Recent Research in Kidney Transplantation

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The main theme of my discussion concerns some recent research of our group in Paris, in two different fields: the selection of the transplant donor, and the immunosuppression of the transplant recipient. These are the two types of approach that have brought us from the dark ages of the 1950s, when the transplantation of organs from one man to another was thought to be a hopeless task, to the present stage in which renal transplantation is successful in the majority of cases. For example, in our series of 130 patients transplanted before July 1969 there were 99 cases of prolonged tolerance. Of these renal transplants, 77 have good function; 17 patients have now kept their allograft for five to more than ten years. This means, however, that there was a number of cases with early or late failure, and that the situation cannot be considered entirely satisfactory, but recent results from our group may lead to some improvement in the understanding of these problems.

**DONOR SELECTION**

One important advance in donor selection has been the discovery of a major histocompatibility system, now called HL-A. Since the description by Jean Dausset of the first HL-A antigen, in 1958, the antigen Mac, some twenty antigens have been discovered, and now they are always looked for in the leucocytes and platelets of prospective donors and recipients in order to choose the most compatible donor. Most centres claim that typing donors and recipients in such a way has resulted in a clear cut improvement of the clinical results (Hamburger, 1969).

On the other hand, some authors are not so enthusiastic. It has been said that in cadaver kidney transplantation there is no evidence that leucocyte typing makes any difference. In the case of living donors, there is undoubtedly a statistical correlation between the results of typing and the outcome of the graft. However, this rule has its exceptions. Some cases without known antigenic differences show poor results, and some cases with several major
|                          | Number of cases | Maximum observation time (yrs) | Successful cases | Totally successful cases | Rejection | Non immunological failure | % of successful cases | % of totally successful cases |
|--------------------------|----------------|-------------------------------|------------------|--------------------------|-----------|--------------------------|-----------------------|--------------------------|
| HL-A identical siblings  | 15             | 10                            | 15               | 13                       |           | 100                      | 87                    |                          |
| HL-A semi-identical siblings | 15             | 7                             | 12               | 10                       | 3         | 80                       | 67                    |                          |
| HL-A semi-identical parent-child | 28             | 5                             | 22               | 9                        | 3         | 79                       | 32                    |                          |
| Total HL-A semi-identical | 43             | 7                             | 34               | 19                       | 6         | 79                       | 44                    |                          |
| Total HL-A different in both haplotypes | 33             | 2                             | 16               | 8                        | 14        | 48                       | 25                    |                          |
| Siblings HL-A different in both haplotypes | 2              | 2                             | 1                | 1                        | 1         |                          |                       |                          |
differences show excellent clinical results. The reason for these discrepancies may be found in some recent personal observations.

The HL-A antigens, as determined by serological methods, depend on a single genetic locus. This means that the HL-A antigenic phenotype depends on two alleles only, or in the modern nomenclature, two ‘haplotypes’, one inherited from the father and the other from the mother. Each haplotype is a complex system with at least two subloci, each sublocus corresponding to one out of no less than ten possible antigens. This makes it most unlikely that two unrelated persons will have one haplotype in common. Thus, the situation in a family will be that a parent–child pair will always have one haplotype in common, but rarely, if ever, more than one. Siblings may have received the same haplotype from each parent (there is a 25 per cent chance of this) and they may then be termed HL-A identical. In 50 per cent of the cases a sibling pair will have only one common haplotype, and they may be termed semi-identical. In the last 25 per cent of the cases siblings will have no common haplotypes.

We have now reached the point where we know enough about the HL-A genetic structure to be able, in most cases, to express the results of leucocyte typing in terms of haplotypes, provided that the members of the family, including the parents, are typed. This has been possible in practically all our recent cases, and the results have been rather striking (Table 1). All transplantations in HL-A identical siblings have been uniformly successful. Moreover, the function and biopsy appearances of 87 per cent of these grafted kidneys are normal. On the other hand, with non-identical siblings the results are the same as those of parents or any other related donors. This correlation between the clinical tolerance of the graft and what might be called ‘haplotyping’ is far better than the correlation obtained with the conventional comparison of antigens. In our experience, all the discrepancies I have mentioned with the latter method disappear when the typing is expressed in genetic terms. It is easy to understand this, for when we have enough clues to state that haplotypes are identical, all the corresponding antigens must be identical, whereas when we only compare antigenic differences one by one, we probably overlook antigens as yet unknown in the HL-A phenotype.

However, HL-A identical donors will always be rare, because they can be found only among siblings. Hence, it remains necessary to find some method of selection between the other possible donors. In this respect I would like to report unpublished data that we have found in using another procedure, the mixed lymphocyte culture. Five years ago, Miss Bain in Montreal, (Bain et al., 1964) and Fritz Bach in New York (Bach and Hirschhorn, 1964) showed that if lymphocytes from two different individuals are mixed and cultured, some
lymphocytes are transformed into large cells of blast type. The less histocompatible the two individuals tested, the greater the percentage of cells transformed. In the technique used in Paris (Hamburger, 1969) results under 5 per cent are termed negative, since they may be found when mixing cells taken from the same individual or from identical twins. Table 2 shows the results obtained in 23 patients. It is remarkable that all patients whose renal function is normal have given negative results with their donor, whereas cases with a creatinine clearance under 70 ml/min are dispersed between negative and highly positive results. This difference is quite significant. A later analysis of 36 cases shows that the clinical results are more significantly correlated with the results of mixed lymphocyte cultures \((p < 0.001)\) than with those of leucocyte typing or even haplotyping. This is, as far as I know, the first clinical evaluation of the lymphocyte culture test in kidney transplantation, and I must confess that we were very surprised to find such well-grouped negative results in successful cases, because until then we had been rather sceptical about this technique.

It may be interesting to compare more closely the results of mixed lymphocyte culture with those of HL-A typing. All HL-A identical siblings have a negative mixed culture. The comparison between antigenic differences and the percentage of cell transformation has been studied in 140 pairs (Debray-Sachs et al., 1968). A positive correlation was found, but our results suggest that known leucocyte groups are not entirely sufficient for predicting the percentage of transformation in mixed lymphocyte cultures. For example, if we compare unrelated and related pairs that have the same number of known antigenic differences, unrelated pairs have a higher transformation average, whatever antigen is considered.

| Table 2. Mixed Donor-recipient Lymphocyte Culture (two-way technique) and Renal Function of the Transplanted Kidney in 23 Patients Observed from 1st January 1966 to 5th January 1969 |
|---------------------------------|---------------------------------|
|                                 | Impaired renal function          | Normal renal function            |
|                                 | (Creatinine clearance of less than 70 ml/min) | (Creatinine clearance of more than 70 ml/min) |
| Less than 5% transformed cells  | 2                               | 13                               |
| (negative)                      |                                 |                                  |
| More than 5% transformed cells  | 8                               | 0                                |
| (positive)                      |                                 |                                  |

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Two explanations may be given for these results. It may be that undetected antigens explain the cases where the HL-A antigen typing is not consistent with the mixed lymphocyte culture and/or the clinical results. If this is true, the situation will be clarified by increased knowledge of leucocyte antigens. But another explanation may be found in an entirely different, and as yet unexplored field; that is, the specific capacity of each recipient to react against one or several given antigens. I shall come back to this new approach to the problem in relation to the immunosuppression of the recipient.

**Immunosuppression of the Recipient**

The most commonly used immunosuppressive agents nowadays are 6-mercaptopurine (6-MP), azathioprine and antilymphocyte globulins (ALG).

Azathioprine is, of course, a powerful agent, but is also responsible for a number of adverse effects and complications. We have found a positive correlation between the use of this drug and the appearance of various complications, including viral and bacterial infections, hepatitis, and thrombopenia or leucopenia induced by bone marrow depression (Crosnier, 1969). On the other hand, ALG is the most powerful agent in the experimental animal, but in the human it is still difficult to judge its clinical value. In our last 28 patients we have used ALG in every other patient, in order to obtain a fair comparison between the results obtained with azathioprine alone and those obtained with azathioprine together with ALG. The difference has not yet been significant. This is perhaps because our doses in man are usually smaller than in the experimental animal. In any case, there is general agreement that none of our present means of immunosuppression is entirely satisfactory.

It is true that we use these agents in a rather blind fashion because so little is known about the pharmacology of immunosuppression. The metabolism of 6-MP and azathioprine is poorly understood because, until recently, it was not possible to measure the level of these drugs in the blood. On the other hand, ALG has been difficult to study because there is not just one ALG but an infinity of varieties and because there is not yet a satisfactory method for ALG standardisation.

These problems may be somewhat clarified by the research of Dr J. F. Bach in our laboratory. Before I present these findings, I might recall the principle of the rosette phenomenon. If a mouse is immunised against sheep erythrocytes, approximately 10 to 15 per thousand of its lymphocytes, when mixed with these red blood cells, will form rosettes. These may be termed the 'immune' rosettes. When the lymphocytes of a non-immunised mouse are mixed with sheep red blood cells, there is still the formation of a small number of rosettes, approximately 0.3 to 1 per thousand lymphocytes, and this process may be
called the 'spontaneous' rosette phenomenon. Formerly, the study of spontaneous rosettes was neglected, except as an inconvenient background when counting rosettes. It was an unexpected finding to see that these spontaneous rosettes were much more sensitive than immune rosettes to azathioprine or 6-MP (Bach et al., 1969a). For example, the inhibition of spontaneous rosette formation is obtained with less than 10 µg of 6-MP/ml, whereas the immune rosettes are 50 times, and the cytotoxicity test 300 times less sensitive. This remarkable sensitivity has made it possible to measure the curve of immunosuppressive activity induced by the drug in the blood of the animal. The study of this curve also led to an unexpected conclusion. If the peak of the blood activity is compared with the effect of azathioprine in vitro, and if the results are expressed in terms of azathioprine concentration reached in the blood, the result appears absurd. The calculated concentration is so high in relation to the amount given to the animal that the diffusion space of the drug would be less than the volume of the plasma. The only possible explanation is that the active substance in the blood is not azathioprine, but some of its metabolites, which are much more powerful than the drug itself.

Another advantage of this test is that it may be used in all animals, including man, whereas no test requiring an active immunisation would be acceptable in the human. We can now follow the activity reached at any time in human blood. For example, Bach has found in our patients that giving 150 mg of azathioprine daily, in one single dose, or in three separate doses of 50 mg each, results in two different curves of activity; it will be necessary to find out which is the more satisfactory. Another highly controversial question was: 'Does renal or liver failure modify the effect of azathioprine?' We may now give the answer (Bach and Dardenne, 1969). Renal failure does not change the curve at all. This was demonstrated in two anephric patients and in four patients with renal failure. Hence, the dosage should not be reduced in the uremic patient, at least with regard to immunodepressive effect. This, of course, does not mean that drug toxicity may not be increased in uraemia, since the toxic metabolites are probably not identical to the active ones. Incidentally, the separation of active and toxic metabolites will also be made possible by the rosette test, which is one of the most exciting items in future research plans.

The study of liver failure also led to a somewhat surprising result (Bach and Dardenne, 1969): in 8 patients, 5 with cirrhosis of the liver and 3 with a chronic post-viral hepatitis, the blood immunosuppressive activity resulting from the administration of 3 mg/kg bodyweight of azathioprine was greatly decreased. In 3 of these patients with severe liver failure, no activity at all was found during the 48 hours following the same dose of azathioprine. This strongly suggests that the transformation of azathioprine into active metabo...
lites takes place in the liver. What this means, from a clinical point of view, is that azathioprine becomes useless in transplanted patients with severe liver complications.

We have also studied the correlation of the rosette inhibition test with the in vivo prolongation of grafts. In mice, a series of twenty-three different preparations of ALG showed that the inhibition titre and the survival of skin allografts have a high positive correlation. Moreover, the inhibition titre obtained with serial dilutions of the same ALG is proportional to the effect on graft survival. This also suggests a satisfactory relationship between graft survival and rosette inhibition.

A joint study of human ALG was carried out by Dr Balner’s group in Holland and in our laboratory in Paris (Bach et al., 1969b). Dr Balner tested the ALG through skin grafts in monkeys. This test is demanding but admittedly the best in vivo test. In Paris, we tested the same ALG in vitro by means of the rosette test. More than fifty specimens of ALG from all parts of the world were submitted to both groups. Each group tested the serums without knowing the results of the other. Dr Balner grouped the ALG into three classes, poor (graft survival of less than 21 days), reasonably effective (graft survival from 3 to 5 weeks) and excellent (graft survival over 5 weeks). It turned out that the in vitro rosette test resulted in exactly the same classification, whereas no correlation was found with the leuco-agglutination test and there was a mediocre correlation with the cytotoxicity test. As the in vivo test in large monkeys is costly and time-consuming, whereas the in vitro test described by Bach is easy and rapid, it may well be that the rosette test is the answer to the problem of ALG standardisation. In our group we have already begun to express the ALG dosage in terms of rosette inhibition units instead of millilitres.

It is obviously important to discover the full significance of these spontaneous rosettes. The fact that they correlate well with graft survival, whereas the classical immune rosettes do not, has made this phenomenon the basis of a convenient new test, and it might also prompt some useful speculations on transplantation immunity. Is it a coincidence that immunosuppressive agents inhibit spontaneous rosettes in proportion to their inhibition of the graft rejection? It could mean that the one out of 2,000 lymphocytes which forms rosettes plays some special role in the mechanism of immunity. These cells must be different from other lymphocytes; they might represent the antigen-recognising lymphocytes.

This hypothesis is supported by the fact that many features attributed to the antigen-recognising cells (or ARC) are also those of spontaneous rosette-forming cells (SRFC). Both ARC (Gowans and MacGregor, 1965) and SRFC (McConnell et al., 1969) are small lymphocytes. Both ARC (Mitchison, 1968)
and SRFC (Laskov, 1968) bear antigen-specific sites. Both are found in non-immunised animals; both may react with antigens not previously phagocytosed by macrophages; both no longer react in the presence of an anti-immunoglobulin serum. Some authors object to the identity of rosette-forming cells as antigen-recognising cells, because there is no correlation between the number of rosette-forming cells and the intensity of the immune response (Biozzi et al., 1968). However, the immune response may well depend on many other factors involved in the antibody formation. It is true that the number of spontaneous rosette-forming cells is slightly higher than that of the cells capable of recognising one antigen, as calculated by various authors; but in the rosette test the erythrocyte may well represent more than one antigen. In summary, it may well be that the spontaneous rosette-forming cells are antigen-recognising cells, whereas immune-rosettes or plate-forming cells may primarily represent the antibody-forming lymphocytes.

If these conclusions are correct, the effect of ALG or 6-MP on the spontaneous rosette would somewhat clarify the mechanism of their action in the prevention of graft rejection.

It has been suggested by Miller and Mitchell (1969) and many others that several populations of lymphocytes are involved in the immune response. There is reason to believe that the bone-marrow cells may recognise the antigens but are capable of forming antibody only after the action of a factor dependent on the thymus. The spleen lymphocytes are able to recognise the antigens and to induce the proliferation of specific antibody-forming cells, sometimes called memory-cells. If the thymus has been removed at birth, spleen cells may still recognise the antigens but their capacity to create antibody-forming cells is grossly impaired. Spontaneous rosettes are not formed by thymus lymphocytes, but by all cells considered capable of antigen recognition, that is bone-marrow and spleen cells, even in the thymectomised animal.

It must be noted that ALG and 6-MP inhibit spontaneous rosettes formed by spleen cells but only slightly inhibit rosette formation by bone marrow cells or by the spleen of thymectomised animals. This may suggest that ALG and 6-MP hardly interfere with antigen-recognising cells until these have been transformed into recognising and reacting cells under the influence of the thymus. Later, when these cells have proliferated, ALG and 6-MP again lose most of their power. The model offered by the rosette phenomenon would then contribute to the understanding of the precise and rather narrow link at which ALG and 6-MP exert their major inhibitory power.

The main value attached to this type of over-simplified hypothesis is its possible provocative effect on further research. It is quite clear that we are now entering into a stage where a purely clinical approach becomes insufficient.
for the follow-up of patients who have received transplants. We shall have to create the tools to explore the immunological situation hidden in the sum of clinical facts. In that respect, the search for circulating antibodies is of limited value; testing the cells which are, in fact, responsible for the immune response will probably be the only effective solution. We must learn how to explore their power of HL-A recognition, their reactivity, and their immune memory. We must devise methods of making an immunological diagnosis as we now make a clinical one. Only then shall we be able to predict clinical events before they actually occur. I hope that some of the results I have presented may help to reach this target.

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