

13 Immunology of Contraception

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General Preface to Series

The impact of immunological thought on medical practice has been increasing at a steady rate now for nearly twenty years. There appear to be very few fields to which the immunologist cannot contribute. Initially the immunological approach was limited to assistance in diagnosis and in sera and vaccine production. New approaches in the field of therapy are not only in the use of vaccines, sera and immunosuppressive agents, but also in the more rational use of conventional therapeutic agents. Immunological knowledge is especially necessary in the field of tumour therapy, particularly in the balanced use of surgery and radiotherapy. Moreover, immunological knowledge in other fields has allowed us to understand more readily the mechanisms whereby a single aetiological agent can produce a wide range of different clinical manifestations. Different disease patterns occur depending on the nature of the immunological reaction causing tissue damage. A completely different symptom complex from reactions involving soluble immune complexes reacting with the complement cascade will be found in those involving the reaction of specifically sensitized lymphocytes with antigen as part of a cell-mediated or delayed hypersensitivity reaction.

As a massive amount of new scientific material accumulates in this field, the clinician is frequently left behind and perplexed. Each year a new scientific journal is published specializing in fields as diverse as immunogenetics, immunochemistry or immunological techniques. We have journals emanating from continents as well as countries. The wealth of material is often bewildering. Simple textbooks of immunology are often too simple, whereas review articles may be too complicated for the specialist physician or surgeon who wants a treatise on those aspects of the subject particularly relevant to his own field of interest. It is hoped that this series will fulfil some of these needs by giving comparatively short reviews that will lay emphasis on immunological subjects which should appeal to both clinicians and those working in clinical laboratories. The aim is to provide the busy clinician in a particular field of medicine with a short volume relevant to his practice written by a specialist. It should introduce the reader to the immunological approach to his subject and indicate how modern immunological thought might influence his day-to-day work in the wards or clinical laboratory.

John Turk

The Royal College of Surgeons of England
London

Preface

The idea of a monograph on this subject originated from the alert mind of the Editor of the Series John Turk, who must receive credit, if it succeeds in fulfilling a purpose. I readily accepted the assignment, partly because I was already 'involved' in it. It seemed an interesting adventure to fathom through a subject which is old (the first experiments in the area date back to 1899) and yet so new and emerging (a new *Journal of Immunology of Reproduction* started publication only in 1979).

My own fascination with it began about eight years back. That year I was appointed a member of the Executive Council of an old, famous and sprawling university and as such was required to visit Varanasi every alternate month. This ancient city situated on the banks of the holiest of the holy rivers Ganga evoked in me each time a mixed feeling. Here was a fertile and alluvial soil with plenty of river and subsoil water and yet the people were so undernourished. Their diminutive appearance bore testimony to the dwindling resources with each generation. The numbers were increasing but not the land. The rate at which productivity progressed was in no way consonant with the mouths to feed. Family planning, though officially promoted as a policy by the State was not making progress with its existing facilities, nor did it seem to have a better prospect in the visible future. This picture may not be special to Uttar Pradesh, but is representative of the scene in many countries of the world where population explosion is taking place. It took 1830 years for the global population to increase from two hundred and fifty million to one billion. The second billion mark was attained in 100 years, the third in 30 years, the fourth in 15 years and the fifth billion mark is expected to be reached by 1987.

Could one not devise a method which may be applicable on a mass scale and minimally dependent on external supply? Why not mobilize the body's own defence mechanisms to control fertility? This idea may not be a mere fancy of mind, but has a *raison d'être* on grounds of basic developmental events and physiological processes of pregnancy. The immune system is coded to treat the body constituents made during the fetal life as 'self' entities. Those which develop in the postnatal years are intrinsically 'foreign' to it. The onset of spermatogenesis and ovum development takes place in puberty, and all new

molecules appearing at this stage, distinct from those present in other tissues, can be reacted against by the immune system. Similarly, development of a new tissue, the placenta, begins with pregnancy, and counteracting it or its products by special techniques breaking tolerance, will be incompatible with the establishment and maintenance of pregnancy.

Were these hypotheses valid, they should have been expressed sometime or other by natural events! A cursory glance at the literature reveals indeed the existence of unexplained cases of infertility, where the basis has been shown to be immunological. A large percentage of men undergoing vasectomy operations make antisperm antibodies. Immunology thus has relevance to both the management of sterility (of a type) as well as the control of fertility.

The scope of the book is wide and is reflected by the Table of Contents. Basic issues such as the mechanisms by which the conceptus, 50 per cent of which is paternal genetic information, is protected against immunological rejection by the mother are discussed, as also is the influence that hormones and contraceptive steroids exercise on the immune response. The emphasis, however, is on analysis of current research aimed at developing antifertility vaccines. The subject is viewed from the angle of one who is himself deeply engaged in research in this area. The treatment is not limited to literature review and recount of actual happenings, although these are by no means ignored. Questions are posed and answers sought to the many riddles and unsolved problems. Ideas are offered and preferences indicated, from personal working experience, of the paths likely to be fruitful at crossroads with many directions. These traits distinguish this work from a mere anthology of current literature, with the obvious attendant advantages and disadvantages. The book has a flavour, but could be limited by the vision, right or wrong, of the author.

Antifertility vaccines are conceptually new modes of contraception. No previous experience of their use or of related drugs is available. The safety requirements of contraceptives as a class are very stringent and several years of safety testing and product development will be required before any new contraceptive sees the light of day for general use. However, a case is made for the possible early application of immunological methods in cancers and veterinary species, to which special chapters are devoted.

It is expected that the readership of this book will be professionals belonging to different disciplines: immunology, reproductive biology, endocrinology, obstetrics and gynaecology, veterinary sciences and others. Individuals and organizations concerned with family planning programmes and population control measures, as well as those in industry may also be interested in acquainting themselves with the current state of affairs in this area. Professional jargon is minimized and an attempt has been made to communicate in a language understandable to most. To meet the needs of research scientists, original references are provided for ready consultation of the details.

The book has drawn extensively from a review that I wrote for the World Health Organization – Regional Office for South-East Asia in 1976. I am grateful to Dr V. T. H. Gunaratne, the Regional Director, and Dr B. K. Anand, Assistant Director General in charge, for permission to utilize this material for the present purpose.

Many of my co-workers and students have helped me in various ways in the

preparation of the manuscript and figures and I would like to express my particular thanks to S. Ramakrishnan, Satish Gupta, Sudhir Paul, Dr C. Das, R. K. Naz, Navreet, S. Pandian, Suman, J. Sandhu, A. Tandon and Mrs. Bhatia. Rajesh Malhotra deserves credit for typing and H. K. Sharma for the figures. The book is dedicated to all investigators, wherever they are working in different parts of the world, who are endeavouring to utilize the techniques and concepts of two disciplines to make possible, one day, a new range of methods of the control of fertility.

New Delhi, 1979

GPT

Common Abbreviations and Glossary

Allogeneic	In which donor and recipient are different members of a genetically heterogeneous population of the same species.
Antigen	A substance capable of generating an immunological response.
Autologous	Within the same individual.
CMI	Cell mediated immunity.
Epitope	A defined antigenic determinant.
FCA	Freund's complete adjuvant.
HCG	Human chorionic gonadotrophin.
LHRH	Luteinizing hormone release hormone.
Syngeneic	Genetically identical.
Xenogeneic	Between members of different species.

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1

Why Search for a Vaccine for the Control of Fertility?

The need

The world population is increasing at an alarming rate. Futurologists estimate that the global population will exceed the six billion mark by the close of the century. In India, the current growth rate is about 2.1 per cent, e.g. 13 million people are added in this country alone in a year, a figure equal to the total population of Australia. The situation in some other developing countries of Asia, Africa and Latin America is even more telling.

With the introduction of immunoprophylaxis and health care measures, mortality due to diseases has shown a steady decline. Data in Fig. 1.1 indicate that the rate at which the decrease in birth incidence has taken place in the last seven decades in India is outstripped by the rate of decline in mortality during the same

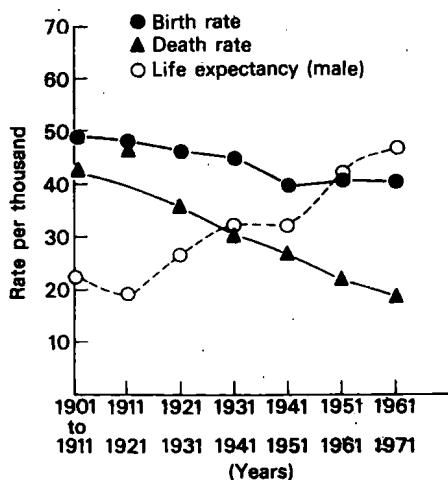


Fig. 1.1 Census figures on the birth rate, death rate and life expectancy as recorded in India during the past seven decades of this century.

period. The slope of the curve for the latter parameter is more steep than for the former, and if these trends continue, the gap between them will widen further. Thus, unless effective measures are taken to equalize the two, the population problem will become more accentuated with the expansion of social and preventive medicine care in communities.

Although a number of contraceptive methods is available, none of them is ideally suited to the basic needs of the people, where the population problem is pressing. Condoms are not available at the time and place where they are needed. Moreover, men do not always enjoy wearing them. The intrauterine devices entail extra blood loss, which an anaemic population can hardly afford, thus the expulsion rate is nearly as high as the insertion rate. Steroidal contraception has its own drawbacks. Besides those noticed in the western countries, there is the risk of liver toxicity in people with low protein intake and high parasitic load. Abortions are not universally legal, nor cheaply available means. Surgical sterilizations of male and female are, by and large, irreversible procedures and have low acceptability, especially in a milieu where survival of children is not assured. There is thus a need to evolve additional methods for the control of fertility, which may not have these shortcomings, and which may be better applicable and more acceptable.

Immunological methods have features which can meet some of the requirements. They require periodic and not continuous intake. They can be administered on a mass scale. They can be delivered by medical or para-auxiliary personnel without the requirement of sophisticated surgical or hospital facilities. They can even be self administered. Vaccines, by virtue of the good that they have done for curbing plague, small pox, polio and other communicable diseases, have established acceptability in urban and rural communities. There is thus little doubt that if a safe and effective antifertility vaccine became available, it would have an important impact on the family planning programmes.

The utility of the antifertility vaccines may not be confined to the developing countries. A vaccine, reversible in action, with fewer side-effects than the steroidal pills, can provide a useful alternative to oral hormonal contraceptives in the developed countries. It would spare the system from constant 'drugging' by non-natural steroids. Moreover, steroidal contraceptives exercise their effect by blocking ovulation, e.g. by interfering in a physiological process. Antifertility vaccines, on the other hand, can be designed to act at a step beyond the hypothalamic - pituitary - gonad axis, leaving the physiological processes to operate normally.

The principle

In an immunological approach, the rationale is to mobilize the body's endogenous capabilities to counteract a critical step in the reproductive process. The aim is to use the cells and the molecules by which the immune system ensures the defence of the body against unwanted bacteria, viruses and pathogens for intercepting reproduction. The immune system has traits of specific recognition of determinants on molecules and of discrete conformations in the macromolecules. It is normally tolerant to all 'self' components and reacts

only against those which are either 'foreign' to it or whose ontogenesis take place at a developmental stage subsequent to its own maturation, e.g. sperms in the post-natal years. Ways and means are, however, available today to break tolerance and make the system respond to and react against a chosen 'self' component. These advances have enabled the undertaking of research directed towards the development of 'vaccines' for the control of fertility. Once the target molecule critical to the success of reproduction is identified, methods can be evolved to mount an immune response against it. Antibodies and cell-mediated immunity will then act by neutralization of the biological activity of the target molecules and/or by exercising a cytotoxic and lytic action against the cell with which the target antigen is associated. Figures 1.2 and 1.3 illustrate the various ways in which this takes place.

One can question whether the search for immunological methods to control a vital and complex process such as reproduction can indeed be a feasible undertaking. The answer is provided, at least in part, by Nature's experiments, by the existence of clinical cases of infertility in couples where everything else is apparently normal and where the block is due to immunological factors. How the block emerged in a given individual is another question, the answer to which is not available, but the antigens against which it is directed in different clinical cases are becoming defined.

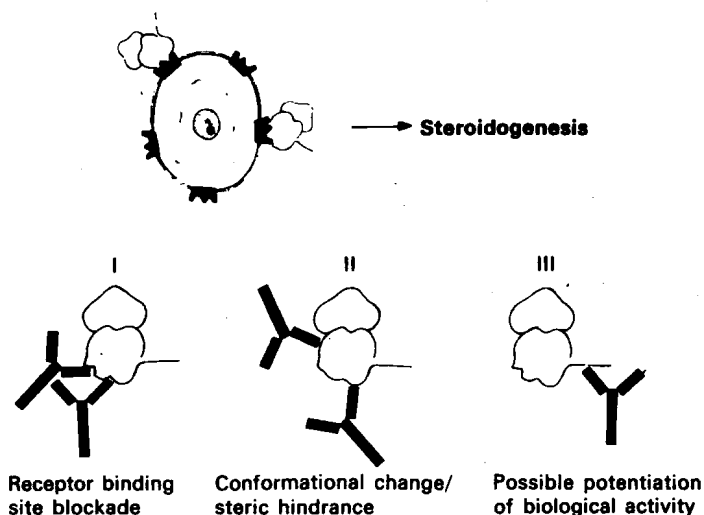


Fig. 1.2 Neutralization by antibodies of the biological activity of gonadotrophins. The example given is of Leydig cells, which, like the luteal cells, possess membrane-bound receptors for gonadotrophins. Hormone receptor interaction leads to steroidogenesis. Three eventualities are envisaged. Two of them, represented by schematic models I and II, cause the block by the antibodies of gonadotrophin-induced steroidogenesis. Experimental evidence is also available for situations where antibodies fail to neutralize the hormonal activity by combining at a site not interfering with the action of the hormone (schematic model III). The antibodies may in fact potentiate the biological activity by slowing down the degradation and excretion of the hormone. (Talwar, G. P. (1979). *Journal of Reproductive Medicine* 22, 61.)

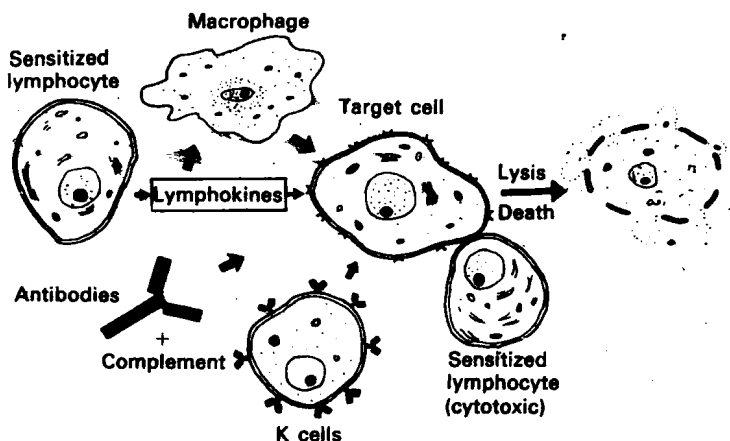


Fig. 1.3 Schematic representation of the manner in which sensitized cells alone, or with antibodies, exercise a killing or lytic effect on a cell bearing target antigens.

Can we induce at will an immune response against a selected protein of the reproductive system? Furthermore, will this lead to the control of fertility? In experimental animals, including sub-human primates, active immunization against a variety of reproductive system hormones and proteins has been demonstrated to be effective in regulating fertility. That the active agents in such situations are indeed the antibodies has been confirmed by simulation of the effect by passive administration of preformed antibodies of defined specificity. There is thus no basic theoretical argument against the development of antifertility vaccines. No wonder that this field of research has expanded enormously in recent years. Whether a satisfactory workable vaccine, which is effective, reversible, and devoid of undue side-effects in short and long range use, can materialize in the near future will depend on the ingenuity of the researcher to conceive of the right 'target' molecules and on the progress in our ability to manipulate successfully the factors that regulate the immune response. In subsequent chapters, the current status of this pertinent field of endeavour is briefly reviewed.

2

Natural Sterility due to Immunological Factors

The recognition that infertility in some couples may be due to immunological factors is both of practical and theoretical interest. To the practising clinician, indication is provided for yet another parameter to be studied for diagnosis. Once the cause is ascertained, a rational management of the case is possible. To the investigator, valuable clues are given on the components of the sperm and the egg, which are antigenic, and immune response against which can block fertility. Proper study of the clinical history of these cases can also furnish information on pertinent issues, such as (a) the reversibility or permanence of the immunological block against a given antigen and (b) the side-effects and accompanying aberrations, if any, on the metabolic, organ and endocrine functions.

Clinical reports on antispermatozoal antibodies in infertile couples

In 1954, two independent observations by Rumke and Wilson revealed the presence of antibodies reacting against spermatozoa in sera of infertile men. Wilson reported the incidence of massive autoagglutination of sperms in ejaculates of two male patients examined by him. Clinical and laboratory investigations excluded any anatomical or physiological abnormality in their wives. It was concluded that the cause of infertility in these couples was perhaps due to the presence of antisperm antibodies in the male partners. Rumke noted similar situations in two patients with oligospermia. Sera from some patients were effective in agglutinating sperm from other donors.

Further data incriminating immunological factors in fertility were published by Nakabayashi, Tyler and Tyler (1961) and by Segal *et al.* (1961). Rao and Sadri (1960) employed a haemagglutination technique with antigen derived from freeze-thawed spermatozoa and found that sera from either the wife or husband of 18 per cent of the 368 infertile couples investigated by them gave a positive immunological reaction in their test system. In 1960, Behrman *et al.* presented evidence for a possible marginal role of ABO blood group incompatibility in fertility. Franklin and Dukes (1964) reported that 31 out of 43 women with a two-year history of non-pregnancy had in their sera sperm-agglutinating antibodies,

while in a control group of 35 fertile women, only 2 had sperm agglutinins. The agglutinins disappeared in two to six months of avoiding contact with sperm and 9 out of 10 women in the former category became pregnant. These observations pointed to the relatively short duration of natural antibody response to sperm antigens and also to the requirement of constant antigenic stimulus for the sustenance of agglutinins. It is perhaps for this reason that the incidence of sperm-reacting antibodies has been noted to be particularly high in prostitutes, 35 out of 48, or 72.9 per cent, had agglutinins in their serum reacting with spermatozoa (Schwimmer *et al.*, 1967).

Antisperm antibodies were seen not only in the serum of male or female partners, but there was also evidence for the presence of antibodies in the reproductive tract, in particular in the cervical mucus extracts. Parish *et al.* (1967) found cytotoxic antispermatozoal antibodies in the cervical mucus of 3 out of 48 infertile women tested by them. D'Almeida and Eyquem (1978), employing a variety of methods, found that about 50 per cent of the 83 infertile women studied, contained sperm-reacting antibodies in cervical mucus. There was dissociation between the presence of antibodies in the serum and in the mucus, only 14 had antisperm antibodies in the cervical mucus. Isojima *et al.* (1978) have reported a case in which very high titres of sperm-immobilizing antibodies were present in the serum but the penetration of sperm in preovulatory cervical mucus was unhindered, perhaps due to the lack of the C₃ component of complement in the cervical mucus. There were also cases in which sperm-immobilizing antibodies were present in the cervical mucus but not in the serum.

The antibodies in the cervical mucus can originate both from systemic circulation (IgG) and from local synthesis (IgA). It seems logical that it is only the antibodies and/or cell-mediated immunity in the local environment of sperm deposition, transport, or sites of fertilization, and implantation of the blastocyst, which will be effective and will have relevance to the interruption of fertility.

Types of antibodies reported in infertile couples

The foregoing review together with the work of other investigators (Shulman and Shulman, 1971; Menge, 1970; Jones *et al.*, 1976; Husted and Hjort, 1975) leads to the conclusion that there are three types of immunological reactions identified in infertile couples. These are summarized below:

In males: autoagglutinins

The presence of antisperm antibodies in males causes agglutination of the sperm. The autoagglutinins, if massive, can render the subject infertile. Sperm antigens develop late in ontogeny and man has, perhaps, no immunological tolerance to these antigens. They are normally secluded in the reproductive tract but in the event of breakage of the blood-testis barrier, autoantibodies are formed to these antigens.

In females

In the female, antibodies against husband or donor sperm, i.e. the antibodies