PRESENT STATUS OF THE NORMAL LYMPHOCYTE TRANSFER TEST

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The rationale for selecting human kidney donors on the basis of tissue histocompatibility is founded on experiments using inbred strains of mice.

It has been well documented that the smaller the histocompatibility differences between donor and recipient, the easier it is to break the immunological barrier to graft acceptance (Snell et al., 1953; Berrian and McKhann, 1960; Billingham, Brent and Medawar, 1956; Main and Drehn, 1956; McLaren, 1961; Medawar, 1963).

Little is known with regard to the number and relative strengths of tissue antigens in man, but that a similar situation to the mouse exists, is suggested by the results of human renal transplantation which clearly indicate that the chances of success are better when donor and recipient are closely related (Murray, 1964; Cepellini, 1965). Both Simonsen (1965) and Cepellini (1965) have postulated on the basis of the human survival figures that a quite small number of strong alleles may exist in man.

Numerous in vivo and in vitro studies have clearly indicated that cells of the lymphoid series are responsible for the destruction of tissue homografts and the death of target cells in tissue culture (Mitchison and Dube, 1955; Billingham, Silvers and Wilson, 1963; Elkins, 1964; Govaerts, 1960; Wilson, 1963).

To the extent that the inflammatory lesion produced by the intradermal (I.D.) injection of viable homologous human lymphocytes (Normal Lymphocyte Transfer Test-N.L.T.) reflects a graft versus host reaction (G.V.H.), it is a measure of the response of the recipient’s lymphocytes on contact with the donor’s antigens.

Some points in the performance and interpretation of the test will be discussed. Evidence bearing on the G.V.H. versus H.V.G. nature of the test will be presented. The sensitivity of the test as a means of selecting the least incompatible donor from a panel has been examined by skin graft experiments and also by trying to correlate the post-operative course of a few patients whose donors have been selected on the basis of the test.

MATERIALS AND METHODS

Preparation of lymphocytes

A number of different methods which are equally effective have been described (Brent and Medawar, 1963; Amos, Nicks, Peacock and Sicker, 1965). Essentially platelets and granulocytes are removed from whole blood by defibrination or by passing the blood through a nylon column and the red cells are then removed by rapid sedimentation.

Some workers (Bridges, Nelson and McGeown, 1964; Amos et al., 1965) have found no clear correlation between the intensity of reactions obtained and the percentage of lymphocytes in the suspension. However, Gray et al. (1965) and Amos et al. have shown that granulocytes and platelets not infrequently produce inflammatory reactions of a non-immunological nature even in the autologous situation and therefore it is better to exclude these elements as far as possible.
Technique of injection

It is important to ensure that the cell suspension is injected wholly intradermally as inadvertent subcutaneous injections of even part of the suspension will give discrepant results and may account for some of the negative reactions reported. Two reports from the Nuffield Unit of Medical Genetics, Liverpool (Moorhead and Patel, 1964; Harris, Clarke and Jones, 1965) bear on this point. In the second report, when these workers used a more precise technique to ensure accurate intradermal injections, few negative N.L.T. reactions occurred as opposed to 33% negative reactions in the first report.

Recording

Reactions are apparent at 24 hours but frequently at this time a weal and flare or erythema alone are also seen (Gray et al., 1963; Amos et al., 1965; Harris et al., 1965). These non-specific reactions, however, usually fade leaving more easily defined lesions at 48 hours. Induration is then recorded as the mean of two diameters: the mean in some 200 transfers being 7-9 mm (Gray et al., 1965). Simonsen (1962) has shown that in mice strong antigens give a much more violent G.V.H. reaction per unit number of normal cells and Cepellini (1965) has shown in man a straight line correlation between cell dosage and percentage positive reactions. Both these authors have suggested that the N.L.T. should be performed by giving three different dose levels of donor cells simultaneously at different sites and simply recording the reactions as positive or negative; strong antigens would be detected by positive reactions at all three dose levels whereas weak antigens would be detected only by the higher dosage of cells.

Biological nature of the N.L.T. reaction

For the N.L.T. to be of value in selecting a tissue donor the visible reaction must be the result of immunological activity by the injected lymphocytes stimulated by the cell recipient's antigens; i.e., it must be G.V.H. in direction. Evidence in support of the G.V.H. nature of the reaction has been presented by Gray et al. (1965). In essence this evidence is (1) The N.L.T. is biphasic, an early 48 hour reaction and a recrudescence flare at 8-11 days. (2) The order of intensity of reactions produced by cells from different sources is not the same at 10 days as it is at 2 days. (3) Killed cells consistently failed to produce initial reactions but were on occasions involved in the late inflammatory reaction. (4) Evidence for host sensitization as shown by the ability to reject a skin graft in an accelerated manner is not apparent until 6-8 days after the lymphocyte injection. (5) The late reaction is exaggerated when the signs of rejection of a concomitantly placed skin graft are at their peak. (6) Cells from uraemic patients which are believed to be fully antigenic produce smaller reactions than normal cells.

Experiments by Brent and Medawar (1964) on inbred strains of guinea pigs also support the G.V.H. hypothesis. They showed that cells from an F1 hybrid reacted feebly in a parent whilst parental cells reacted vigorously to the F1; also X-irradiation of the recipient animals did not abolish the reaction but on the contrary caused them to be larger.

There are, however, several reports to suggest that the 48 hour N.L.T. reaction is in part at least H.V.G. in nature. Hattler and Amos (1965) have reported that the 48 hour N.L.T. reaction is related to the clinical condition of the recipient; cells from normal subjects provoked minimum induration in patients with advanced cancer. Aisenberg (1965) reports that the skin reaction produced by transferred Hodgkin's lymphocytes is only slightly impaired at 48 hours but is markedly depressed at the second or late phase. On this basis he concludes that host factors are important in the 48 hour reaction whereas the late reaction is G.V.H. However, in the same report he shows that the 48 hour reaction in Hodgkin's and non-Hodgkin's patients, when normal cells are used, is almost the same. Finally Ramsier and
Streilein (1965) have shown that the inflammatory reactions produced in the skin of hamsters by homologous lymph node cells, are markedly reduced by pre-irradiation of the host animal. A possible explanation of this finding is that the hamster has an extremely thin skin which makes true I.D. injections almost impossible. This would mean that the 48 hour reaction was produced not against host skin antigens but possibly against circulating antigen, e.g. lymphocytes and the reduction of this form of antigen by irradiation would explain the small reactions in these animals.

SENSITIVITY IN DONOR SELECTION

The discriminatory ability of the N.L.T. reaction to select the least incompatible donor from a panel has been tested by skin graft experiments (Gray et al., 1965). Lymphocytes from source A were injected into a volunteer group of men; skin was taken from a pair of cell recipients who showed a difference in N.L.T. reaction and grafted on to the cell donor with the prediction that the skin from the individual showing the greater reaction would be rejected before that from the individual showing the lesser reaction.

Sixteen pairs of grafts have been carried out; in nine cases the prediction of order of rejection was correct, in two cases the order was the reverse of that predicted, and in five instances the grafts were rejected simultaneously.

A fairly good correlation between N.L.T. intensity and skin graft survival was observed. A similar correlation between skin graft survival and N.L.T. intensity has been observed by Cepellini (1965).

The best way to determine the value of the N.L.T. test would be to use it to select good and bad kidney donors and to compare the clinical courses of the two groups of patients; clearly no one would be willing to undertake this experiment. In the Department of Surgery (Dr. Paul S. Russell), Massachusetts General Hospital, a number of donors have now been selected on the basis of the N.L.T. test. Space does not permit a detailed account of each patient. Approximately 50% of these patients have done well, the three with the best matches are now surviving with normal renal function at 29 months, 20 months and 18 months. Others who apparently were well matched on N.L.T. test have been unsuccessful. However, there are many factors which affect the course of a transplanted kidney of which histocompatibility matching is only one, so that no definite conclusions one way or the other can be made on the basis of these results.

CONCLUSIONS

The normal lymphocyte transfer test as read at 48 hours is a measure of genetic disparity between donor and recipient and is primarily G.V.H. in nature. Nevertheless host factors are involved and this element must reduce the sensitivity of the test. As judged by reciprocal skin graft experiments its discriminatory power is not high but skin is probably not the best tissue in which to test the N.L.T. There are not sufficient numbers of patients whose donors have been selected by this means to warrant a conclusion on its value in selecting human kidney donors.

At the present time, the test has some value at least in excluding particularly poor donors on grounds of histocompatibility.

REFERENCES


181


MEDAWAR, P. B. (1963): The use of antigenic tissue extracts to weaken the immunological reaction against skin homografts in mice. Transplantation, 1, 21.


DISCUSSION

The Chairman: Thank you. Je crois que nous pouvons ouvrir la discussion.

Is there not an apparent contradiction between the general feeling of Dr. Parsons suggesting that there is no large difference between cadaver and related donors and the fact that Dr. Simonsen's approach is based on the fact that there is a difference? Individual statistics apparently raise the same problem. For example, in our group, if we use the definition of successes and failures as proposed by Dr. Simonsen, we have as many successes with unrelated cadaver kidneys as with related living donors. I wonder whether this is not due to a wrong definition of the success of a renal graft. Our clinical impression is that the fact of being alive after more than 6 months does not mean much. I mean that there is a huge difference between people who are alive after one year or even two years with a very poor function, and other people who can accidentally die after not more than 6 months when the transplanted kidney itself is functioning satisfactorily.

So the question I should like to bring up is: is there the possibility of giving Dr. Simonsen valuable data to test his approach? Is the duration of life a good test? Would not the functional and pathological situation of the transplanted kidney at a chosen time be a better criterion?

Dr. Morten Simonsen (East Grinstead): First, the question as to whether there is or is not a difference between the cadaver cases and the family cases. I do not think there can be any doubt from the World Registry data that there is such a difference, whereas there is no difference between cadaver cases on the one side and live, unrelated donors on the other side.

The Chairman: May I suggest that this World Registry is a mixture of—I shall say it in French because I do not know the English words—C'est un mélange assez informe de statistiques de valeur et de significations très différentes. (Laughter).

Dr. Morten Simonsen (East Grinstead): All right, let me put a question to you. When you say that you have got 70% long term survival with your cadaver cases, is that after trying to select the best cadavers, or do you just take any cadaver?

The Chairman: We take any cadaver except for A.B.O. incompatibility. May I add that the proportion of cases surviving after 6 months or 1 year is as high with these cadaver transplantations as with the others, but biopsies of the transplanted kidney usually show more alterations with unrelated than with related donors. So I agree that cadaver kidneys are not at all as well tolerated as kidneys from related donors but I do not think that the survival of the patient after a given number of months does mean much from a clinical point of view.

Dr. Morten Simonsen (East Grinstead): It is quite possible that 6 months is too short to label the patient as a long term survival. It might be that one ought to take 12 months, or 15 months, or something else as the criterion on which to distinguish long term from short term. I would still maintain that, in spite of your material, which is after all not quite big enough to allow you to take the statistical comparison of cadaver with the living unrelated donor.

The Chairman: Of course, ...

Dr. Morten Simonsen (East Grinstead): I would bet you 100 : 1 that there is a difference! (Laughter)
DISCUSSION

The Chairman: Of course there is a difference. The problem is: how can you evaluate it. You should come, Dr. Simonsen, to any of the centres in which there are 20 to 30 living persons and see them. By comparing several of them at the same stage, I mean for example after one year or two years (we have 8 now for more than two years), you would be convinced that there are very strong differences between one case and another.

Dr. Morten Simonsen (East Grinstead): According to my favourite hypothesis among the four I have discussed, I do prefer the one locus with a few alleles, you ought to get a fair amount, about \( \frac{1}{4} \), of long term survivals in your random cases, whether they are cadavers or alive. Is the fact that you got 7 out of 10 in one series incompatible with that hypothesis? I am not sure off hand.

The Chairman: That is correct.

Dr. Morten Simonsen (East Grinstead): Dr. McGown, you showed a slide with some dose-response relationships. It was not clear to me whether different doses of injected cells referred to the same individuals. I mean, did you inject various doses of the same cells into the same individuals?

Dr. M. McGown (Belfast): The slides I showed you relate to all the lymphocyte transfers, which were some 236. But in a number of instances we did multiple injections on the same subject, using doubling doses from 2 million to 10 million lymphocytes from the same suspension. But the over-all slide which I showed you contained the results of all our lymphocyte transfers compared with the number of lymphocytes.

The Chairman: We tried, on your suggestion, 5 different amounts of lymphocyte (from 1 to 5 millions) on the same patient. We had no 'negative' or 'positive' results but a progressive reduction in the reaction with no clearcut limit allowing to use a 'Yes' or 'No' interpretation instead of a quantitative one.

Dr. M. McGown (Belfast): You have to draw the line somewhere...

The Chairman: But where are you going to put a line?

Dr. Morten Simonsen (East Grinstead): It is a pity you did not go below 1 million; maybe 0.5 million would have given you negative results in some combinations but not in others.

The Chairman: The test was so progressively fading that with low concentrations it seemed arbitrary to call the result positive or negative.

Dr. Morten Simonsen (East Grinstead): I understand. I am just suggesting that your lowest dose was too high. You should have graded them, perhaps, from 200,000 to 2 million rather than from 1 million to 10 million. This is easy to say after the experiment, but still it is a valid comment.

The Chairman: Who has some suggestions for these difficult problems?

Qui a quelque suggestion à apporter sur ce problème de la sélection du donneur? Il est clair que, à l'heure actuelle, nous ne sommes pas en possession d'une méthode sûre dans la sélection du donneur. Malgré le grand nombre de travaux et de suggestions théoriques, il demeure difficile de faire la preuve de la valeur d'un quelconque des tests proposés.

I do not think there is any evidence now that any of these tests have been proved to be related with the clinical success. But that does not mean that they are not interesting.
DISCUSSION

Dr. Morten Simonsen (East Grinstead): I believe that Dr. Terasaki had a good correlation between his cytotoxic test and the clinical results from Dr. Starzl's group.

The Chairman: Have you been to Leiden?

Dr. Morten Simonsen (East Grinstead): I have not been to Leiden, but I have heard reports from Leiden.

The Chairman: We could ask Dr. Dormont, for example, who was in Leiden to give a brief report of the conclusions of the meeting.

Dr. J. Dormont (Paris): Le Dr. Paul Terasaki a présenté à la réunion de Leiden (Août 1965) deux séries de sujets ayant subi à Denver une homotransplantation rénale. Dans l'une des séries les donneurs étaient apparentés au receveur. Dans l'autre ils ne l'étaient pas, mais avaient été sélectionnés comme relativement compatibles d'après le test de cytotoxicité sur les leucocytes. Les résultats semblent très comparables dans les deux séries de cas, mais avec la réserve que le recul n'est pas encore important.

Mr. A. E. Kutilake (Hammersmith, London): For the past 18 months we have used a different test from that described today. In trying to select live donors we have done a skin graft from a prospective donor group to the recipient and put the recipient on half the dose of immuran for 7 weeks. Within this period, if the patient's uraemia was severe enough we have done intermittent dialysis.

In the past 18 months we have done 3 transplants on the basis of this selection. One was a non-identical twin, from the sister to the brother; he is still living after 17 months. We had another from a sister, but the recipient was Rh negative while the donor was Rh positive, but both were of the same blood group. She died after 4 months from infection. We now have another one who has had a kidney received from the wife, but has been alive for only 6 weeks so far.

So from the tests so far it appears that it is a reasonable test to try out in large numbers.

The Chairman: Are you not afraid of pre-immunisation by this skin graft?

Mr. A. E. Kutilake (Hammersmith, London): No, if there is rejection we do not do the graft, therefore there is no question of pre-immunisation.

The Chairman: I am not sure that this will be accepted by all immunologists. Anyhow, thank you. We have to pass to the second session, which could be called the immunological and humoral approach and I should like to call Professor Calne to introduce this session.