Histocompatibility and Immunologic Competence in Renal Homotransplantation

Abstract. *Cultures of peripheral lymphocytes may be used to assay histocompatibility. The lymphocytes from potential kidney recipients are cultured with those from potential donors and the percentage of large cells and mitoses stimulated is determined. The immunologic competence of cultured lymphocytes from recipients receiving immunosuppressive therapy is evaluated by determining their responsiveness to phytohemagglutinin. Suppression of response appears to be correlated with the absence of clinical signs of graft rejection.*

Circulating human lymphocytes grown in tissue culture may be stimulated with phytohemagglutinin (PHA) to form large cells and to undergo mitoses (*1*). These cells also produce γ-globulin when stimulated by PHA or by a specific antigen to which the lymphocyte donor has previously been sensitized (*2*). Two of us (F.B. and K.H.) have recently shown that when lymphocytes of two genetically disparate individuals are grown together in culture, up to 80 percent of the cells will enlarge and divide (*3*), and that when lymphocytes of identical twins are grown together, the cells do not stimulate each other (*4*). If a person is sensitized to the white blood cells of either of two unrelated subjects, and shows an accelerated rejection of the skin graft of the other, the lymphocytes of the two subjects, when cultured together, show a low mitotic rate and few large cells. The degree of stimulation appears to be related to histocompatibility, as determined by the skin graft studies. Skin testing with lymphocytes has been suggested as a method of determining histocompatibility by Brent and Medawar (*5*), and the results of such tests correlated closely with homograft survival in guinea pigs.

We have investigated lymphocyte matching in vitro as a guide to the selection of renal homograft donors from members of the same family. Furthermore, after transplantation, the ability of the recipients' lymphocytes to enlarge and divide when stimulated with PHA was investigated to assess the efficacy of immune suppression by cytotoxic agents.

Lymphocyte matching in vitro was performed for two female patients with terminal renal disease according to the method of Bach and Hirschhorn (*3*). Both of these patients (J.R. and D.F.) have since received renal homotransplants. Only the cells of J.R.'s mother were used for matching in this manner. The cells of both J.R. and her mother showed a normal response to PHA. A mixture of their cells, cultured together for 7 days, showed only 9.0 percent large cells plus mitoses. More extensive studies were made in the case of D.F. Cells from the patient, her mother, father, and sister all responded normally to PHA. The percentages of large cells plus mitoses in the mixtures cultured for 8 days are presented in Table 1. Although other considerations led to the choice of the patient's father as donor, the presence of only 29 percent large cells plus mitoses in the mixture of their lymphocytes was still low. As stated previously, this value may be as high as 80 percent for two unrelated individuals. The relatively low degree of stimulation in the mixture of the father's and mother's cells, while presumably unusual in a family study, could be explained by known patterns of inheritance. Four months have now elapsed since J.R.'s transplantation, and 3 months since D.F.'s transplantation, and they have both shown minimal evidence of graft rejection. Both have had bilateral nephrectomy and splenectomy, and are being treated with cytotoxic drugs and steroids. The major agents used have been azathioprine and steroids. Puromycin, which has been shown to suppress human lymphocyte growth and antibody formation in vitro (*6*), has also been administered.

Table 1. The percentage of large cells and mitoses in mixtures of cells from patient D.F. and her relatives. The cells were cultured together for 8 days.

Source of lymphocytes	Large cells plus mitoses (%)
D.F. and father	29.0
D.F. and mother	8.5
D.F. and sister	27.0
Father and sister	7.5
Father and mother	9.0
Mother and sister	6.0

Cells taken from patients being treated with these drugs and cultured with PHA respond to a variable degree. We have made quantitative estimates of the response by determining the percentages of mitotic, large, medium, small, and degenerate cells in cultures stimulated by PHA and examined after 72 hours. The lymphocytes were washed three times before being placed in culture, fresh PHA was used each day, and lymphocytes from normal individuals were cultured simultaneously. Diminution of the PHA response appears to be correlated with the apparent effectiveness of suppression of the immune response in vivo and the lack of any major clinical evidence of homograft rejection, at least during the early course of the patients (Fig. 1). Although neither of these patients has had

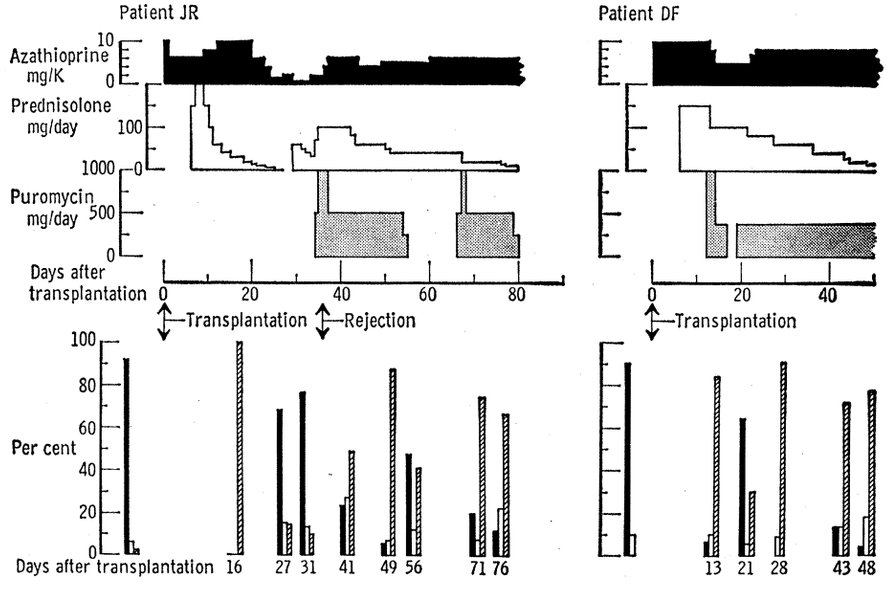

Fig. 1. The course of two patients, J.R. and D.F., after they received homotransplants. The solid bar indicates the percentage of large cells plus mitoses; the open bar, the percentage of medium cells; and the striped bar, the percentage of small and degenerating cells.

severe rejection crises, J.R. had mild but definite signs of rejection, probably starting on day 36. Several days before the apearance of clinical evidence of rejection, there was a significant increase in the number of large cells plus mitoses in PHA-stimulated cultures of her lymphocytes. Raising the dosage of immunosuppressive drugs decreased the response in vitro and eliminated signs of rejection.

This technique of lymphocyte culture offers a new approach to the problem of donor and recipient typing for renal homotransplantation. It may also provide a way of assessing the degree of immunologic suppression effected by cytotoxic agents. Use of this technique should allow further insight into the problem of histocompatibility and the nature of drug-induced suppression of the immune response.

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References and Notes

1. J. Hastings, S. Freedman, O. Rendon, H. L. Cooper, K. Hirschhorn, *Nature* **192**, 1214 (1961).
2. K. Hirschhorn, F. Bach, R. L. Kolodny, I. L. Firscheim, N. Hashem, *Science* **142**, 1185 (1963).
3. F. Bach and K. Hirschhorn, this issue.
4. K. Hirschhorn and F. Bach, unpublished data; B. Bain, M. R. Vas, P. H. Lowy, *Blood* **23**, 108 (1964).
5. L. Brent and P. O. Medawar, *Brit. Med. J.* **1963-II**, 269.
6. K. H. Stenzel and A. L. Rubin, unpublished data.
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Glycogen Synthetase: Its Response to Cortisol

Abstract. *Glycogen synthetase activity in the livers of starved mice is stimulated by the glucocorticoid, cortisol.*

Greengard *et al.* (*1*) and Weber *et al.* (*2*) recently reported that liver glycogenesis induced by cortisone in starved rats is inhibited by puromycin and actinomycin, two agents that interfere with protein synthesis. It was concluded that the formation of an enzyme protein induced by cortisone and required for glycogenesis is interrupted. The enzymes investigated were aldolase, lactic dehydrogenase, glucose-6-phosphatase, fructose-1,6-diphosphatase, and tyrosine transaminase. In addition, transaminase has been implicated in cortisol-induced glucose precursor synthesis by Rosen *et al.* (*3*).

As part of a study (*4*) being conducted on hepatotoxic agents and glycogenesis, we found that yet another enzyme, glycogen synthetase (uridine-diphosphate-glucose–glycogen-1,4-transglucosylase) is stimulated in vivo by the glucocorticoid, cortisol. This observation now becomes relevant to the question of the identity of the enzyme protein whose antibiotic-inhibited synthesis prevented glycogenesis by cortisone.

Three-month-old A/Jax male mice (20 to 25 g) in groups of six were killed at the intervals indicated in Table 1. A 10-percent homogenate of the freshly excised liver in each case was prepared in a medium composed of 0.25*M* sucrose, 0.001*M* EDTA, and 0.1*M* NaF solution. The homogenate was assayed for glycogen synthetase according to Leloir and Goldemberg (*5*). Liver glycogen was extracted from a weighed specimen of fresh liver with hot 30 percent KOH solution. It was precipitated by ethanol and measured as glucose units by Dische's cysteine-sulfuric acid reaction (*6*).

Table 1. Liver glycogen and glycogen synthetase during starvation; effects of cortisol and deoxycorticosterone acetate (DOCA).

Expt. No.	Fasting interval (hr)	Steroid (1 mg)	Glycogen (g/100 g liver) (Mean±S.D.)	Glycogen synthetase* (specific activity) (Mean±S.D.)	Comparison of Expt. No.	Comparison of Expt. No.	*P*
1	0		3.35 ± 0.35	2.02 ± 0.13			
2	12		0.05 ± 0.04	1.18 ± 0.04	2	1	<0.01
3	24		0.05 ± 0.01	1.20 ± 0.20	3	1	<0.01
4	12	Cortisol	3.02 ± 0.32	1.70 ± 0.11	4	2	<0.01
5	12	DOCA	0.02 ± 0.02	1.09 ± 0.03	5	2	>0.05
6	24	Cortisol	0.69 ± 0.39	1.77 ± 0.11	6	3	<0.01
7	24	Cortisol (last 12 hours)	2.70 ± 0.63	1.86 ± 0.21	7	3	<0.01

* Micromoles of uridine diphosphate formed per hour per milligram of protein in the presence of glucose-6-phosphate as described by Leloir and Goldemberg.

Table 1 shows that (i) liver glycogen depletion by 12 and 24 hours of fasting is accompanied by a significant reduction in glycogen synthetase (expts. 1, 2, and 3); (ii) the administration of cortisol (expt. 4) but not deoxycorticosterone acetate (expt. 5) prevents this phenomenon at 12 hours; (iii) 24 hours after administering cortisol, the glycogen value is depressed but glycogen synthetase activity is not; and (iv) the administration of cortisol at the 12-hour interval restores the values of both glycogen and glycogen synthetase to normal in 24 hours.

The present results agree with the conclusion of Dorsey and Munck (*7*) that changes in blood glucose level alone in fasted adrenalectomized rats cannot explain quantitatively the rate of deposition of glycogen soon after cortisol injection. The alternatives suggested were that cortisol either (i) provided more substrate in the form of blood glucose, (ii) acted directly on the liver by mechanisms such as enzyme activation, changes in physiology of circulation, and so forth, or (iii) acted directly on extra-hepatic tissues to produce glucose, activator, and substrate. Our findings support a direct action of cortisol on the liver.

It is clear that the activity of glycogen synthetase is influenced by glucocorticoid and not by mineralocorticoid. Moreover, a decrease in glycogen concentration is not always accompanied by a drop in glycogen synthetase (expt. 6). The central role of glycogen synthetase in glycogenesis, therefore, justifies its inclusion in evaluations of individual enzymes responding to cortisol stimulation much along the lines reported by Hilz *et al.* (*8*).

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