicular to the long axis of the uterus. One section in every 50 was mounted and stained with PAS/diastase and haematoxylin.

The number of mononuclear cells in the maternal blood channels in the TGC layers was counted in two tangential sections of each conceptus, one section at each end. As before, the number of giant cell nuclei in the same sections was used to standardize for variations in the area of the sections. A comparison of numbers of MNC in conceptuses from B10 X B10.A second pregnancies and in those from B10 X B10.A first pregnancies (Chapter 2) was made with the non-parametric Mann-Whitney U-test (Siegel, 1956).

RESULTS

Tangential histological sections showing MNC in the maternal blood channels in the trophoblastic giant cell layer of conceptuses from B10 X B10.A first and second pregnancies are similar to Figures 2.3 and 2.2, respectively.

The numbers of MNC at 9 a.m. on the 10th day, standardized per 50 giant cell nuclei, in B10 X B10.A first and second pregnancies are given in Table 4.1.

Table 4.1

Standardized numbers of mononuclear cells at 9 a.m. on the 10th day of gestation in conceptuses from first and second pregnancy B10 X B10.A matings.

The number in brackets is the actual number of mononuclear cells counted. Each entry represents one conceptus.

B10 X B10.A 1st pregnancy	B10 X B10.A 2nd pregnancy
411 (977)	29 (101)
205 (935)	26 (87)
485 (2165)	127 (281)
101 (336)	47 (186)
117 (150)	18 (75)
354 (771)	
205 (455)	
265 (509)	
119 (299)	
125 (435)	

The numbers found in the B10 X B10.A second pregnancies are significantly lower than those found in the B10 X B10.A first pregnancies ($p < 0.01$).

DISCUSSION

This preliminary study has been done in an attempt to observe the effects of a second pregnancy, identical in genotype to the first, on the high numbers of MNC in the TGC layer that are seen in conceptuses that possess maternal-conceptual H-2 disparity. The results of the current investigation indicate that the numbers of MNC in a B10 X B10.A second pregnancy are significantly lower than those found in a B10 X B10.A first pregnancy (Chapter 2).

The proposal of an explanation for this decrease in the number of MNC in the B10 X B10.A second pregnancies is, at present, difficult. However, the decrease is consistent with the hypothesis that the MNC accumulation represents a primary immune response, which would then be modified during a secondary response.

In the introductory chapter, a description was given of the two main types of graft rejection process. The so-called first set response occurs almost 10 days after a first graft from an unrelated

donor and the second set response occurs in about 7 days in an animal which had previously received a graft from the same unrelated donor (Weir, 1977). This phenomenon of first and second set rejection can be compared to primary and secondary immunization in which accelerated secondary response is brought about by stimulation of an already sensitized immune system.

Evidence exists that pregnancy alters maternal immune responsiveness. For example, successive pregnancies result in an impairment of the ability of female mice to reject grafts of the paternal genotype (Prehn, 1960; Breyere & Barrett, 1961; Breyere & Burhoe, 1963). This observation is compatible with the hypothesis that mechanisms similar to tolerance or enhancement may be operative during pregnancy. Two questions are now raised. 1) Is there a cellular immune response to pregnancy? 2) If so, is it also affected by successive pregnancies?

The answer to the first question appears to be yes. Support for this comes from reports on the enlargement, due to cellular proliferation, of regional lymph node and spleen during pregnancy (e.g. Maroni & de Sousa, 1973; Beer et al., 1975) and the work presented in Chapters 2 and 3 of this thesis. The answer to the second question also may be yes, as seen from the results of the present experiment.

The question of the type of cell(s) involved in the MNC response and their function is now raised. The possibility does exist that

these cells are lymphocytes. A cellular reaction, resembling a classical rejection reaction involving lymphocytes, is seen during the regression of equine endometrial cups, which are thought to be outgrowths of trophoblast (Allen & Moor, 1972; Allen, 1979). Rejection is apparently more rapid both in successive pregnancies, which could be due to a second-set rejection response, and in hybrid pregnancies between the horse and donkey, which could be explained through the wider antigenic differences between mother and fetus (Edwards & Coombs, 1975). If the phenomenon of MNC accumulation in the TGC layer of the mouse represents some type of graft rejection response, the low numbers of MNC at 9 a.m. on the 10th day of gestation in a second pregnancy may be due to occurrence of the accumulation at some other time, possibly earlier as may be found in a second-set rejection response. In order to investigate this question further, it will be necessary to search for MNC accumulation in second pregnancies from the time of implantation on the 5th day through to day 10.

It would also be interesting to study the accumulation in successive pregnancies of B10 females mated with males of new H-2 haplotype in every third pregnancy to see whether the accumulation reappears at 9 a.m. on the 10th day with each new antigenicity.

Even though the reason for modification of MNC response during second pregnancy remains unknown, the results of this experiment are consistent with the hypothesis that the MNC accumulation in the TGC

layer of conceptuses heterozygous for H-2 and present in a mother that is homozygous for H-2 is an immune-type response.

The next two chapters of this thesis will be devoted to another cell type, the metrial gland cell, which also may have some function in the maternal immune response to the presence of a conceptus. The first of these (Chapter 5) will cover the pertinent literature on these cells while the second (Chapter 6) will contain a study of the effects of histoincompatibility and strain genotype on the numbers of these cells in the placental labyrinth.

CHAPTER 5

Metrial gland cells and pregnancy: a general introduction

The metrial gland, which is located between the inner circular and outer longitudinal muscle layers in the mesometrial triangle of the gravid uterus, has been described in early literature on the placenta (Duval, 1891; Weill, 1919). It has been found in rats (Dickson & Bulmer, 1961), mice (Smith, 1966), guinea pigs and rabbits (Asplund et al., 1940).

One cell type found in the metrial gland is called the metrial gland cell (MGC). A typical fully developed MGC exhibits four major features: (1) it is large (18-35 μm in diameter), (2) it is binucleate (Velardo et al., 1953) and (3) it exhibits a perinuclear aggregation of acidophilic PAS-positive, diastase-fast granules that are (4) surrounded by a rim of apparently clear cytoplasm with a high glycogen content. Cells that are histochemically and morphologically similar to those of rodent metrial glands have been described in the human (Dallenbach-Hellweg & Nette, 1964; Dallenbach-Hellweg et al., 1965) and the monkey (Dallenbach-Hellweg et al., 1966) uterus. Metrial gland cells are also found in the decidua basalis. As a matter of fact, they are present there earlier than in the metrial gland in both

the rat (Dickson & Bulmer, 1961) and the mouse (Stewart & Peel, 1978). The name of these cells is therefore inappropriate.

The origin of these cells has been the subject of considerable debate. It has been suggested that they may be derived from fusiform stromal cells of the uterus (Jenkinson, 1902; Dickson & Bulmer, 1971; Larkin, 1972). Another suggestion was that they may arise from mesenchymal cells or fibroblasts (Velardo et al., 1953; Ellis, 1957; Larkin & Schultz, 1968; Larkin & Cardell, 1971) of maternal origin (Baker, 1948; Dallenbach-Hellweg et al., 1965).

Histochemical and autoradiographic studies on metrial gland formation (Larkin & Schultz, 1968) indicated that the metrial gland is formed by proliferation of cells in the mesometrial triangle. This finding seems to negate the suggestion that decidual tissue migrates into the mesometrial triangle to form the gland (Selye & McKeown, 1935). The results of experiments (Bulmer & Peel, 1974) on nuclear labelling associated with development of the metrial gland are in agreement with those of Larkin & Schultz (1968). However, the studies of Bulmer & Peel (1974) also revealed that proliferation of undifferentiated cells, first in the central zone of the mesometrial decidua and later in the mesometrial triangle, is followed by differentiation into characteristic granulated cells. This finding may be interpreted as evidence in support of differentiation of metrial gland cells in the decidua basalis.

A further proposal was that the precursors of metrial gland cells were endothelial and adventitious cells of vessels, cells which migrate into the mesometrial triangle and smooth muscle layers of the uterine wall (Selye & McKeown, 1935). Others have taken the opposite view that metrial gland cells may pass through the endovascular plasmodium-lined walls of the placental supply arteries and migrate in the latter to lodge in the junctional zone of the placenta and become its glycogen cells (Seyle & McKeown, 1935; Pritchard, 1947; Bridgman, 1948a,b). Dickson & Bulmer (1961) stated that metrial gland cells appear to be of maternal origin, but this is by no means certain, it being quite possible to interpret the morphological evidence as indicating a trophoblastic origin from the endovascular plasmodium.

A current and perhaps the most interesting theory on the origin of metrial gland cells is that, in the mouse, they arise from cells of the lymphocytic series, indicating a maternal origin (Smith, 1966). Electron microscopic studies have supported Smith's proposal, since cell types which morphologically exhibited intermediate stages from a cell identical in appearance to a small lymphocyte (Zucker-Franklin, 1969) to a characteristic granulated MGC have been found in the metrial gland (Peel & Bulmer, 1977).

The function of the metrial gland and its typical granulated cells has been a matter of much speculation, but no hypothesis has found wide acceptance. It has been suggested that these cells (1)

provide nutrients to the developing embryo (Selye & McKeown, 1935; Selye et al., 1942; Bridgman, 1948a, b); (2) liberate a holocrine secretion facilitating disruption of the muscle coat during the expansion of the uterine wall (Bloch, 1964) or (3) produce relaxin (Velardo et al., 1953; Dallenbach-Hellweg et al., 1965; Wislocki et al., 1957). None of these ideas, however, has been proven or is considered generally acceptable.

The most convincing evidence in favour of relaxin production by metrial gland cells was its demonstration in these cells by immunofluorescence (Dallenbach-Hellweg et al., 1965). However, the relaxin might have been simply a storage material derived from a primary source elsewhere rather than a hormone synthesized by the metrial gland cells (Bulmer, 1968b). Furthermore, the cytoplasmic granules appear to consist of an alkaline protein conjugated with a mucopolysaccharide (Wislocki et al., 1957) whereas relaxin is a simple protein (Frieden & Hisaw, 1953). It was concluded from negative bioassay studies, even at high concentrations of metrial gland extracts, that the metrial gland cells were neither a significant source of, nor a store for, biologically active relaxin during pregnancy (Bloom et al., 1958; Kroc et al., 1959; Larkin, 1974).

A luteotropic function for the acidophilic granules in these cells has also been suggested (Dickson & Bulmer, 1961). Light microscopic studies demonstrated the presence of lysosomal enzymes in

metrial gland cells (Bulmer, 1967, 1968a,b).

Perhaps the most attractive hypothesis for the function of metrial gland cells is that they may have an immunological role that is related to the survival of the fetus as a homograft. Support for this hypothesis is the evidence in favour of derivation of metrial gland cells from lymphocytes (Smith, 1966; Peel & Bulmer, 1977). In addition, cytoplasmic immunoglobulin is found in these cells (Bulmer & Peel, 1977). This may be due to synthesis in situ or to endocytosis of immunoglobulins that are produced elsewhere, although absence of any evidence of endocytotic activity suggests the former (Bulmer & Peel, 1977; Sharma & Peel, 1979). Also, metrial gland cells are equipped to synthesize and package a protein for secretion (Larkin & Flickinger, 1969; Meier & Cardell, 1968) and morphological evidence of granule release from these cells has been demonstrated (Larkin, 1972). Previous studies (Baker, 1948; Larkin & Schultz, 1968), however, suggested that granules are not actively secreted by intact metrial gland cells but rather that their release is accomplished by the breakdown of the cells in atretic areas during involution of the metrial gland.

Although an immunological role has been suggested for metrial gland cells, the differentiation of the gland is not necessarily associated with the presence of a conceptus, for it develops in association with deciduomata in induced pseudopregnancy in the rat and

displays morphological and cellular components that are very similar to the metrial gland of pregnancy (Velardo et al., 1953; Ellis, 1957). Furthermore, there is an orderly sequence of events in decidual development and involution during pseudopregnancy (Velardo et al., 1953). The reaction occurs first in the antimesometrial region, then the mesometrial area and lastly in the mesometrium. Involution occurs in the same order so that at termination of prolonged pseudopregnancy the only apparently functional decidual tissue is the metrial gland.

The presence of metrial gland cells in the lumina of blood vessels has been a matter of dispute. These cells were observed entering the lumina of maternal vessels supplying the placenta and migrating along them on the 9th and 10th days in the rat (Bridgman, 1948a,b). However, Larkin & Cardell (1971) could find no such evidence. Metrial gland cells were found very infrequently in these vessels and not at all in the placenta of the rat, although they were found in the ectoplacental cone (Bulmer & Dickson, 1960; Dickson & Bulmer, 1961). They were also observed in the process of passing through the endovascular plasmodium-lined wall (Dickson & Bulmer, 1961).

In a recent study (Stewart & Peel, 1978), in mice, granulated metrial gland cells were commonly seen after day seven of gestation in the blood vessels of the metrial gland, in the lumen of blood vessels of the decidua basalis, in the maternal vessels of the labyrinth, and in the maternal vascular spaces around the early conceptus. Metrial

gland cells were also seen apparently traversing between endothelial cells of blood vessels in the decidua basalis and the metrial gland (Stewart & Peel, 1978). The position of the granulated cells in the mesometrial aspect of the decidua and their association with, and appearance in, maternal vessels provide them with an easy and efficient access to the placenta. Stewart & Peel (1978) further state that metrial gland cells are seen in the lateral sinusoids which carry blood away from the implantation site (Holmes & Davies, 1948).

In random bred Swiss-Webster/ALAS and C57BL/HPB mice, comparative estimates were made of the number of metrial gland cells in maternal vessels of the chorio-allantoic placenta and maternal lungs of both strains from the 11th to the 16th day of gestation (Dickson, 1980). There was a greater accumulation of these cells in the maternal labyrinthine vessels in Swiss-Webster than in C57BL mice and the accumulation in these vessels rose to a higher peak on the 15th day in the Swiss-Webster strain. The fate of these cells after entering the blood vessels is uncertain. Some pass to the lungs (Dickson, 1980). Moreover, more metrial gland cells were found in sections of lung from C57BL mice than from Swiss-Webster mice on day 11 of gestation (Dickson, 1980). After day 11 they were not common in lungs of either strain. The significance of these observations is unknown.

It is possible that the greater number of MGC in the Swiss-Webster labyrinth than in the C57BL/HPB labyrinth is associated with

the greater genetic heterogeneity of the former, as was proposed in the previous studies dealing with MNC accumulations. If this were the case, it would be consistent with the hypothesis that metrial gland cells may have some immunological function during pregnancy. In the following chapter an experiment will be presented which examines the effects of maternal-conceptual histoincompatibility and strain genotype on the numbers of MGC in the placental labyrinth.

CHAPTER 6

Genetic control of the differences in the numbers of metrial gland cells in the placental labyrinth

INTRODUCTION

As mentioned previously (Chapter 5), metrial gland cells, which are found in the placental labyrinth of the pregnant mouse, may be derived from a cell of the lymphocyte series (Smith, 1966; Peel & Bulmer, 1977; Stewart & Peel, 1977). They may possibly have a role in the immunology of pregnancy, perhaps in the capacity of carrying a blocking antibody to the trophoblast (Bulmer & Peel, 1977).

It was noted (Stewart & Peel, 1978) that, in the mouse, the maternal vessels of the labyrinth commonly contain metrial gland cells on the 11th day of gestation (equivalent to the twelfth day in the present study). Swiss-Webster random bred mice have, overall, more metrial gland cells, from the 11th to the 16th day of gestation, in the maternal interchange vessels of the chorioallantoic placenta than do C57BL/HPB female mice (Dickson, 1980). Consistent with the hypothesis that these cells may play a role in the immunology of

pregnancy, it was postulated that this difference in numbers may reflect different degrees of conceptual antigenicity of paternal origin (Dickson, 1980; Dickson & Krcek, 1981).

The current study was undertaken to test the hypothesis that, like the numbers of MNC in the TGC layer (Chapters 2 and 3), the numbers of metrial gland cells in the maternal vessels of the placental labyrinth may also be affected by maternal-conceptual H-2 disparity. A strain survey was included to determine the effects of strain genotype since the possibility exists that differences in numbers of MGC may be solely a strain-related phenomenon.

It has been suggested that maternal-conceptual genetic disparity may account for the finding of a heavier placenta in inbred interstrain matings than in corresponding inbred matings (Billington, 1964; Hetherington, 1971, 1973). Furthermore, it has been suggested that placental weight may be influenced by H-2 disparity between mother and conceptus (Billington, 1964; James, 1965). However, increased placental weight was not found when maternal-fetal disparity existed only at the H-2 or H-3 locus (Finkel & Lilly, 1971; Hetherington, 1973) and genotype-dependent differences in placental weight could not be accounted for simply by the presence or absence of maternal-conceptual H-2 disparity (Blakley, 1978). The measurement of labyrinthine areas is included in this study since the numbers of MGC should be standardized in relation to placental size (Dickson, 1980). This provided

the opportunity to observe the effects of maternal-conceptual H-2 disparity and strain genotype on the size of placenta, specifically its labyrinth, as measured in histological sections.

The effects of H-2 disparity were studied using the B10 and B10.A histocompatibility congenic resistant strains, which differ only at the H-2 locus (i.e. $H\text{-}2^b/H\text{-}2^b$ and $H\text{-}2^a/H\text{-}2^a$, respectively), while the effect of strain genotype was investigated by comparing isogenic matings of the B10 and B10.A strains with the SWR/J and DBA/2J strains, which, according to Taylor (1972), are genetically distinct from each other.

MATERIALS AND METHODS

Mice of the SWR/J and DBA/2J strains were obtained from the colony of Dr. F. G. Biddle at the University of Calgary. The congenic strains B10 and B10.A were obtained from the Jackson Laboratory (Bar Harbor, Maine). The animals were maintained as described previously (Chapter 2).

Female B10, B10.A, SWR/J and DBA/2J mice were mated with B10, B10.A, SWR/J and DBA/2J male mice, respectively. Female B10 and B10.A mice were also mated with B10.A and B10 males, respectively. Three

females from each mating were killed at noon from the eleventh to the sixteenth days of gestation.

The histological and quantitative methods that are described below are based on similar methods employed in a previous investigation of this nature (Dickson, 1980). The uterine horns were excised and fixed in a gluteraldehyde-formalin solution (see Chapter 2). One randomly selected conceptus per mouse was dehydrated in a graded series of ethyl alcohol solutions, embedded in paraffin wax and sectioned at 5 μm perpendicular to the long axis of the uterus. One section in every 50 was then mounted and stained with PAS/diastase and hematoxylin.

Metrial gland cells were identified by observation of at least two of the following characteristics: large size (Bridgman, 1948a); binuclearity; circumnuclear ring of PAS-positive, diastase-fast granules (Wislocki et al., 1957); the presence, adjacent to the nuclei and inside the ring of granules, of a structureless area that by comparison with electron micrographs (Larkin, 1972) appears to be the Golgi region.

Metrial gland cells in the maternal labyrinthine vessels of the placenta were counted in 5 PAS/diastase stained sections spaced 250 μm apart. The middle section transected the attachment of the umbilical vessels.

In order to make comparisons between the numbers of metrial gland cells in the various labyrinths, the counts were standardized in relation to placental size (Dickson, 1980). The area of the placental labyrinth in each section in which metrial gland cells were counted was measured, in an image projected at a known magnification by a camera lucida, with an electronic planimeter (Numonics). Major maternal and embryonic vessels and large tongues of junctional zone trophoblast projecting into it were excluded. The areas of these sections were added together to give, for each conceptus, the total area of labyrinth in which metrial gland cells were counted. The number of metrial gland cells per labyrinth, taking into account the area measured, was then standardized in accordance with the formula

$$\frac{\text{Number of metrial gland cells X } 10^3}{\text{Sum of areas (sq.mm.) of all 5 sections}}$$

The multiplier 10^3 was included to make the quotients whole numbers.

The non-parametric Mann-Whitney U-test (Siegel, 1956) was used for statistical analysis. A graph of the mean standardized number of metrial gland cells per day, from the eleventh to the sixteenth days, for each strain mating is included to aid in the interpretation of the data.

RESULTS

An example of a metrial gland cell in a maternal labyrinthine vessel is seen in Figure 6.1.

The numbers of metrial gland cells in the maternal labyrinthine vessels from the eleventh to the sixteenth day of gestation are found in Table 6.1. One labyrinth, indicated by an asterisk in the table, has been omitted because the development appeared to be retarded. No comparisons were made using the figures from Table 6.1 because, if metrial gland cells were uniformly distributed in the same density in the labyrinth, the number counted in a fixed number of consistently placed and equally spaced sections through a labyrinth would depend on its size (Dickson, 1980). Therefore, the sums of the areas of the labyrinth measured in the central and two sections on either side of it (each 250 μm from the central one), in which metrial gland cells were counted, are presented in Table 6.2. Also included is a table of the means, for each strain mating, for each day (Table 6.3) and a graph of these means (Figure 6.2).

When the areas of the B10 X B10 labyrinths are compared with those of the B10 X B10.A labyrinths, the latter have a larger mean area on all days except the eleventh and thirteenth, when the former

Figure 6.1

An example of a metrial gland cell (MGC) in a maternal labyrinthine vessel. X 1000

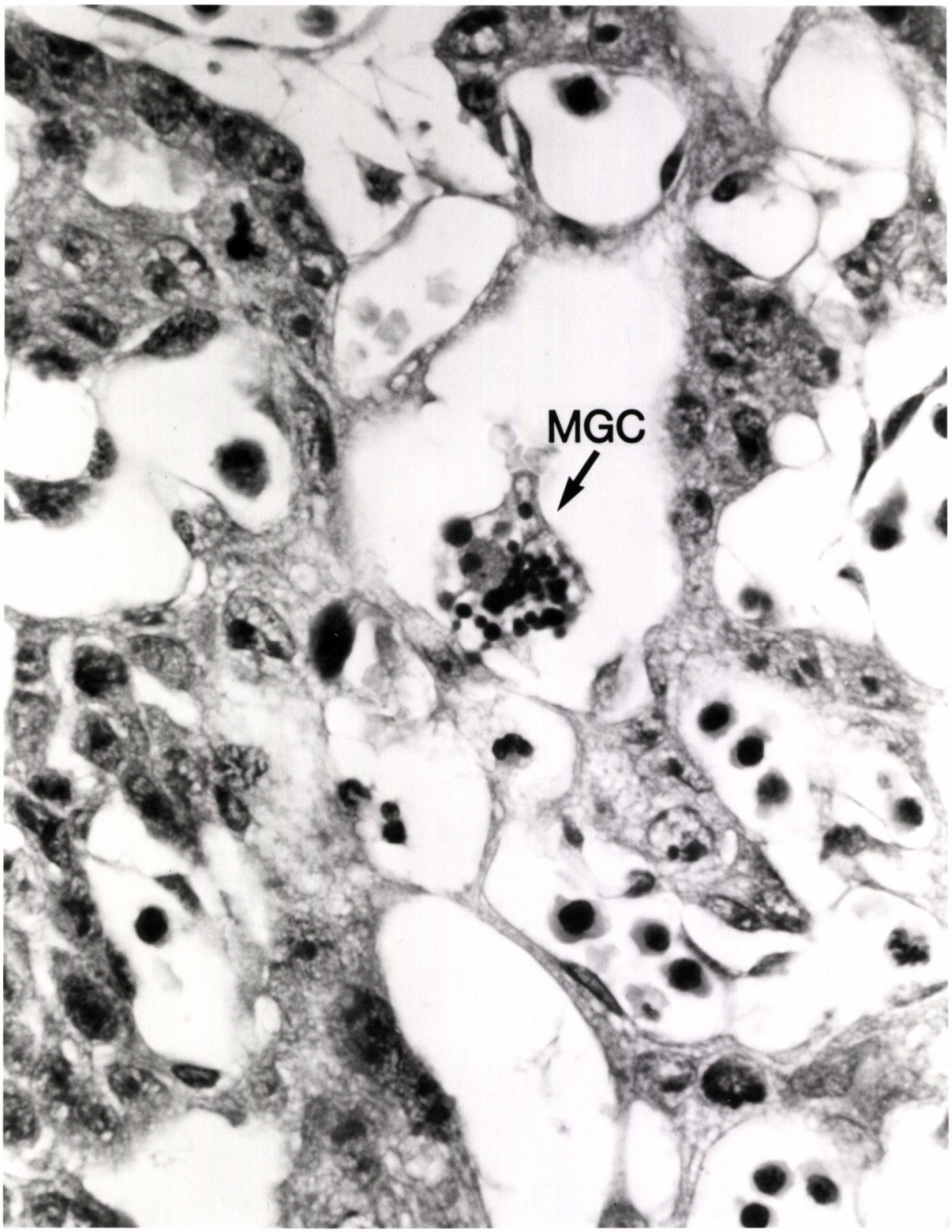

MGC

TABLE 6.1

Number of metrial gland cells in maternal blood vessels of the placental labyrinth from the eleventh to the sixteenth day of gestation. Each entry refers to one mouse

Day	B10 X B10	B10 X B10.A	B10.A X B10.A	B10.A X B10	SWR/J X SWR/J	DBA/2J X DBA/2J
11	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	*	0	0
12	1	2	1	0	1	2
	2	0	0	0	0	7
	5	0	0	0	0	2
13	3	2	2	0	16	0
	5	3	4	0	16	3
	2	3	0	0	9	1
14	3	1	3	6	7	7
	3	4	7	2	38	1
	12	2	8	1	18	12
15	19	0	2	20	56	7
	1	15	17	3	44	0
	26	22	7	9	12	8
16	13	16	8	11	11	5
	19	15	0	1	8	1
	14	18	17	6	9	0

* possible delayed development

TABLE 6.2

Sum of areas (sq. mm.) of the five sections of placental labyrinths in which metrial gland cells were counted. Each entry refers to the mouse in the corresponding position in Table 1.

Day	B10 X B10	B10 X B10.A	B10.A X B10.A	B10.A X B10	SWR/J X SWR/J	DBA/2J X DBA/2J
11	128.1	67.5	36.4	68.4	89.7	66.1
	136.0	122.3	37.5	46.8	96	24.3
	60.0	76.1	35.5	*	65.4	137.9
12	89.0	152.8	82.5	150.6	124.4	340.9
	92.2	140.5	84.1	141.6	157.9	172.2
	181.0	124.9	93.3	125.5	119.3	156.9
13	183.6	208.4	274.5	288.8	382.3	196.9
	272.2	239.4	209.6	185.1	324.5	199.5
	229.9	233	235.9	291.0	386.7	206.7
14	250.4	461.6	314.9	471.2	474	451.4
	308.5	281.5	312.3	262.7	489.5	604.5
	377.5	337.3	364.6	437.2	454.7	457.9
15	237.2	509.1	322.7	389.4	498.2	631.6
	453.5	361.9	378.1	408.2	406.8	446.8
	407.2	372.2	416.4	451.4	492.9	768.2
16	458.1	553.5	390.4	303.6	591.9	461.4
	334.7	407.1	341.3	468.2	647.7	494.5
	311.5	421.2	350.9	286.8	498.7	392.5

* possible delayed development

TABLE 6.3

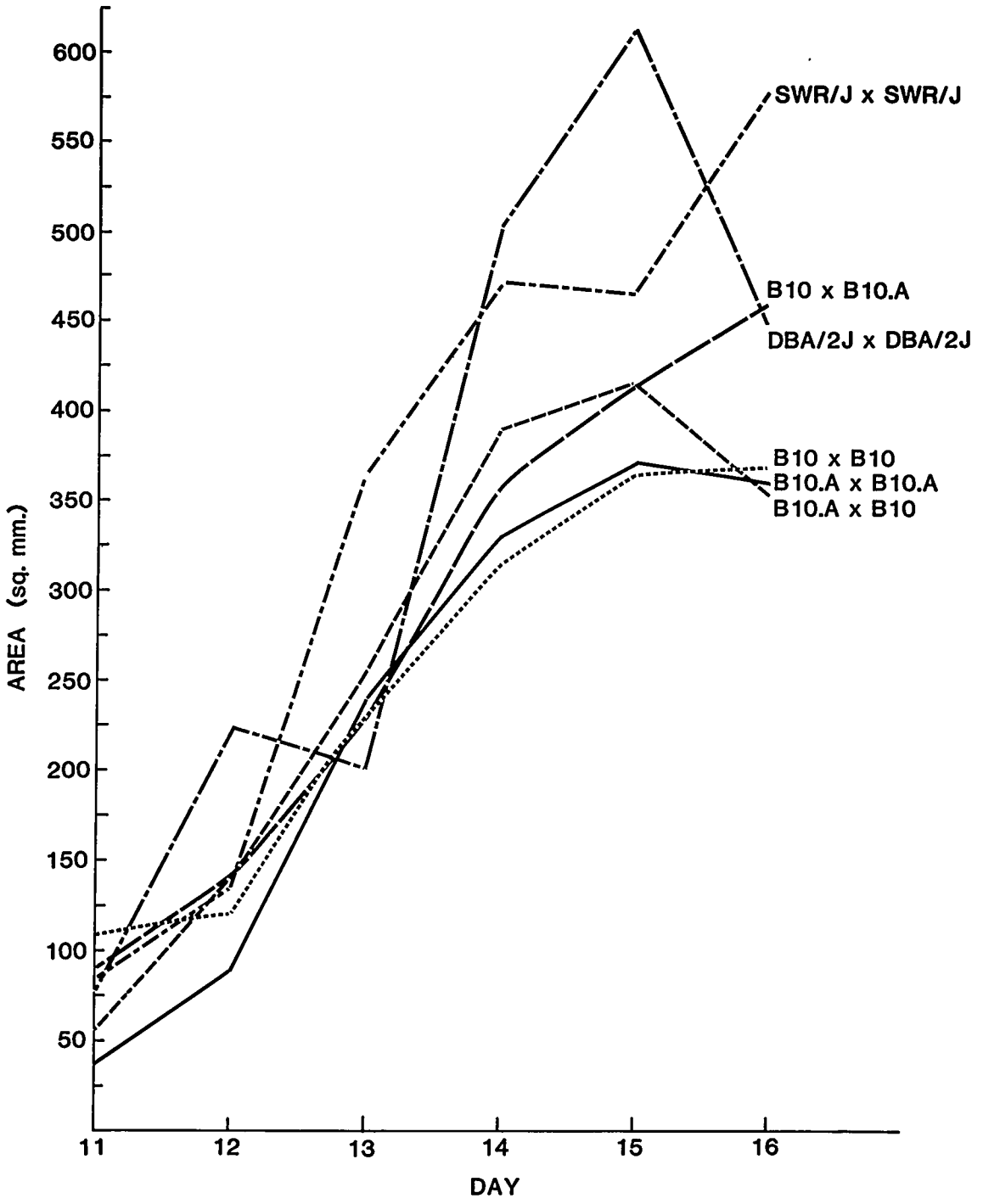
Mean of areas (sq. mm.) of the three placental labyrinths in which metrial gland cells were counted.

Day	B10 X B10	B10 X B10.A	B10.A X B10.A	B10.A X B10	SWR/J X SWR/J	DBA/2J X DBA/2J
11	108.0*	88.6	36.4	57.6	83.7	76.1
12	120.7	139.4	86.6	139.2	133.8	223.3*
13	228.5	226.9	240.0	254.9	364.5*	201.0
14	312.1	360.1	330.6	390.3	472.7	504.6*
15	365.9	414.4	372.4	416.3	465.9	615.5*
16	368.1	460.6	360.8	352.8	579.4*	449.4

* largest mean area for day of gestation

Figure 6.2

The mean area, in square millimetres, for three labyrinths (one per mouse) for each day from the 11th to 16th days of gestation


600
550
500
450
400
350
300
250
200
150
100
50
AREA (sq. mm.)
11
12
13
14
15
16
DAY
SWR/J x SWR/J
B10 x B10.A
DBA/2J x DBA/2J
B10 x B10
B10.A x B10.A
B10.A x B10

exhibits the larger area (Table 6.3; Figure 6.2). When the B10.A X B10.A labyrinths are compared with B10.A X B10 labyrinths, the latter is seen to exhibit larger mean areas on all days except the sixteenth (Table 6.3; Figure 6.2).

Comparison of the overall means of areas for all the strain matings reveals that the largest area is exhibited by the SWR/J X SWR/J labyrinths.

Finally, in Table 6.3, the largest mean area, for each day, of all the strain matings is indicated by an asterisk. It is seen that the largest mean size on days 12, 14 and 15 is seen in the DBA/2J X DBA/2J mating, on day 13 and 16 in the SWR/J X SWR/J mating and on day 11 in the B10 X B10 mating.

The number of metrial gland cells per labyrinth, taking into account the area measured, is seen in Table 6.4. A graph of the mean standardized numbers of metrial gland cells per day for each strain is presented in Figure 6.3. Inspection of Table 6.4 and Figure 6.3 suggests that, from the eleventh to the fifteenth day, the number of metrial gland cells in the placental labyrinth increases in all strain matings except the isogenic DBA/2J mating. In the last, the number of metrial gland cells appears to decrease slightly on the fifteenth day. Furthermore, from the fifteenth to the sixteenth day, there is a decrease in numbers of metrial gland cells in all strain matings except

TABLE 6.4

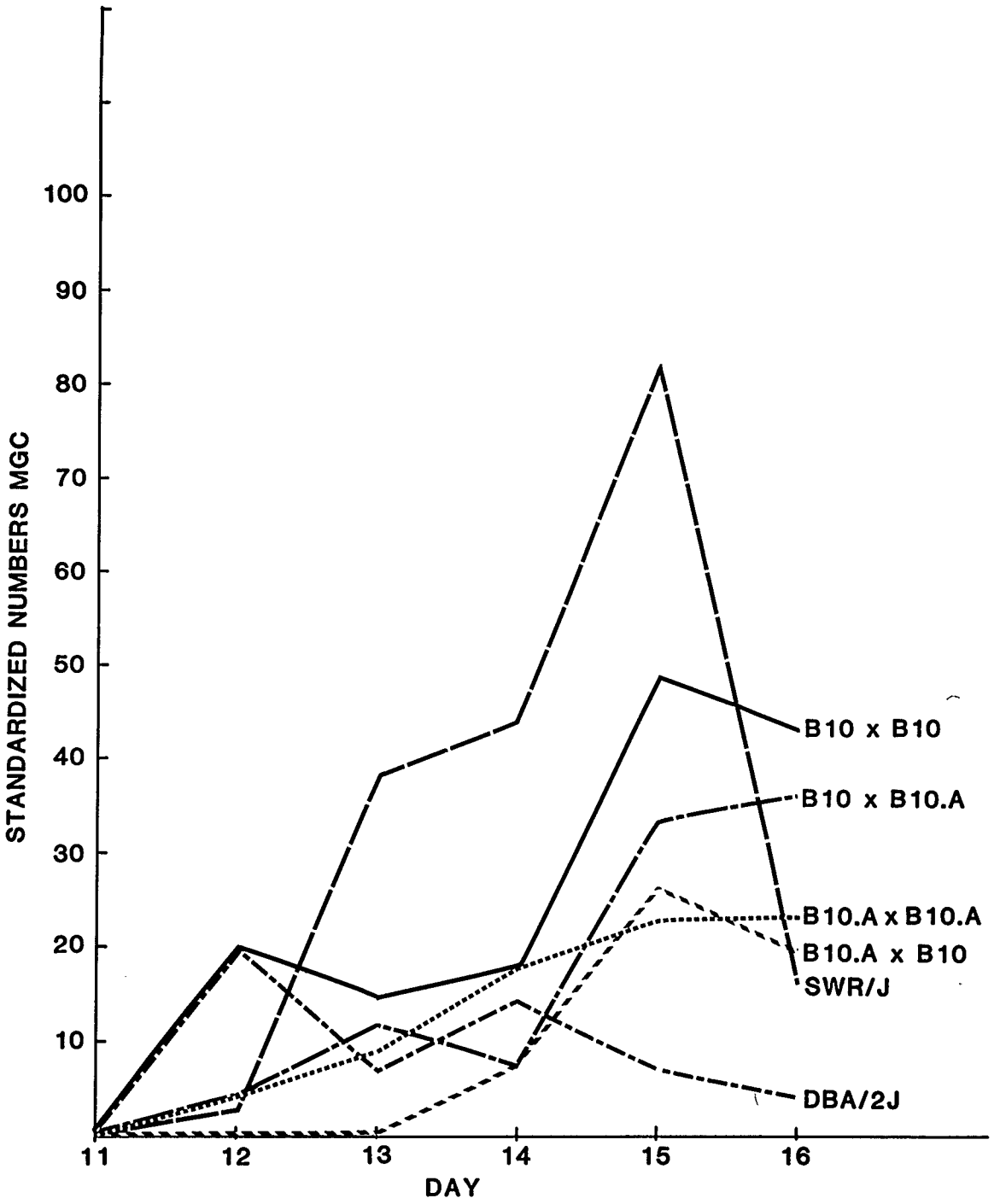
Number of metrial gland cells in the placental labyrinth standardized for area. Each entry refers to the mouse in the corresponding position in Tables 1 and 2.

Day	B10 X B10	B10 X B10.A	B10.A X B10.A	B10.A X B10	SWR/J X SWR/J	DBA/2J X DBA/2J
11	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	*	0	0
12	11	13	12	0	8	6
	22	0	0	0	0	41
	28	0	0	0	0	13
13	16	10	7	0	42	0
	18	13	19	0	49	15
	9	13	0	0	23	5
14	12	2	10	13	15	16
	10	14	22	8	78	2
	32	6	22	2	40	26
15	80	0	6	51	112	11
	2	42	45	7	108	0
	64	59	17	20	24	10
16	28	29	21	36	19	11
	57	37	0	2	12	2
	45	43	49	21	18	0

* possible delayed development

Figure 6.3

Standardized numbers of metrial gland cells, from the 11th to the 16th days of gestation, in the maternal vessels of the placental labyrinth. Each day is represented by the mean for three labyrinths (one per mouse).


STANDARDIZED NUMBERS MGC
100
90
80
70
60
50
40
30
20
10
11
12
13
14
15
16
DAY
B10 x B10
B10 x B10.A
B10.A x B10.A
B10.A x B10
SWR/J
DBA/2J

except the isogenic B10.A mating, in which the number seems to remain approximately the same. In the B10.A X B10 mating, metrial gland cells were not found in the labyrinth until the fourteenth day. Overall, when the mean numbers of metrial gland cells are compared in all the strain matings, from the eleventh to the sixteenth days, the SWR/J isogenic matings appear to exhibit the highest number, followed by the B10 X B10, B10 X B10.A, B10.A X B10.A and B10.A X B10 matings. The DBA/2J isogenic matings appear to have the lowest number. Similarly, when the numbers on the fifteenth day are compared, the same order is seen.

Upon further inspection of Table 6.4 it appears that, in all matings except the DBA/2J, a difference in numbers of metrial gland cells may exist between the first half of the period of study (i.e. days 11, 12 and 13) and the second half (i.e. days 14, 15 and 16). Indeed, lower numbers were found in the first half than the second half in the following matings: B10 X B10 ($p < 0.025$), B10 X B10.A ($p < 0.025$), B10.A X B10.A ($p < 0.01$), B10.A X B10 ($p < 0.001$) and SWR/J X SWR/J ($p < 0.025$). However, no difference existed in the case of the DBA/2J X DBA/2J matings ($p > 0.05$).

DISCUSSION

It has been shown (Chapter 2) that maternal-conceptual H-2 disparity, but not strain genotype, appears to affect the number of MNC in the TGC layer of murine conceptuses. It was also suggested (Chapter 4) that these cells may be involved in the maternal immune response to pregnancy and may even play a role in the non-rejection of the fetal allograft.

MGC may also be part of the maternal immune response and may also have a role in the maintainance of the fetal allograft. The current study was therefore constructed so that a similar search for the effects of H-2 disparity and strain genotype on numbers of labyrinthine MGC and, concomitantly, size of the placental labyrinth could be carried out. The use of three mice per group tended to reduce the chance of misleading sampling of the population. However, it must be recognized that wide fluctuations existed within some groups pertaining to a particular day.

The results of comparisons between groups were interpreted conservatively. The non-parametric Mann Whitney U test was employed for comparisons.

The placing of the sections and the number of sections per labyrinth were selected to reflect the size of the disc-shaped labyrinth and to take into account such factors as deviations from perfection of the disc shape and non-parallelism of the disc axis with the plane of section. The sum of the areas of the central section of the labyrinth and two sections on either side of it (spaced 250 μm apart), employed in the present investigation, were considered to be appropriate indicators of labyrinth volume.

The use of the histocompatibility congenic resistant strains used in this study specifically tested the effect of maternal-conceptual H-2 differences (that is, conceptual heterozygosity at the H-2 complex) on otherwise isogenic and homozygous maternal and conceptual backgrounds.

Substrains of inbred strains are genetically more similar to each other than they are to other strains and certain substrains are indistinguishable (Taylor, 1972). When the object of an experiment is to detect variation, a survey should include relatively distinct strains such as SWR/J, one of the C57-family of strains and DBA/2J (Taylor, 1972).

Gene effects on placental weight are not well understood (Blakley, 1978). The mammalian conceptus differs antigenically from its mother except in matings between highly inbred strains of animals

(James, 1965; Hetherington, 1973). Although some researchers have reported that anitgenic differences, including H-2, result in increased placental weight (e.g. Billington, 1964; James, 1965; Hetherington, 1971), others have failed to demonstrate this correlation (Finkel & Lilly, 1971; Hetherington, 1973). In the current investigation it was possible to observe, by measurement of areas of histological sections, the effects of maternal-conceptual disparity and genetic background on the size of the placental labyrinth; shrinkage following fixation was assumed to affect all conceptuses equally.

In agreement with the finding of increased placental weight with maternal-conceptual H-2 differences mentioned above, it appears that, in the current study, H-2 disparity between mother and conceptus results in a larger placental labyrinth. Furthermore, it is interesting to note (Table 6.3) that variations in the size of the placental labyrinth appear to exhibit strain specificity. The SWR/J X SWR/J matings, although inbred and isogenic exhibit, overall, the largest mean labyrinthine area. The DBA/2J X DBA/2J matings, also inbred and isogenic, exhibit the largest labyrinths on three of the six days of study. Even though careful attention was paid to consistency in orientation of conceptuses during histological processing the possibility still remains that an inconsistently and unusually large labyrinth may be a reflection of excessive tilt of the plane of section away from the central axis of the placenta. This would be unlikely to affect all specimens of one strain.

The effect of embryonic sex on placental weight (Blakley, 1978) may also affect the size of the labyrinth. In order to make this correlation it would be necessary to know the sex of the embryos.

The decline in labyrinthine size on day 16 in some of the matings (or day 15 in the case of the SWR/J X SWR/J mating) may reflect placental weight loss late in pregnancy (McLaren, 1965). Differential placental weight loss (Blakley, 1978) may be responsible for the occurrence of this decline in some groups and not in others.

Turning now to labyrinthine metrial gland cells, it appears that the numbers found in the B10 X B10, B10 X B10.A, B10.A X B10.A and B10.A X B10 labyrinths are similar. However, it is noteworthy, although as yet unexplained, that metrial gland cells were not found in the B10.A X B10 labyrinth until the fourteenth day.

Bearing in mind the proposal that metrial gland cells may be involved in the immunology of pregnancy (Bulmer & Peel, 1977; Dickson, 1980; Dickson & Krcek, 1981) and the finding that maternal-conceptual H-2 disparity increases the size of the labyrinth, it might be expected that a similar effect (i.e. increase) would be seen with numbers of labyrinthine metrial gland cells. However, this does not appear to be the case. Rather, the results suggest that a factor other than maternal-conceptual H-2 disparity exerts an effect on the numbers of metrial gland cells.

As mentioned earlier, higher numbers of labyrinthine metrial gland cells were found in Swiss-Webster mice than in the C57BL/HPB strain (Dickson, 1980). It was postulated that maternal-conceptual genetic differences may be responsible for this difference. The results of this study, although by no means unequivocal, appear to support a different hypothesis, namely, that differences in numbers of labyrinthine MGC may be caused by a strain-specific trait under genetic control. Overall, similar to the C57BL/HPB strain and Swiss-Webster mice, the number of labyrinthine metrial gland cells in all the matings of the B10 and B10.A inbred strains are lower than the number seen in the SWR/J strain (derived from Swiss-Webster mice). The SWR/J mice are all virtually genetically identical and therefore they would be expected to have numbers of labyrinthine metrial gland cells similar to those found in other inbred strains if genetic heterogeneity was responsible for higher numbers of these cells. The higher number in the SWR/J strain does not meet with this expectation. Furthermore, the number in the DBA/2J strain, also inbred and therefore presumably virtually genetically identical, would also be expected to be similar to other inbred strains. Again, this does not appear to be the case, since the DBA/2J strain has, overall, the lowest number of labyrinthine metrial gland cells.

The current findings also do not accord well with the report of greater numbers of metrial gland cells in the labyrinth of outbred matings (LAC 129 X obese) when compared with inbred matings (LAC 129 X

LAC 129) as reported by Jbara (1980). Unfortunately, actual numbers and other details were not given.

There is no evidence to indicate the underlying basis for the accumulation of metrial gland cells in the labyrinth (Dickson, 1980). The greater numbers in the latter half of the period of study, as seen in the current investigation, may be due to an increase in migration with the advance of gestation (Dickson, 1980; Dickson & Krcek, 1981). This may be a reflection of varying rates of migration to the labyrinth during the first and second halves of the periods of study. However, any wish to suggest a plausible explanation for this should, at present, be resisted. In order to acquire further information in this regard, knowledge of the migration of these cells in the placental vessels would permit formulation of clearer hypotheses.

This investigation has been a search for the possible association of differences in numbers of labyrinthine MGC with maternal-conceptual H-2 dissimilarity and strain specificity. It is impossible to deduce any unequivocal conclusions from the results due to the particular number of samples employed in this study but it does appear that certain trends and consistencies, which merit further careful examination, are evident.

CHAPTER 7

CONCLUDING REMARKS

Three topics will be addressed in concluding the work that has been presented in this thesis: 1) the reason for MNC accumulations occurring at 9 a.m. on the 10th day; 2) the question of cell type involved in the MNC accumulations; 3) effects of the major histocompatibility complex and strain genotype on MNC and MGC. All of the above will include suggestions for future studies.

A somewhat puzzling observation, the occurrence of the MNC accumulations at 9 a.m. on the 10th day of gestation, was first documented by Dickson (1979) and has been found consistently throughout this study. It has been shown, from the work presented in Chapter 2, that these accumulations do not appear to occur during any time throughout the 9th, 10th or 11th day of gestation other than at approximately 9 a.m. on the 10th day. The maximal accumulation may, however, occur at some time, other than 9 a.m., between 6 a.m. and 12 noon (for example, 8 a.m. or 10:30 a.m.). An experiment involving collection of the conceptuses every half-hour between 6 a.m. and 12 noon would allow more accuracy in locating the specific time of maximal MNC accumulation. It is interesting that a significant increase in T cell proliferation,

occurring on the 10th day, in iliac and popliteal lymph nodes of allogeneically mated female rats has also been reported (McLean et al., 1980).

The significance of this particular time is, as yet, not apparent. It is possible that the accumulation is a response to an antigenic stimulus, occurring near midgestation, such as the expression of auto-antigens associated with trophoblast (Beer et al., 1972) and/or phase-specific fetal or developmental antigens (Baldwin & Vose, 1974; Chatterjee-Hasrouni et al., 1980).

Another possibility is that the outgrowth of the conceptus from the decidua capsularis, which results, before midgestation, in contact with the decidua basalis, may allow more direct exposure of the maternal immune system to trophoblastic antigenicity.

A study that may provide some insight would be the examination of another species, for example the rat, for the presence and timing of a mononuclear cell accumulation like the one that has been examined in the mouse in this study. Appropriate congenic rat strains are available for this purpose (Altmann & Katz, 1979).

The question of cell type(s) found in the MNC accumulation was alluded to in the discussion of Chapter 4. This knowledge would facilitate the proposal of a possible function for these cells.

Preliminary experiments involving characterization of these cells have been carried out. Suspensions of MNC from B10 X B10.A conceptuses, collected at 9 a.m. on the 10th day, were obtained by centrifugation on Lympholyte-M (Cedarlane Laboratories, Hornby, Ontario) and on Ficoll-Hypaque (Pharmacia, Duval, P.Q.). The cell suspensions were then divided into two aliquots. One was incubated with affinity-purified rhodamine-conjugated IgG (heavy and light chain specific) (Cedarlane Laboratories) and the other with fluorescein-conjugated monoclonal anti-Thy 1.2 antibody (Cedarlane Laboratories). Examination by fluorescence microscopy revealed that some cells were labelled positively in each case, indicating the presence of B-cells and T-cells, respectively. Appropriate positive and negative controls were employed in each case.

In order to establish whether or not monocytes are present in the MNC accumulations, they should be subjected to a latex phagocytosis test (Johnsen & Madsen, 1978) or examined by a histochemical method for detection of peroxidase (Karnovsky, 1965). A subcharacterization of the B-cells could be done employing fluorescein or rhodamine labelled $F(ab')_2$ fragments to mouse IgG, IgM and IgA. The T-cells could be subcharacterized by employing appropriately labelled Ly antisera. The fluorescent antisera could also be applied to cryostat sections of conceptuses for in situ identification of cell types.

While it might be advantageous to use cytotoxic assays to

characterize the MNC (Nakayama et al., 1979), it is not certain whether the numbers of cells recoverable will be sufficient to allow this.

It is possible that these cells may serve some suppressor function and protect the fetal allograft from cell-mediated immune rejection. The presence of a non-T suppressor cell population within the uterus of allogenically mated C3H and DBA mice has recently been reported (Clark & Slapsys, 1982).

A noteworthy finding, resulting from this study, is that, unlike the MNC accumulations, the numbers of MGC in the maternal labyrinthine vessels do not appear to be affected by maternal-conceptual H-2 disparity. They do however appear to be related to strain genotype, whereas the MNC accumulations are not. This finding does not entirely disprove the hypothesis that MGC may have some function in the maintenance of the fetal allograft. It does however indicate that MGC may be under the control of some factor(s) other than the major histocompatibility complex. In addition to the numbers of MGC in the labyrinth, the numbers of MGC in the decidua, metrial gland and placental supply and drainage vessels in the strain combinations employed in this study need to be investigated in order to obtain an overall picture of MGC activity.

Further investigations of the genetic control of both MGC

activity and MNC accumulations should include the effects of maternal-conceptual minor histocompatibility locus disparity as well as non-histocompatibility disparity (for example, coat colour or some other trait) using congenic resistant strains.

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