« G. Mendel » Institute of Medical Genetics and Twin Research, Rome
(Director: Prof. Luigi Gedda)

An Outline of the Problems of Immunogenetics and Reproduction

L. Gedda, M. Milani-Comparetti

1. Introduction

While general genetic problems in sterility and infertility have been rather extensively considered by the senior author (Gedda, 1968) a more extensive treatment of the special relationship of sterility and infertility with immunogenetics seems to be worthwhile in order to assess the present state of our knowledge and to visualize the most promising areas for further research.

This introductory treatment of the subject is justified by the fact that a large percentage of the patients applying for genetic counseling at our Institute represent cases of sterility, infertility and hypofertility, and it appears to the authors that immunogenetic etiology in this field is probably one of the areas in which most progress is yet to be made.

Before going into the subject it may be useful to recall a few condensed definitions:

— Individual sterility is the diagnosed inability of one individual (male or female) to attain conception.

— Sterility of the couple is the inability to attain conception by mating partners without assessed individual sterility in either partner.

— Infertility is the inability to carry any product of conception to independent life.

— Hypofertility is the inability to carry to independent life the products of subsequent conceptions.

— Immunogenetics is the study of the ability by any organism to discriminate between " self " and " non-self " (" self " being all those substances that belong to the genetically induced normal structure and function of the organism).

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— Antigen is any substance that, when identified as " non-self " by any organism, can elicit a *specific* immune reaction.

— Antibody is a substance that any organism may produce to react *specifically* with an antigen (or with its haptenic moiety).

— Hapten is the specific moiety of an antigen that reacts with the corresponding antibody, yet lacks the ability to elicit by itself the immune response. Any hapten, conjugated with the proper " support ", acquires immunogenicity and thus becomes an antigen.

Immune reactions are the result of the activity of the body's complex apparatus of specific defense and self-control, governing the control of compatibility with individual structure and function, as genetically determined, of any substance that may be found in the body itself.

It may be worth mentioning that, biologically speaking, any substances that may be found in the respiratory or digestive tracts are to be considered as " outside " the body as long as they do not cross the epitelial membranes.

Immunological " maturation " may occur before or after birth. In man it is prenatal; in the mouse, for instance, it occurs around the twentieth day of independent life.

2. The Basic Rule of Immunology

Any antigen that is present in an organism (and in potential contact with its lymphatic system) at the time of immunological maturation is recognized as " self " and will be subsequently tolerated. Any other antigen will normally elicit, in one way or another, an immune response.

This fundamental rule may have exceptions in either direction. An excess of immune reaction occurs in " autoimmune " diseases, where the body fails to identify as self any antigen that belongs to its normal structure and function (Hashimoto's disease, Lupus, etc.). Deficiencies of immune defences are those of " immune tolerance " and " immune paralysis ".

Apparent exceptions to the fundamental rule of immunology occur whenever:

— A substance lacks immunogenicity, i.e. it lacks the ability to elicit an immune response (it is not an antigen, as in the case of haptens).

— An antigen is identical to another previously recognized as " self ".

— An antigen is not accessible to the afferent branch of the immune reaction, i.e. it cannot reach the immunocompetent structures of the host.

— An antigen is not accessible to the efferent branch of the immune reaction, i.e. it may not be reached " in situ " by the host's antibodies.

— An antigen is located in a part of the host that lacks lymphatic drainage.

— Before reaching the host's immunocompetent structures the antigen reacts with preformed antibodies; the resulting antigen-antibody complex loses immunogenicity.

3. Male Sterility

Taking now into consideration male individual sterility, we find that it may be due to autoimmune phenomena. The products of male gonads and of associated glands are isolated from the body's internal environment and thus escape identification as "self" upon immunological maturation.

Several studies have been carried out on antigen specificities of the spermatozoa and seminal plasma and especially on the SCA (spermatozoal-coating antigen, of seminal plasma origin) and the spermatogenic antigen (Weil et al, 1956; Weil and Rodenburg, 1960; Rao and Sadri, 1959; Katsh and Katsh, 1961).

The possibility that males may have autoimmune reactions against seminal or spermatozoal antigens has been verified in part (Rümke and Hellinga, 1959).

Much further research is desirable (applying the newest techniques of immunology) on autoimmune phenomena concerning spermatozoa and seminal plasma as well as the various related structures and cells (Sertoli, Leydig), since immunological damage to any component of this complex system may impair the fertilizing ability of the sperm.

4. Female Sterility

Female individual immunological sterility may also be envisioned as a consequence of autoimmunization, but the situation is different from that of males in view of the fact that the product of female gonads is not directly excreted.

Immunization against sperm antigens is more likely, and in fact seems to be a frequent occurrence. Franklin e Dukes (1964) conducted a very significant study in this respect. Comparing four groups of women (I. sterile without demonstrable organic cause; II. sterile with demonstrable organic cause; III. fertile controls; IV. random sample including unmarried women) they found that agglutination of a sperm suspension by the sera of these four groups gave the following distribution of positive reactions: I 78.9%; II 10.4%; III 11.8%; IV 4.3%. This finding is all the more interesting since it only reveals agglutinating antibodies, while blocking antibodies may be independently present and even more efficient in preventing fertilization.

Several studies have been made of ABO incompatibility and sterility in secretor and non-secretor individuals on the assumption that A or B antigen-bearing sperms would incur agglutination or coating by antibodies in the female genital tract. The findings are contrasting, and no clearcut statistical evidence seems to support differential fertility as related to blood groups in this respect.

Studies on histocompatibility antigens and HLA sensitization are yet lacking in this field, but the fact that the presence of antileukocyte antibodies is more frequent in multiparous (even if polyabortive) women seems to indicate that histocompatibility involves the postzygotic rather than the prezygotic phases of reproduction.

A separate analysis might be devoted to the problem of mutual acceptance between egg cell and sperm, on the basis of stereochemical combinations resembling antigen-antibody specificity (see Lillie's fertilisin-antifertilisin system). Yet the Authors prefer to consider such rigorous specificities as preordained (in order to provide species-specific reproduction and to limit fertilization to intact gametes); thus the phenomenon exceeds the definition of an immune reaction.

Perhaps it is easier to visualize the involvement of this specificity as the secondary effect of a primary immune reaction involving the gametes: coating of gamete surface antigens by blocking antibodies would in fact interfere with mutual recognition of egg and sperm, thus preventing fertilization. In this sense it is well worth advocating further studies on incomplete (non-agglutinating) antibodies in the female genital tract. This would seem to involve a Coombs or immunofluorescence test on spermatozoa retrieved from female salpinges.

5. Infertility

While previous considerations apply (without pretence of exhausting them) to possible prezygotic immunological factors leading to either individual or couple sterility, postzygotic factors of an immunological nature may also lead to infertility or at least hypofertility.

We know all too well that the body responds specifically to the introduction of foreign antigens, even within the species, and transfusion or grafting problems are the result.

Pregnancy is the obvious exception to the rule: the fetus, with about 50% of its antigens derived from the father and thus foreign to the maternal organism, is not ordinarily rejected.

The main classical explanations invoked for such non-rejection are:

a) The contact between fetal and maternal tissues is established through the trophoblast, whose syncytial character prevents the exfoliation of individual cells and the resulting catabolism by the maternal organism. It is generally accepted, in fact, that the first step in the immune reaction — the identification of the foreign antigen — must be the catabolic phagocytosis by the host.

b) The trophoblastic contact surface is lined by a non-antigenic fibrinoid layer. Also, the surface of this layer consists of negatively-charged carboxylic terminals that repel the similarly charged maternal lymphocytes (Currie and Bagshave, 1967).

c) No lymphatic drainage seems to occur in the area where fetal and maternal tissue meet, while it has been proved that lymphatic drainage is a *condicio sine qua non* for immune reactions including graft rejection (Barker and Billingham, 1967).

d) Even if primary antibodies are formed, the fibrinoid-trophoblast barrier prevents contact with fetal antigens in situ (efferent block in the immune response). Only microglobulinic antibodies can cross the barrier, and even this may only occur as the result of a selective active-transport process.

e) If small amounts of antibodies are present in the maternal blood, they

may coat the antigenic sites of occasional fetal tissue fractions entering the maternal circulation (following possible microtraumas). Thus antigen-antibody complexes are formed, and immune elimination follows, preventing further maternal sensitization.

These and possibly other explanations may, individually or collectively, contribute to the complex mechanism nature has adopted to protect the fetus against maternal immune reactions.

A general picture thus emerges in which many instances of sterility and infertility may be associated with either excesses or deficiencies of the immune response.

Several possible instances have been mentioned, and these involve genetic problems on two different levels.

The most obvious level of genetic involvement is that of antigen inheritance, and the importance of blood group genetic analysis for immunology and reproduction is the natural example.

But another area of genetic research is currently gaining importance: we refer to the study of genetic variability in the immune response to antigenic stimulation.

The ability to respond to antigenic stimulation is under genetic control, in terms of both generalized and specific response. We refer the reader to the paper by Fudenberg (1966) on genetically determined abnormalities in antigen-antibody interaction.

A study of the possible differential incidence of sterility and infertility in families exhibiting defects of immunologic response would contribute to an understanding of the problem, and the genetic counseling service at our Institute is currently engaged in such a study.

6. The Role of Natural Antibodies and Genetic Polymorphism in the Protection of the Fetus

The foregoing treatment of possible relationships between immunology and human reproduction follows the accepted concepts of immunology, trying to focus attention on a few points where immunogenetic studies may contribute to the understanding of the pathology of reproduction.

Yet we deeply share the feeling expressed by many immunologists (e.g. Behrman, 1965) that some basic questions of vital importance in immunology have yet to be answered. Thus we beg our readers to follow and possibly assist us if, in the following context, we try to clear our own minds by what we might call " thinking aloud ".

— The main task of immune responses by any organism is the recognition and rejection of whatever is foreign (" non-self ") to its normal structure and function.

— In order to provide variation among living beings, nature has devised a rearrangement of genetic material in germinal cell lines and subsequent blending of paternal and maternal material to produce descendants. Thus the products of germinal cell lines are foreign to the respective bodies, and the product of conception

is foreign to the maternal body. (For the sake of simplicity, in the following text we shall generally refer only to fetal substances, cells or tissue).

— In order to avoid rejection of the fetus, nature had to provide appropriate exceptions to the basic rule of immune response. These exceptions are obtained by a careful choice of many immune mechanisms, within the simplest possible classification of immune responses: 1) no immunization; 2) primary immunization; 3) secondary immunization.

— The finding that fertility is associated with a rigorous balance of immune mechanisms, while infertility is often associated with either deficiencies or excesses of the immune response, seems to suggest a central role of primary immunization in the protection of the fetus, with the levels of no immunization and secondary immunization representing deficiency and excess in this respect.

— Primary immunization is generally associated with the production of antibody macroglobulins, while secondary immunization is generally associated with the production of antibody microglobulins.

— The barrier between maternal and fetal tissues in the placenta is generally impervious to primary antibodies.

— The shift from primary to secondary immunization (and thus from macroglobulins to microglobulins) requires an interruption in the contact of the corresponding antigen with the immune system. Thus sustained supply of the antigen generally involves sustained production of macroglobulins, unable to cross the placenta.

— Mother and fetus are antigenically different, yet there must be metabolic exchanges between them. How can this be achieved without immunological rejection? The answers are given on pages 6-7. The placental barrier is pervious to metabolites that are immunologically acceptable by both mother and fetus. The barrier itself is nonantigenic. Thus the danger of maternal immune response is minimized at the " no immunization " level of response. Yet the placental barrier may be impaired, especially by traumas. In that case fetal matter may enter the maternal body. Since such an event is not so improbable, the appropriate disposal system must be foreseen before it produces secondary immunization.

— The most obvious disposal system would appear to involve the immune mechanism itself, by means of appropriate antibodies. Such antibodies would have to be: (*a*) preexisting in the maternal serum; (*b*) unable to cross the placental barrier; (*c*) directed against the expected antigens of fetal matter but not against those of the maternal " self ".

— These conditions are fulfilled in nature by the so-called " natural antibodies " (see Boyden, 1965), of which the best-known example are human α and β hemagglutinins. Preexistence of such antibodies is assured by ubiquitous presence of the corresponding antigens in nature. The same ubiquitous presence of antigen ensures sustained *primary* stimulation, resulting in continued presence of macroglobulinic antibodies, unable to cross the placenta. The normal mechanism of " self " recognition upon immunological maturation would have avoided the production of such antibodies if the corresponding antigens were present in the mother.

— The placental barrier between mother and fetus divides the respective blood streams. Thus the fetal matter most likely to enter the maternal organism upon rupture of the barrier would be fetal blood.

— Any component of fetal blood accidentally entering the maternal blood should find there natural antibodies against *some* of its antigenic determinants. Reaction with such antibodies would result in disposal by the maternal organism before its *other* antigenic determinants succeed in immunizing the mother.

— Proof of the existence of such a system is provided by the natural α and β hemagglutinins. These are elicited by ubiquitous diffusion of the corresponding antigens, providing sustained production of macroglobulins. Therefore ABO- incompatible fetuses are safely carried by their mothers despite the latter's primary sensitization. If ABO incompatible fetal cells enter the maternal blood, they are eliminated following reaction with the corresponding antibody without sensitizing the mother to their other antigens. The protection of ABO incompatibility in respect to Rh-Hr sensitization is an obvious example, and this probably also underlies the finding by Issit (1965) that when no Rh sensitization occurs other blood group sensitizations are also generally absent.

— The fact that the frequency of individuals homozygous for the amorph O gene tends to 50% seems to represent the ideal for the statistical probability of this mechanism to be used by mothers in the protection against immunization by other red blood cell antigens.

7. Conclusions

The authors have dealt rather extensively with one particular system of antigens and antibodies because, if their interpretation is correct, it may possibly be taken as one example of a much wider system.

Many genetic polymorphisms in serum factors that are currently being discovered would thus find their justification in the natural economy, as protective systems for immune disposal (by corresponding maternal natural antibodies) of the respective fetal serum components before they are recognized as foreign.

What the authors propose is a central interpretation of the role of natural antibodies and genetic polymorphism in the natural economy as a mechanism intended to protect fetal antigens against immune rejection by the maternal organism.

The central concept may be extended to the protection of prezygotic, germinal antigens and possibly to the protection of "sequestered" antigens against autoimmunity.

Such extensions would provide further justification for the existence of the same mechanism in males. Yet the fundamental justification for the existence of the same immunological mechanism in both sexes is probably to be found in the more general aspects of natural economy.

The implications are extensive not only in immunology but in several related fields, such as population genetics, and the Authors intend to conduct appropriate test. Yet they chose to formulate their interpretations in this introductory note as a contribution to the effort of understanding the complexities of immunology.

Summary

The authors trace an outline of the problems involved in the relationship between immunogenetics an reproductive pathology, indicating the need for further specific research.

The authors formulate a "central interpretation" of several aspects of immunology, based on the role assigned by nature to primary sensitization in the protection of germinal and fetal antigens against immunological rejection. The implications of such an interpretation may be far-reaching in such fields as reproductive pathology, autoimmune diseases, anthropology and population genetics.

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RIASSUNTO

Gli autori passano in rassegna i problemi relativi ai rapporti fra immunogenetica e patologia della riproduzione, indicando la necessità di ulteriori ricerche specifiche.

Gli autori formulano una « interpretazione centrale » di diversi aspetti della immunologia, basata sul ruolo assegnato dalla natura alla immunizzazione primaria nella protezione degli antigeni germinali e fetali contro il rigetto immunologico. Le implicazioni di tale interpretazione possono essere assai vaste in campi diversi, quali la patologia della riproduzione, le malattie autoimmuni, l'antropologia e la genetica di popolazioni.

RÉSUMÉ

Les auteurs considèrent les problèmes concernant les rapports entre immunogénétique et pathologie de la reproduction, en indiquant l'exigence d'ultérieures recherches spécifiques.

Les auteurs formulent une « interprétation centrale » de plusieurs aspects de l'immunologie basée sur le rôle assigné par la nature à la sensibilisation primaire dans la protection des antigènes germinaux et fétaux contre le rejet immunologique. Les implications d'une telle interpretation seraient assez vastes dans plusieurs domaines tels que la pathologie de la reproduction, l'auto-immunisation, l'anthropologie et la génétique de populations.

ZUSAMMENFASSUNG

Verf. liefern einen Überblick über die Probleme, die sich aus den Beziehungen zwischen Immungenetik und Reproduktionspathologie ergeben, und betonen, dass weitere spezifische Untersuchungen auf diesem Gebiet notwendig sind.

In einer « Zentralinterpretation » fassen die Autoren verschiene Aspekte der Immunologie, auf Grund der Rolle die Natur der Primärimmunisierung erteilt hat, nämlich Keim und Foetus durch Antigene gegen die immunologische Abstossung (Rejection) zu schützen. Diese Auslegung der Verf. gestattet weitegehende Verknüpfung mit verschiedenen Forschungsgebieten, wie der Reproduktionspathologie, den autoimmunen Erkrankungen, der Anthropologie und der Bevölkerungsgenetik.