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Reproductive Immunology News*

NEW DEVELOPMENTS

The NICHD once again has provided the AJRI with grant summaries of projects in reproductive immunology awarded grant funding for fiscal year 1988. In each of the following 1989 issues, we will feature descriptions of some of those grants. The information given in each summary was provided in the grant application by the principal investigator.

The Editorial Board of the AJRI would like to thank the NICHD for its cooperation in regard to this feature of the Reproductive Immunology News Section of the Journal. It is their hope that continuing to publish this information will continue to facilitate increased communication among investigators in the field of reproductive immunology.

Norbert Gleicher
Editor-in-Chief

REPRODUCTIVE IMMUNOLOGY

Title of project: Immunoregulatory factors in the testis
Principal investigator: Michael D. Griswold
Affiliation: Washington State University, Pullman
Award amount: $108,151
Summary: Fluids from the male reproductive tract contain immunoregulatory substances that protect sperm from attack by the immune system. These immunoregulatory substances have been shown to be potent inhibitors of complement action, lymphocyte activation, and antibody production. Sertoli cells from the testis secrete the components of the fluid of the seminiferous tubules and contribute to the composition of seminal fluid. Sertoli cells in culture secrete a number of glycoproteins that are normally constituents of this fluid. The secretion products of the Sertoli cells contain similar immunoregulatory activities to those described for seminal fluids. It is the long-term goal of this proposal to isolate, purify, and characterize the factors in the Sertoli cell secretions that are responsible for this immunoregulatory activity. The immediate goals of the research include the following specific aims: (1) To determine the complete spectrum of immunoregulatory activities present in the Sertoli cell-secreted proteins. Assays will be run to determine if the secreted proteins inhibit antibody production, macrophage function, suppressor cell function, cytotoxic activity, as well as lectin-induced mitogenesis and complement-mediated lysis. (2) The factors responsible for the immunological regulatory activities will be isolated and purified by standard biochemical methods including HPLC. (3) The mechanism(s) by which these purified factors exert their activities will be examined in a series of assays designed to examine in more detail the activities described in specific aim 1.

Results from these studies could be important in our understanding of the basis of immunological infertility and genital tract-related diseases.

Title of project: Immune response in sperm surface antigens
Principal investigator: Paul Primakoff
Affiliation: University of Connecticut, Farmington
Award amount: $175,830
Summary: This proposal is based on the application of concepts from basic research on the cell surface and gamete interaction to the clinical problem of immune infertility. Antisperm antibodies might lead to infertility in vivo in a variety of ways. Proposed mechanisms include (1) sperm agglutination, (2) sperm cytotoxicity in the presence of complement, (3) inhibition of cervical mucus penetration, (4) inhibition of discrete steps in gamete interaction, and (5) enhancement of phagocytosis of sperm. Our first goal is to test one of these proposed mechanisms using the guinea pig as an animal model. We will test the hypothesis that antisperm antibodies can block fertility in vivo by blocking function of two specific sperm surface antigens required in guinea pig sperm-egg interaction.

Since surface antigens of human sperm that function in gamete interaction have not been identified, this hypothesis cannot currently be tested with human sperm. To remedy this situation, our second aim is to identify at least some of the human sperm surface antigens that function in gamete interaction.

The third related goal is to biochemically identify the human sperm surface antigens recognized in individuals with suspected immune infertility. This study will answer to what degree the antisperm antibodies from different individuals recognize diverse or common sperm surface antigens. Antigens recognized in common by sera from all patients or groups of patients will be identified. A collection of monoclonal antibodies will be produced that recognizes these common auto- and isoantigens of the sperm surface. The monoclonal antibodies will be useful for structural characterization of individual auto- and isoantigens. Also, the monoclonal antibody collection to the common antigens could be used in new, highly specific, and sensitive tests for diagnosis of immune infertility. In the long term, the common autoantigens could be purified using the monoclonal antibodies. The purified antigens could be used for treatment in a subgroup of autoimmune men. Purified sperm surface antigens could be added to the ejaculate to absorb out the autoantibodies, thus preventing their binding to sperm.

*This section aims to provide information on contemporary active research, relevant national and international meetings, and new developments in reproductive immunology. Foundations and interested persons should send pertinent information to Norbert Gleicher, M.D., Editor-in-Chief, *American Journal of Reproductive Immunology (AJRI)*, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center of Chicago, California Avenue at 15th Street, Chicago, IL 60608.

Title of project: Postthymectomy oophoritis and orchitis
Principal investigator: Kenneth S. Tung
Affiliation: Washington University, St. Louis, Missouri
Award amount: $168,830
Summary: The purpose of this collaborative study is to investigate neonatal (D3) thymectomy (D3TX)-induced autoimmune oophoritis and epididymovasitis as models of human infertility: the former corresponding to idiopathic secondary amenorrhea, and the latter, to men with sperm granuloma and those with vasal constriction and focal orchitis. Immunopathologic studies will test the hypothesis that D3TX depletes gonad-specific suppressor T cells, whose thymic or postthymic differentiation is antigen dependent and that effector T cells, of which the differentiation is also antigen dependent, emigrate from the thymus before day 3. Antigen-specific T-cell response to ovarian antigens will be quantitated by T-lymphocyte proliferation and interleukin 2 production. By studying ovarian antigen from mice of different ages, the ontogeny of immunogenic ovarian antigens will be determined. The disease-inducing and disease-suppressing properties of thymic cells from mice of days −4, −2, 0, 3, and 7 will be analyzed, with or without further clonal expansion, for their ability to adoptively transfer disease to neonates and to suppress disease in D3TX recipients. Clonal expansion will be achieved by stimulation with ovarian antigens in vitro or in vivo in irradiated syngeneic recipients (with or without marrow reconstitution or thymectomy). Next, prenatal or neonatal ovariectomized D3TX mice reconstituted with ovarian homogenates will be studied in order to determine antigen dependency of effector and suppressor cell induction. Adoptive transfer of oophoritis to the syngeneic or allogeneic recipients will be studied with respect to possible involvement of donor and recipient Ia+ antigen-presenting cells, and hence for the requirement of MHC restriction. To investigate the possibility that chronic epididymovasitis can lead to vasal constriction, sperm granuloma, and infertility, the fertility capacity of 6-month-old D3TX males will be studied by mating, and the results correlated with testicular histopathology. Endocrinologic studies will examine the hypothalamus-pituitary-testis axis of D3TX mice. Sham TX, D3TX, and D7TX mice will be compared, before and after disease onset, for serum testosterone and gonadotropin levels; testicular receptors for LH and PRL; pituitary LH, FSH, PRL, GH, GnRH, and dopamine receptors; and hypothalamic LHRH, tyrosine hydroxylase, and somatostatin. For these studies, radioimmunoassay and immunocytochemistry will be employed.

Title of project: Infertility and testicular autoimmunity
Principal investigator: Cory Teusher
Affiliation: University of Pennsylvania, Philadelphia
Award amount: $141,883
Summary: The long-range objectives of this proposal are to elucidate and define the various regulatory (immunologic, anatomic, physiologic, and/or endocrinologic) mechanisms, which are (1) operative in normally preventing infertility due to autoimmune responses to either the gonads and/or gametes and (2) involved in eliciting the pathologic state of infertility when such abnormal responses do occur. The specific aims of this proposal are to define the relationships between the pathology, immunology, and genetics of idiopathic male infertility believed to be associated with an autoimmune orchiepididymitis syndrome using the murine model of experimental allergic orchitis (EAO). The fertility status of each strain of the BXH series of recombinant inbred (RI) mice derived from the disease-susceptible strain C57BL/6J and the disease-resistant strain C3H/H3J will be determined at various times following immunization. We will concomitantly study the underlying testicular lesions, i.e., orchitis, aspermatogenesis, epididymitis, and vasitis, as well as examine the male reproductive tract for the presence of immune deposits and quantitate the accompanying autoimmune responses to sperm- and testis-specific autoantigens. The fertility status of experimental and control groups of each RI line will be established by test breeding each male with three female C57BL/6J animals for three weeks following which both the number of pregnancies and the number of concepti per pregnancy will be determined. Testicular lesions will be assessed histologically and the presence and distribution of immune deposits will be determined by IF. The levels of the various isotypes of circulatory antisperm and anti-TSDA autoantibodies will be carried out using an ELISA on synergeic epididymal sperm and testicular cells. Immunochemical analysis of the various sperm autoantigens will be performed by isotype-specific immunoprecipitation of radiolabeled sperm autoantigens and SDS-PAGE. Cell-mediated autoimmune responses to testicular cell autoantigens will be determined using the in vitro lymphocyte proliferation assay with estimation of the number of independently segregating gene loci involved in controlling such infertility as well as allowing for the determination of genetic linkage, characterization of multiple inheritances, and investigation of the genetic reassortments involving infertility and the associated autoimmune abnormalities.

Title of project: Seminal lymphocyte and fluid-induced immunosuppression
Principal investigator: Steven S. Witkin
Affiliation: Cornell University, New York
Award amount: $111,272
Summary: None provided.

Title of project: Genetic studies of pregnancy-specific β1 glycoprotein
Principal investigator: Wai-Yee Chan
Affiliation: Oklahoma Medical Research Foundation, Oklahoma City
Award amount: $97,225
Summary: Pregnancy-specific beta 1 glycoprotein (SP1), besides being a good index for monitoring abnormal pregnancies and fetal well-being and a marker for trophoblastic tumors, may also play a very important role in implantation of the embryo and the subsequent growth of the fetus. The long-term objective of this proposal is to understand the functional roles of SP1 in human pregnancy with the view to enhance our knowledge of factors that affect the well-being of the growing fetus. The major obstacle to achieving this goal in the past has been the occurrence of molecular heteroge-

neity of the SP1 protein, making all the immunochemical assaying methods for its quantitation unreliable. This proposal specifically will (1) try to solve the question of molecular heterogeneity of SP1 by cloning the cDNA of the protein. SP1 cDNA clones will be obtained by screening a human placental cDNA expression library using labeled antibody. The identity of the clones obtained will be confirmed by techniques including Western blot hybridization, genomic blot hybridization, and DNA and protein sequencing. (2) Investigate the validity of cultured human skin fibroblasts, cultured placental cells, with mouse, rat, and baboon serving as models for studying SP1. The SP1-like molecules in these systems will first be compared with human SP1 by Northern blot hybridization. Further comparison will be made by cloning the cDNA of these molecules and comparing them with human SP1 cDNA by restriction enzyme mapping and, when needed, by DNA sequencing. (3) Investigate the mechanism of enhanced SP1 production in Meckel's syndrome. Cultured fibroblasts derived from a patient will be used. The production of SP1 in these fibroblasts will be studied by immunoprecipitation. If expression of the genetic defect in the fibroblast is obvious, the mechanism of SP1 overproduction will be further investigated by quantitating the mRNA of SP1 with dot blot hybridization. The cDNA will then be cloned and examined by restriction enzyme mapping and eventually DNA sequencing. (4) Determine the gene structure of SP1. This will be achieved by screening a human genomic library with SP1 cDNA probe. The clones hybridizing to the probe will be picked and sequenced. The gene structure of SP1 will be compared with that of human placental lactogen and human chorionic gonadotropin beta chain. With techniques such as chromosomal walking, genomic blot hybridizing, etc., the arrangement of these genes on the human genome will also be determined. (5) Lastly, investigate the properties of SP1-like molecules in tumor tissues using hydatidiform mole as an example. Techniques used will include Northern blot hybridization to identify the presence of SP1, dot blot hybridization to quantitate the quantity of mRNA produced, cloning restriction enzyme map, and DNA sequencing to determine the structure and nature of the SP1 protein in tumor.

Title of project: Treatment of lupus anticoagulant in pregnancy
Principal investigator: Susan F. Cowchock
Affiliation: Thomas Jefferson University, Philadelphia
Award amount: $142,430
Summary: Patients with the "lupus anticoagulant," a clotting abnormality associated with circulating antibodies to phospholipids, are at increased risk for both venous and arterial thromboses. Pregnant women with such antibodies are at high risk for fetal loss, presumably due to thrombosis at the level of the spiral arterioles or within the maternal floor of the placenta. Affected women with repeated pregnancy loss have been treated prophylactically in subsequent pregnancies with immunosuppression and anticoagulation. Pregnancies at risk permit comparison of the effectiveness of prophylactic immunosuppression or anticoagulation in the prevention of thromboses over a limited period of time with a specific treatment end point. We plan a prospective, randomized collaborative trial of immunosuppression vs. anticoagulation for pregnant patients at risk for this complication and will compare both fetal outcome and maternal safety of these two regimens. A specific immunoassay will be used for measurement of maternal serum levels of antibody to tailor immunosuppressive therapies for the maintenance of normal serum antibody levels.

Title of project: Cell interactions in endometriosis
Principal investigator: Jouko K. Halme
Affiliation: University of California, Los Angeles
Award amount: $189,469
Summary: The cause-and-effect relationship between ectopic endometrial cell growth and characteristic local inflammatory response is unknown but is probably of fundamental importance in endometriosis (E). We are proposing to investigate this relationship and have formed two interrelated hypotheses to be tested. According to the first, the distinct macrophage response in the peritoneal cavity in patients with E may be responsible for enhanced survival of endometrial cells. The second hypothesis assumes that an intrinsic biologic difference may exist in either all or only in ectopic cells of endometrial origin in patients with E as compared to those of normal women (N). This "abnormality" may increase their survival in the peritoneal environment and directly lead to a distinct macrophage response. Our recent studies have identified differences in macrophage membrane function and in vitro differentiation in N and E patients. In order to study the biologic relevance of these phenomena we will determine if macrophages from N and E secrete factors stimulating endometrial cell growth and whether this can be influenced by the stage of macrophage maturation or by sex steroids/danazol. Biologic characterization of endometrial cells will be attempted since these cells in E may have a basic alteration in growth regulation and this may be reflected either by the response to exogenous growth factors or by altered expression of the normal growth-regulatory cellular oncogenes. In order to test this hypothesis, proliferative response of endometrial cells of N and E patients to several known growth-stimulating factors will be tested and tissue specimens will be analyzed for enhanced or suppressed expression of critical oncogenes. If enhanced expression of an oncogene is found in either ectopic or eutopic cells in E, gene amplification and the possibility of genomic alterations will be assessed.

Due to the lack of availability of large amounts of human cells and variability of individual specimens, it is imperative that we produce long-term cell lines. We will attempt to establish immortalized macrophage and endometrial cell lines from N and E patients by viral transfection. These would provide a unique resource for detailed characterization of the various factors, cellular growth requirements, and interactions between the two cell types. They would ultimately permit a direct assessment of the role of altered oncogene expression.

Title of project: Lupus anticoagulant and recurrent pregnancy loss

Principal investigator: David W. Branch, Jr.
Affiliation: University of Utah, Salt Lake City
Award amount: $118,117
Summary: Lupus anticoagulant (LAC) is an autoantibody that is associated with recurrent first-trimester abortion and second-trimester fetal death, with 80% or more of pregnancies in women with the anticoagulant ending in fetal wastage. In pregnancies that progress beyond mid-gestation, severe or early preeclampsia and fetal growth retardation are common. LAC is commonly detected by its prolongation effect on phospholipid-dependent coagulation, and the antibody has been identified as an antiphospholipid. The pathophysiologic effect of LAC has been attributed to the inhibition of vascular prostacyclin production that has been observed in vitro in the presence of the antibody. We propose to study two aspects of LAC. First, we will evaluate the specific antigen-antibody interactions for LAC using an immunologic assay that we have developed. Our preliminary results suggest that the antigen is the phospholipid phosphatidylserine. Second, we will study the mechanism of LAC using a prostacyclin generation assay and mouse and rabbit models of reproduction. The animals will be actively immunized with various phospholipid preparations or passively immunized with purified human LAC preparations or human or mouse monoclonal LAC, and their reproductive performance evaluated.

Title of project: A molecular approach to gonadotropin receptor regulation
Principal investigator: Jayantha Wimalasena
Affiliation: University of Nebraska, Omaha
Award amount: $99,002
Summary: We have successfully developed procedures to solubilize LH/hCG receptors (LHR) from porcine and rat ovaries in high yield. Porcine and rat LHR have been purified to apparent homogeneity by a two-step hCG-Sepharose affinity chromatographic procedure. The predominant subunit of LHR so purified has a molecular weight of 70,000–80,000 daltons under denaturing reducing conditions. During the proposed grant period, the physicochemical and biological properties of purified rat and porcine LHR will be compared. Antibodies to porcine LHR will be developed in rabbits, and rat LHR will be injected into mice with the goal of producing a monoclonal antibody to rat LHR. Antibodies will be characterized by immunochemical techniques and their biological properties will be tested using isolated receptors, membranes, cultured cells, and the whole animal. The molecular basis of receptor regulation by hormones will be analyzed through direct measurements of rates of synthesis and degradation of LHR by specific immunoprecipitation methodology.

Receptor antibodies will provide a superb analytical tool for investigation of the molecular biology of LHR synthesis, processing, and turnover. LHR antibodies will not only be critical tools for investigation of ovarian physiology at the molecular level, but also may have significant practical application in immunocontraception and in understanding infertility of ovarian origin and in the development of monoclonal LHR antibody-drug conjugates which may be useful in therapy of ovarian cancer.

Title of project: Effects of HLA and transferrin on Hutterite fertility
Principal investigator: Carole Ober
Affiliation: Northwestern University, Evanston, Illinois
Award amount: $117,124
Summary: The objective of the proposed study is to evaluate the effects of HLA compatibility, transferrin alleles, and other genes on reproduction. Our population-based study will be conducted in the Hutterites, a religious isolate that lives communally and proscribes contraception. During field trips to Hutterite colonies in South Dakota, blood samples for genetic analyses will be collected from all cooperative adults. All samples will be typed for HLA-A, -B, -C, -DR, -DQ, ABO, Rh, Gm, Km, and transferrin. In selected couples studies of HLA-DP sharing and presence of maternal plasma blocking factors and antilymphocyte antibodies will be performed.

The specific hypothesis tested is whether normal pregnancy requires maternal recognition of paternally derived histocompatibility antigens carried by the fetus (i.e., histoincompatibility). We seek to (1) identify gene(s) or region within the MHC associated with reduced fertility in couples sharing HLA antigens; (2) investigate the relationship between reproductive histories and presence of antilymphocyte antibodies and/or maternal plasma blocking factors in couples sharing and not sharing HLA antigens; (3) examine interactive effects between HLA compatibility and other genes known to affect immune responsiveness or fertility (namely ABO, Rh, Gm, Km, transferrin); and (4) evaluate the effects of HLA compatibility on genotype or haplotype segregation ratios and sex ratios among offspring.

Elucidating the immunological mechanisms involved in normal pregnancy is necessary for understanding immunopathological processes causing spontaneous abortion in humans. Thus our studies may help delineate a subset of women who experience repetitive spontaneous abortions who may potentially benefit from immunotherapy.

Title of project: Maternofetal antigenic disparity and pregnancy outcome
Principal investigator: Charles J. Hoff
Affiliation: University of Southern Alabama, Mobile
Award amount: $267,090
Summary: None provided.

Title of project: Basic studies on endometrial protein
Principal investigator: Sharad G. Joshi
Affiliation: State University of New York, Albany
Award amount: $136,307
Summary: Progestin-regulated uterine proteins are believed to play major roles in pregnancy. Therefore we searched and identified for the first time a "progestagen-associated human endometrial protein" or PEP which is synthesized within the endometrium. It is emphasized that PEP is perhaps the only major human endometrial protein the synthesis of which is dramat-

ically increased during early pregnancy. However, both the hormonal control and the role of PEP are poorly understood. Based on the results of studies on progestin-dependent uterine proteins in animals undertaken by other investigators and our own experience with PEP, we postulate that PEP synthesis is regulated mainly by progesterone and that PEP plays a role(s) in immunosuppression, protease inhibition, or steroid binding. To test this hypothesis we propose to undertake a 3-year study with the following specific aims: (1) to study effects of steroid hormones on in vitro synthesis of PEP by proliferative human endometrium; (2) to purify PEP required to study its role; and (3) to determine the role of PEP in immunosuppression, specifically to test whether PEP interacts directly with leukocytes to alter their proliferation response to mitogens (or antigens) or to impair their ability to synthesize immunoglobulins or whether PEP interacts with placental (fetal) cell membranes (thereby masking the fetal antigens on cell membranes and preventing their recognition by the immune system). PEP will be purified by the conventional protein fractionation techniques. PEP synthesis by endometria and immunoglobulin synthesis by leukocytes will be studied by radiolabeling and immunoprecipitation methods. PEP binding to leukocytes and placental cell membranes will be examined by radioligand technique. Leukocytic proliferation response to PEP will be quantitated by ^{3}H-thymidine incorporation. If the results of these studies fail to support the role of PEP in immunosuppression, efforts will be directed to examine the alternate possibilities that PEP is involved in steroid binding or in the control of placental proteases (which have been implicated in the placental cell invasion of maternal endometrial tissue). We hope the elucidation of hormonal control and role of this newly discovered major human pregnancy protein, namely PEP, will lead to the development of methods for the manipulation or control of human pregnancy.

Title of project: Basic studies on a human endometrial protein
Principal investigator: Sharad G. Joshi
Affiliation: Union University, Albany, New York
Award amount: $19,033
Summary: Progestin-regulated uterine proteins are believed to play major roles in pregnancy. Therefore we searched and identified for the first time a "progestagen-associated human endometrial protein" or PEP which is synthesized within the endometrium. It is emphasized that PEP is perhaps the only major human endometrial protein the synthesis of which is dramatically increased during early pregnancy. However, both the hormonal control and the role of PEP are poorly understood. Based on the results of studies on progestin-dependent uterine proteins in animals undertaken by other investigators and our own experience with PEP, we postulate that PEP synthesis is regulated mainly by progesterone and that PEP plays a role(s) in immunosuppression, protease inhibition, or steroid binding. To test this hypothesis we propose to undertake a 3-year study with the following specific aims: (1) to study effects of steroid hormones on in vitro synthesis of PEP by proliferative human endometrium; (2) to purify PEP required to study its role; and (3) to determine the role of PEP in immunosuppression, specifically to test whether PEP interacts directly with leukocytes to alter their proliferation response to mitogens (or antigens) or to impair their ability to synthesize immunoglobulins or whether PEP interacts with placental (fetal) cell membranes (thereby masking the fetal antigens on cell membranes and preventing their recognition by the immune system). PEP will be purified by the conventional protein fractionation techniques. PEP synthesis by endometria and immunoglobulin synthesis by leukocytes will be studied by radiolabeling and immunoprecipitation methods. PEP binding to leukocytes and placental cell membranes will be examined by radioligand technique. Leukocytic proliferation response to PEP will be quantitated by ^{3}H-thymidine incorporation. If the results of these studies fail to support the role of PEP in immunosuppression, efforts will be directed to examine the alternate possibilities that PEP is involved in steroid binding or in the control of placental proteases (which have been implicated in the placental cell invasion of maternal endometrial tissue). We hope the elucidation of hormonal control and role of this newly discovered major human pregnancy protein, namely PEP, will lead to the development of methods for the manipulation or control of human pregnancy.

Title of project: Isolation of human fetal cells from maternal blood
Principal investigator: James F. Leary
Affiliation: University of Rochester, New York
Award amount: $196,174
Summary: An understanding of the subtle immune interactions of mother and fetus has been hindered by an inability to measure and correlate the frequency and type of fetal cells that have crossed the placenta into the mother's blood with information about the mother's immune system. Such information has been obtained only in a crude fashion and before monoclonal antibodies against B- and T-cell subsets were available. While it has been shown to be theoretically possible to isolate human fetal cells directly from mother's blood by multiparameter cell-sorting techniques, the currently available cell-sorting technology is very slow (less than 5,000 cells/sec) and unreliable for detecting and isolating rare (less than 0.1%) cell subpopulations. Consequently, many hours of cell sorting and/or manual counting of sorter-enriched subpopulations are required to isolate these cell subpopulations. Current cytogenetic techniques are geared for relatively large numbers of cells and consequently are not very suitable for small subpopulations of sorted cells for which expansion in tissue culture is either undesirable or impossible (e.g., fetal blood cells).

Many of the above problems are addressed in this grant. We have developed new high-speed cell analysis and sorting technology capable of accurately and reproducibly analyzing live cells at rates in excess of 100,000 cells/sec and high-speed two-step sorting capable of isolating rare cell populations. Analysis experiments that currently require 5–6 hours of difficult and frequently unreliable work on a conventional cell sorter can be performed in our laboratory in 15 minutes with both

ease and reliability using unique hardware and software on a modified commercial cell sorter. Cell sorting can be performed by new high-speed two-step sorting that will yield in less than an hour the same number and purity of cells that it would take a traditional cell sorter more than 8–9 hours to isolate. In addition we demonstrate the feasibility of obtaining karyotypes on small numbers of rare-cell subpopulations that cannot be expanded in tissue culture.

Title of project: Immune thrombocytopenia in pregnancy
Principal investigator: Neal S. Rote
Affiliation: Foundation for Blood Research, Scarborough, Maine
Award amount: $79,977
Summary: The long-term objective of this proposal is to define the underlying immunologic mechanisms responsible for fetal thrombocytopenia associated with autoimmune thrombocytopenic purpura (ATP) and pregnancy-induced hypertension (PIH). ATP and PIH frequently result in thrombocytopenia, which may be associated with significant maternal morbidity. Whether the cause of platelet destruction is immunologically similar in both conditions is unknown, but fetal thrombocytopenia is a complication of ATP and PIH. Since fetal thrombocytopenia is associated with serious neonatal complications including intracranial bleeding during vaginal delivery, obstetric management of these patients could be selectively altered if the thrombocytopenic fetus could be identified. However, to date, no antepartum maternal characteristics have been identified that can reliably predict fetal thrombocytopenia. Four questions are posed in this application concerning maternal and fetal platelet-associated and circulating antibody, and experiments are detailed to provide the answers: (1) Is the antibody specifically directed against a platelet antigen? (2) If specific binding is occurring, which platelet antigen is being recognized? (3) Do ATP and PIH patients produce antibodies against the same platelet components and do all specificities of antibody cross the placenta? (4) What is the relationship between antibodies in the cord blood and those in the maternal circulation? Antibody specificity will be determined by several methods; our principal approach will be the use of transblotting, but a pattern of antibody reactivity will also be determined by adsorbing samples containing antibody with a battery of typed donor platelets. Using papain and pepsin digests of the antibody, binding via Fc vs. Fab will also be determined. The information in this study will facilitate the understanding of the basic immunologic mechanisms of thrombocytopenia in pregnancy and possibly the development of a reliable antepartum technique to predict fetal platelet destruction. This would allow the selection of appropriate obstetric management and route of delivery in each instance and may lead to the identification and active treatment of fetal thrombocytopenia prior to labor.

Title of project: Evaluation of postoperative adhesion formation
Principal investigator: Gere S. di Zerega
Affiliation: University of Southern California, Los Angeles
Award amount: $91,739
Summary: In our previous studies (NICHD R01 19002) we found that macrophages recruited by surgical injury to enter the peritoneal cavity secreted substances critical to postsurgical peritoneal repair. We observed that supernatants obtained from short-term cultures of normal rabbit (nonsurgical or resident) peritoneal macrophages contain material that modulates fibroblast proliferation, as well as secretion of connective tissue proteins (collagen, glycosaminoglycans). Macrophages removed from the peritoneal cavity of rabbits after surgery contain an increased respiratory burst and secretion of a substance that induces fibroblast proliferation. The preexposure of postsurgical macrophages to cyclohexamide in vitro eliminates or markedly reduces the secretion of these activities in situ or in spent protein synthesis and secretion. It was not clear from these initial observations, however, whether peritoneal macrophage culture supernatants contained one or several active principles. Nor was it clear whether these studies detected a previously unknown substance or whether they were simply describing new functional properties for previously identified macrophage secretory products, such as interleukin 1, MDGF, etc. The purpose of this grant is to address these issues. In addition to obtaining further insight into the functions of postsurgical peritoneal macrophages, the studies outlined here are designed to provide further understanding of the role this postsurgical macrophage plays in peritoneal healing and adhesion formation. Accordingly, the specific aims of this projects are (1) to characterize macrophages present at the site of postsurgical trauma, utilizing those parameters traditionally used to define macrophage activation, including superoxide radical production and tumoricidal activity; (2) to examine the secretory capabilities of macrophages from postsurgical sites, in particular the production of neutral proteases and arachidonic acid metabolites; (3) to characterize ascitic fluids from postsurgical peritoneum for the level of neutral proteases, protease inhibitors, and macrophage activity factors; (4) to examine the role of macrophages in the preparation of fibroblasts for tissue repair and secretion of proteins necessary for matrix formation.

Title of project: Vasovasostomy: morphology, physiology, and immunology
Principal investigator: Stuart S. Howards
Affiliation: University of Virginia, Charlottesville
Award amount: $138,457
Summary: The overall goal of this project is to determine the extent to which morphologic, physiologic, and immunologic alterations after vasectomy are reversed by a subsequent vasovasostomy and to correlate specific alterations with post-vasovasostomy infertility. During the current grant period, we studied Lewis rats that received either a bilateral vasectomy and a later vasovasostomy, a vasectomy alone, or sham operations. Some vasectomy-induced alterations were not reversed by a subsequent vasovasostomy. The first aim of the proposed work is to determine the nature and sequence of the initial alterations after vasectomy in the Lewis

rat and to determine whether the lesions and infertility are reversible by vasovasostomy, provided it is performed early enough. The second aim is to define autoantigens and the role of antisperm antibodies in development of testicular alterations after vasectomy in Lewis rats. Comparisons with respect to autoantigens recognized will be made between sera from animals showing elevated antisperm antibodies with and without testicular alterations and between sera obtained before and after vasovasostomy. The third aim is to determine whether the reversal of reproductive tract alterations, antisperm antibody levels, and fertility after vasovasostomy in Lewis rats can be modified by treatment with immunosuppressive drugs (glucocorticoids and cyclosporin). The fourth aim is to determine the extent to which alterations after vasectomy are reversed by vasovasostomy in Sprague-Dawley rats and, most importantly, to determine the basis for the differences in fertility after vasovasostomy that we have observed in preliminary studies on this strain. Young adult male rats will be subjected to bilateral extrascrotal vasectomy and, at a later time, a microsurgical vasovasostomy will be performed. A second group of animals will receive a vasectomy but no vasovasostomy, and a third group will receive sham operations. The animals will be studied at several intervals after the operations. Methods include light and electron microscopy, measurement of intraluminal hydrostatic pressure by micropuncture, probing of the blood-testis barrier with tracers and freeze-fracture, intratesticular testosterone determinations, measurement of the resistance of the vas deferens to fluid flow, determination of serum antisperm antibody levels with an ELISA, and fertility testing. Postvasectomy autoantigens will be studied by gel electrophoresis, Western blotting, and vectorial labeling techniques.

Title of project: Gonadotropin-related gonadal antigenicity
Principal investigator: James A. Dias
Affiliation: New York State Department of Health, Albany
Award amount: $90,788
Summary: A study is proposed to determine if chronic administration of the pituitary glycoprotein follicle-stimulating hormone (follitropin) results in an autoimmune response to ovarian cell membranes that contain the receptor for follitropin. The experimental animal model will be the female rat. The hypothesis suggests that antibody inactivation of the receptor and/or membrane-associated entities results in gonadal failure. Inactivation may occur by the receptor becoming antigenic by virtue of its association with its hormone, which was involved in a primary antibody response (heterogenization); a secondary response antibody is produced that recognized the first antibody, which was formed to the hormone (anti-idiotype formation). In the latter case, antibody directed against the paratope of the antihormone antibodies would interact directly with the hormone-binding region of the receptor. Isotopic labeling of gonadotropin receptor in granulosa cells will be performed in order to produce an immunodiagnostic reagent to be used for the detection of receptor antibodies in serum. Initially granulosa cell membrane receptor-binding parameters, which characterize the initial event of the hormone-receptor interaction, will be studied in control tissues. Then the ability of putative immune sera to precipitate FSH receptors vs. other membrane antigens and the effect of immune sera on gonadotropin action as well as the cytotoxicity of immune sera will be studied. The effect of administration of follitropin on serum and pituitary lutropin and follitropin levels during treatment will be determined, and ovarian and pituitary morphology will also be examined. In order to directly test the hypothesis, the effect of active immunization with purified antibody to hormone will be studied. Antisera with a positive titer for anti-idiotype will be tested for the presence of antireceptor antibodies. Spleens of rats that exhibit positive titers for anti-idiotypes will be removed and the cells will be fused with myeloma cells. Resultant hybridomas that survive the fusion and are positive for anti-idiotypes will be expanded and used for further studies on the effects of antireceptor antibodies and reproductive function. The current clinical use of gonadotropins for the treatment of infertility emphasizes the clinical significance of these studies. The long-term goal of the grant is to determine whether or not gonadotropin receptors may be implicated in infertility of an autoallergic etiology. Understanding the processes of autoallergy or how they occur may provide new insights into the treatment of infertility and autoimmune diseases.

Title of project: Function of sperm membrane autoantigens in fertilization
Principal investigator: Patricia M. Saling
Affiliation: Duke University, Durham, North Carolina
Award amount: $24,207
Summary: In the proposed research, the function of sperm membrane autoantigens during fertilization will be determined using mouse fertilization, both in vitro and in vivo. A set of intrinsic sperm membrane autoantigens, selected on the basis of an effect upon sperm function during fertilization, will be defined with the aid of monoclonal antibodies produced by syngeneic immunization with mouse testis membranes. Furthermore, the behavior of the specific functional autoantigens during capacitation, zona binding, acrosome reaction induction, zona penetration, and sperm-egg membrane fusion will be assessed by labeled monoclonal antibody probes. Finally, the lipid composition of the sperm plasma membrane will be altered to determine the role of sperm plasma membrane lipid during fertilization. The significance of this proposal lies in its examination of individual sperm membrane autoantigens with defined functions during specific, separable steps of the fertilization process in the mouse.

Title of project: Immunoregulation in pregnancy
Principal investigator: Gary W. Wood
Affiliation: University of Kansas, Kansas City
Award amount: $132,917
Summary: This is a proposal to continue studies into the role of local immune regulation in preventing a maternal antifetal immune rejection. The underlying rationale is that the system may at times fail, leading

to spontaneous abortion. Understanding the mechanisms may be important to decreasing the incidence of such failures. The operating theory is that protection of the developing embryo results from a combination of the immunologically inert physical barrier provided by the trophoblast and a region of local immunosuppression generated in the uterus around the fetus. This environment is generated during the decidual response through a massive increase in numbers of prostaglandin-producing cells, principally macrophages. To explore this hypothesis, it is proposed that a study be performed of (1) control mechanisms influencing macrophage accumulation in the uterus during pregnancy, (2) mechanism(s) responsible for immunoregulation by decidual macrophages, (3) the extent and nature of immunoregulation in uterine regional lymph nodes, (4) a model of indomethacin-induced abortion, which is closely relevant to the proposed immunoregulatory mechanisms. The above specific aims will be studied using a variety of cellular immune techniques in a mouse model system. In conclusion, these studies should lead to an extension of current knowledge of the immunology of pregnancy.

Title of project: A local immune system in the genital tract
Principal investigator: Margaret B. Parr
Affiliation: Southern Illinois University, Carbondale
Award amount: $124,649
Summary: Our studies are concerned with the induction, control, and function of the local secretory immune system in the genital tract. (1) We will study the role of local immunity in protecting the female genital tract against infectious agents by using ELISA to measure naturally occurring antibacterial antibodies in mouse vaginal fluid and anti-Salmonella antibodies after oral immunization with Salmonella. (2) We will use immunolabeling to quantitate IgA and IgG antiferritin plasma cells in four segments of the mouse female genital tract after ferritin immunization by several routes, including oral, vaginal, oral-vaginal, vaginal-vaginal, and intraperitoneal-vaginal. The results should clarify whether plasmablasts migrate from gut lymphoid tissue to the female genital tract. Also, the results would compare the effectiveness of primary immunizations at three sites in enhancing the local genital tract immune response to vaginal challenge. (3) Using adoptive transfer methods, we will localize radiolabeled plasmablasts in the female genital tract 24 hours after transfer of cells from mesenteric, combined iliac-renal, or peripheral lymph nodes. The comparison should determine whether there is selective homing to the genital tract by plasmablasts from local lymph nodes. The results from parts 2 and 3 should clarify the relationships of the local genital tract immune system to the common mucosal immune system. (4) We will use histological methods to describe the occurrence and distribution of lymphocyte-like cells in the epithelium and lamina propria of the rat oviduct, particularly the junctura, in relation to the time of mating. (5) We will study the local immune system of the male rat genital tract in two ways: by localizing IgA, IgG, and IgM plasma cells and secretory component in the genital tract with immunolabeling techniques and by using ELISA to determine whether naturally occurring antibacterial antibodies are present in seminal fluid and whether anti-Salmonella antibodies can be detected in seminal fluid after oral immunization with Salmonella.

Our objective is a better understanding of local immunity in the genital tract and its possible use in fertility control.

Title of project: Studies of maternal and neofetal immunity
Principal investigator: Thomas B. Tomasi, Jr.
Affiliation: New York State Department of Health, Buffalo
Award amount: $151,248
Summary: The overall goal of this proposal is to study the mechanisms underlying the immunologic immaturity of the murine neonate. A variety of physical and serological techniques will be employed to isolate neonate cell populations. From this panel of cells, we will then attempt to characterize the cells responsible for accessory and natural killer (NK) activities, as well as other cell types that inhibit these activities. The methods to be employed include adherence, density gradient centrifugation cell sorting, and in vitro culture with various growth factors (IL-2, IL-3, M0 growth factor), including a stimulatory factor(s) from mouse amniotic fluid (MAF). We propose to study whether suppressor cells that have already been identified in the neonate inhibit via a prostaglandin (PGE)-mediated effect on Ia expression and/or via other soluble factors. The relationship, if any, between M0 suppressors, T suppressors (Ts), and the accessory cell deficiency will be explored. Whether MO, dendritic cells, and/or epithelial cells are the predominant accessory cells found in the neonate thymus will be examined. These latter experiments bear on the role of the thymus in selecting the T-cell repertoire. Neonatal suppression has also been attributed to circulating soluble factors such as alphafetoprotein (AFP). We propose to isolate the suppressive components from MAF and determine whether their activity is mediated via M0 and/or Ts. The phenotypic characteristics of the cells induced by MAF will be compared (using MAbs and flow cytometry) with those isolated from the neonate. The role of PCE as well as non-PGE suppressors will also be explored in this system. Recently, we observed that Cu^{++} modulates suppression by AFP and a series of experiments are outlined on the role of Cu^{++} in neonate immunosuppression. In addition, factors that stimulate the growth of neonate and a small subpopulation of adult spleen cells have been identified in MAF. Experiments are planned to isolate the stimulatory factor(s) by HPLC and to determine the spectrum of cells affected (particularly whether suppressor M0 and/or Ts are stimulated). The work outlined is pertinent to the hypothesis that the decline of circulating inhibitory factors in the postnatal period is responsible for the acquisition of positive immunity in the young adults. These studies are also relevant to immunosuppression in pregnancy and disease states where high circulating levels of AFP are found.