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Immunogenetical Considerations on Corneal Transplantation *

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In the field of immunogenetics a special role is played by experiments and clinical findings concerning homotransplantation. This very important and complex subject is studied by our School also from the angle of clinical and experimental findings provided by corneal homografts.

Our attention has been focused on this subject by a clinical case, the immunogenetical implications of which exceed, in our mind, the boundaries of the case itself. The case, described clinically by Dr. M. L. Restivo, presented the following relevant data:

C. A., aged 24, had undergone bilateral penetrating keratoplasty (in the year 1956 in the left eye and the following year in the right eye), being affected by bilateral keratoconus. The clinical course evolved uneventfully in both operations and a visus of 10/10 O. D. – O. S. was obtained.

The patient led a normal life and married at 23. On February 3rd, 1964 (8 years after the first operation and 7 after the second) when at her second month of pregnancy she entered the hospital with ocular symptoms reflecting the so-called " late clouding "; the disease was diagnosed as follows:

1) lack of general or local morbid manifestations possibly responsible for propagation of a pathologic process to the homografts.

2) Pathologic reaction strictly limited to the transplanted grafts.

3) Regression and disappearance of the symptoms following cortisone therapy.

The child was born normally at term. No other relevant detail was observed.

We use this case as a starting point for a series of considerations that evolve from our immunological interpretation.

Much as we realize that the subject is very well known, we wish, mostly for our own benefit, to trace the pattern of the problems involved in the transplantation and survival of corneal grafts.

As distinguished from nearly all other cases of homo- and heterotransplantation, corneal grafts are often free of immune reactions. The transplantation of corneal grafts (or keratoplasty) can be effected by either the lamellar or the penetrating technique. In the former case (when the endothelial layer of the host is unaffected while

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clouding of the outer layers has occurred) only the clouded outer layers are exchanged for equal layers from a healthy donor. In the latter case a full-thickness corneal section, including the endothelium, is exchanged.

Lamellar homografts almost never involve immune reactions; lamellar heterografts involve a variable percentage of immune reactions, and the same applies, in different proportions, to penetrating homografts, while penetrating heterografts are practically always rejected.

Apart from the possible consequences of peculiar operation techniques, the argument over the very nature of the reaction (immune reaction or other etiology?) has been going on for years, but the consensus now seems to be in favor of the immunological interpretation. A further argument concerns the specificity of the antigens responsible for such immune reactions: species antigens, individual antigens or organ antigens?

However wide the variability of reports, an analysis of available literature tends to emphasize the role of species antigens in heterografts and individual antigens in homografts, while the role of organ antigens seems to be debatable.

A third argument concerns the permanence of antigenic properties in the graft, since it is widely believed that at least most of the endothelium is supplanted by proliferating cells of the surrounding host endothelium. Recent studies however, employing isotope-marked grafts, or grafts from donors of one sex onto hosts of the opposite sex (for sex-chromatine determination) have confirmed the survival of the donor's endothelial cells. Besides, our index case itself seems to prove the permanence of the antigenic properties of the grafted tissue for at least eight years after the operation.

In order to try to explain this case we have formulated the hypothesis that the antigens of the corneal graft may not have stimulated the production of antibodies in the host organism: being isolated in a non-vascularized area, in fact, the antigens may have failed to reach the antibody-synthesizing sites. Eight years later, with the beginning of pregnancy, the fetal villi would have brought into contact with the maternal blood flow some antigenic traits (inherited from the father) that were absent in the maternal organism but were equal or similar to one or more of those originally present in the corneal graft. Thus the antibodies formed against the fetal antigens would be able to " recognize " and to attack the graft's antigens.

It has appeared to us that this hypothesis may well deserve further consideration and extension, beyond the limits of the individual case, in view of the contribution that it may give to the interpretation of immune phenomena involved in keratoplasty. Our hypothesis should be especially valid in the case of lamellar grafts, since 1) the grafted lamella is isolated within a tissue whose basically colloid structure hinders the diffusion of any substance and particularly of large molecules; and 2) the graft itself, being made of the same tissue, seems to have only limited antigenic properties. In the case of penetrating keratoplasties, however, the situation is quite different: the graft has its own endothelial layer (much richer in antigenic properties than the overlying stroma) in direct contact with the aqueous humor where the diffusion of molecules of any size, even if still extravascular, is far easier and faster.

The different implantation of the lamellar and penetrating grafts may thus be involved in the observed fact that heterografts are generally tolerated if lamellar and always rejected if penetrating.

Against this interpretation (which incidentally does not explain the occasional clouding of penetrating homografts) it may be objected that even a percentage of lamellar grafts undergoes clouding. This should not occur if, as we assumed, the production of antibodies were not stimulated because the graft's antigens could not reach the antibody-forming sites.

At this point we may recall that, while it is a fact that antibody production is conditioned by introduction of the corresponding antigens, it can be stimulated not only by the antigens of the transplanted graft but also by other antigens, (identical or even closely similar to those of the graft) introduced separately (see for instance our own case). Particular attention in this respect should be given to a series of experiments carried out over the years, out of which we may mention one by Basu and Ormsby (1956). Experimenting on a technique of lamellar keratoplasty, these Authors found that the incidence of reactions increased from the normal 30% to 60 or 80% if a skin graft from the same donor was effected at the same time. The obvious inference is that the increased incidence of reactions in cases of lamellar keratoplasty associated with a skin graft from the same donor onto the same host should be ascribed to the introduction of circulating antigens from the skin graft, stimulating the production of specific antibodies which would then be able to identify and attack the same antigens on the corneal graft. (This type of reaction, incidentally, tends to rule out organ antigens).

The percentage of reactions occuring even in the absence of a simultaneous skin graft might be explained, rather than by sensitization to the corneal graft's own antigens, to independent sensitization to other antigens (identical or closely similar to those of the corneal graft's donor having been introduced separately).

It may be objected again at this point that such separate immunization, if it took place before keratoplasty, should be connected with an earlier onset of the reaction. A recent study by Sanna and Rivara (1962) indicated, however, that an earlier reaction can take place only if vascularization has already occurred. These Authors conducted the following series of experimental corneal grafts:

a) on animals in which corneal vascularization had been previously induced;

b) on animals in which immunization had been previously induced;

c) on animals in which both corneal vascularization and immunization had been previously induced.

Their findings indicate that in both cases a) and b) the onset of the reaction occurred on the 12th – 15th day, while only in case c) did the onset of the reaction occur as early as 48 hours after keratoplasty. Thus it appears that previous sensitization of the host does not induce an earlier onset of immune reactions in keratoplasty unless previous corneal vascularization has also occurred.

In order to analyze the different factors involved we should now examine the different phases into which the genesis of the immune reaction can be divided:

1) introduction of the graft;
2) release of the antigenic fractions of the transplanted tissue;
3) diffusion of the released antigenic fractions through the surrounding tissues;
4) entrance of the diffused antigenic fractions into the host's vessels, reaching the antibody-forming structures;
5) formation of specific antibodies;
6) migration of antibodies outside the vessels;
7) diffusion of antibodies through the tissues surrounding the transplanted graft;
8) specific antigen-antibody reaction;
9) possible autocatalytic effect due to further release of antigenic fractions following disruption of graft by antibodies.

It is quite obvious that phases 1-4 (transfer of corneal antigens into the host's vessels) and 6-8 (transfer of specific antibodies from the host's vessels to the grafted tissue) may be quite different in cases of lamellar and penetrating keratoplasty.

It is our assumption that phases 2-5 (introduction of antigens and formation of antibodies) may occur independently from phase 1, whenever they derive from separate introduction of antigens, identical or closely similar to those of the grafted tissue. If our hypothesis were to be proved correct, it may contribute to explain a large part of the cases of non-reaction, especially in lamellar keratoplasty. Yet this hypothesis can hardly explain the high variability of the incidence of reactions in both lamellar heterografts and penetrating homografts, even when carried out with the highest possible degree of standardization.

At this point we wish to introduce a second hypothesis, closely related to the first one, concerning phases 6-8 of immune reactions as previously analyzed (these phases concern the transfer of antibodies from the host's vessels to the antigenic loci remaining on the grafted tissue).

Immunological experience tells us that the antibody response of an organism to the introduction of foreign antigens tends to take different routes and forms (as it happens in most of the mechanisms of defence and regulation of the organism). We should therefore discriminate the various *types* of antibodies present and active in different areas in connection with the introduction of different antigens. Our attempts to obtain indications in this respect from existing literature on the subject have failed to provide relevant contributions: reported antibody analyses seem to have been aimed at verifying the presence of antibodies in the anterior chamber of the eye (identifying antibodies as gammaglobulins in general) in order to verify the immune quality of the reaction. Many studies have investigated either the amount of antibodies (especially their serum/aqueous humor ratio) or their specificity.

By comparison with the well-known phenomenon of dialysis of antibodies through the endothelial barrier between mother and fetus, by which only antibodies of low molecular weight can normally reach and attack fetal blood cells, we can assume that a parallel phenomenon may occur in the immune reactions of keratoplasty. This

hypothesis takes on added significance when we consider that in this case the endothelial barrier is doubled or even tripled (endothelium of the vessels, endothelium of the anterior chamber and, in the case of lamellar grafts, corneal endothelium).

The fact that antibodies can have different sizes is well documented, and the studies carried out on " complete " or " bivalent " and " incomplete " or " univalent " antibodies (molecular weight about 1,000,000 and 160,000 respectively) are sufficient proof. The hypothesis that different types of antibodies may undergo differential filtering (dialysis) between blood plasma and aqueous humor (and then again between aqueous humor and stromal layers in lamellar grafts) would explain the majority of the observed differences of behavior and of incidence in the immune reactions following keratoplasty. Assuming, in fact, that different types and sizes of antigens may sensitize different antibody-forming structures of the host, resulting in the formation of different types and sizes of antibodies, one could explain differences of reaction to homografts and heterografts, to stromal and endothelial antigens, up to the point of possibly explaining the apparent paradox of heterografts from species philogenetically more removed sometimes causing lesser reactions than others.

Examining now from a general point of view our hypothesis, let us analyze existing indications concerning the presence of antibodies in the serum, in the aqueous humor and in the corneal stroma respectively. Concerning the serum/aqueous humor ratio, we can refer to a study by Ovary and D'Ermo (1953) indicating that in the immunized rabbit the titre of antibodies has ratios varying between 1:50 and 1:350. This finding indicates that serum antibodies do not diffuse completely into the anterior chamber, and in some cases such diffusion seems to be only minimal. Concerning the ratio of antibodies between the anterior chamber and the stromal layers, in the absence of direct evidence, we can draw some interesting inferences from Maurice's studies on the distribution and origin of different substances in the stromal layer of the cornea (1962).

In the four cases illustrated by Maurice we find that proteins (albumin in the specific case) do *not* diffuse through the endothelium but diffuse minimally through the limbus. Substances of intermediate molecular size hardly diffuse through the limbus, from which they fail to reach the central portion of the stroma (into which a lamellar graft would be placed), while their diffusion through the endothelium is still minimal. With decreasing molecular weight, down to glucose, diffusion through the limbus tends to zero while diffusion through the endothelium tends to be complete.

This consistent pattern of increasing difficulty in the diffusion of substances into the stromal layers of the cornea, in direct proportion to their molecular size, seems to fit quite closely our hypothesis of selective diffusion of the various types of antibodies in the corneal tissue. A special role in this connection should be played by primary and secondary immunization: the authors plan to reviev this subject in a separate paper.

A series of experiments is currently being carried out to test the hypotheses formulated in the present paper.

Summary

A case of bilateral clouding of corneal homografts in a 24-year-old woman 8 and 9 years after the respective operations, coincidentally with the second month of pregnancy, leads the Authors to a series of considerations and hypotheses on the immunological aspects of keratoplasties.

Differential diffusion of antigens to reach the antibody-forming sites, and of antibodies to reach the antigenic loci on the graft, through the endothelial barriers and the stromal layers, is held responsible for the peculiarities of corneal graft survival, especially in view of the different molecular sizes of antibodies and in connection with primary and secondary immunization. A series of experiments is currently being carried out to test the various hypotheses.

Literature

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RIASSUNTO

Un caso di opacamento bilaterale di lembi corneali, ad 8 e 9 anni di distanza dai rispettivi trapianti, in una donna di 24 anni, in coincidenza col secondo mese di gravidanza, conduce gli Autori a formulare una serie di considerazioni ed ipotesi sugli aspetti immunologici della cheratoplastica.

Alla diffusione differenziale degli antigeni fino a raggiungere le sedi dell'anticorpopoiesi, e degli anticorpi fino a raggiungere i loci antigenici sul lembo trapiantato, attraverso le barriere endoteliali ed il tessuto stromale, vengono attribuite le peculiarità della sopravvivenza dei trapianti corneali, soprattutto in considerazione delle diverse dimensioni delle molecole anticorpali ed in rapporto alla immunizzazione primaria e secondaria. Una serie di esperimenti è attualmente in corso per verificare le ipotesi formulate.

RÉSUMÉ

Un cas d'opacification bilatérale de greffes cornéennes, 8 et 9 ans respectivement après leur implantation, chez une femme de 24 ans, en coïncidence avec le deuxième mois d'une grossesse, conduit les Auteurs à formuler una série d'hypothèses sur les aspects immunitaires de la greffe cornéenne.

Les péculiarités du comportement des greffes cornéennes sont attribuées à la diffusion différentielle des antigènes jusqu'aux sièges de l'anticorpopoiese, et des anticorps jusqu'aux loci antigéniques sur la greffe. Cela surtout en considération des différentes dimensions moléculaires des anticorps et en rapport avec l'immunisation primaire et secondaire. Une série d'expériences destinées à vérifier les hypothèses formulées est actuellement en cours.

ZUSAMMENFASSUNG

Bei einer Frau von 24 Jahren trat im 2. Schwangerschaftsmonat im Abstand von 8 bzw. 9 Jahren nach Korneatransplantation beiderseits eine Verschleierung von Korneastellen auf. Die Verf. stellen daher einige Überlegungen und Vermutungen bezüglich der immunologischen Seite von Cheratoplastiken an. Die Eigenart des Überlebens von Korneatransplantationen wird, vor allem, wenn man die verschiedene Größe der Antikörpermoleküle und das Verhältnis zur Primär- und Sekundärimmunisierung betrachtet, der differenzierten Diffusion von Antigenen und Antikörpern zugeschrieben, von denen Erstere biszum Sitz der Antikörperpojesis und Letztere bis zu den Antigenloci auf der transplantierten Korneastelle gelangen.

Es werden zur Zeit Untersuchungen angestellt, um diese Hypothesen zu überprüfen.