ERYTHROPOIETIN RELEASE FROM BABOON RENAL ALLOGRAFTS TREATED WITH SUBCELLULAR KIDNEY CELL FRACTIONS*

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Erythropoietin (ESF) is a hormone active in the regulation of red blood cell production.[1] It is normally of renal origin, although extrarenal sources have been demonstrated.[2,3] Autotransplanted simian[4] and canine kidney grafts[5] release ESF in response to arterial occlusion, hypoxic hypoxia, or bleeding. Baboon and simian kidney allografts also release ESF in large amounts during graft rejection.[6,7] This report describes ESF alterations in untreated baboons and in animals given subcellular kidney cell fractions (SKCF) to suppress rejection.

MATERIALS AND METHODS

Twenty-one healthy adult male and female Cape Chacma baboons, housed and cared for at the University of Stellenbosch Primate Facility at Karl Bremer Hospital, Bellville, CP, were used. Of these 21 animals, 6 were untreated controls, 9 received 2·0 ml. SKCF intramuscularly on alternate days and 6 received 0·2 ml. SKCF intramuscularly on alternate days. Single kidney allografts were inserted in these animals under routine sterile conditions. Five additional unoperated animals were given intramuscular subcellular kidney cell fractions (2·0 ml. every alternate day) similar to the treated allotransplanted animals. All animals were compatible in terms of their major human ABO erythrocyte blood groups. The animals were selected from heterogenic groups from western Cape Province to avoid possible effects of inbreeding. Details of anaesthesia and operative techniques have been described previously.[5,9] Methods of pre- and postoperative measurement of haematocrit, renal plasma flow and creatinine clearance have also been described.[6] All animals were eventually subjected to postmortem examination which included full histopathological studies.

Subcellular kidney cell fractions (SKCF) were prepared as follows: Pooled, whole baboon kidneys were stored in a deep-freeze unit, until time of homogenization, at −20°C. The capsules were removed after thawing, and the kidneys were washed in Hanks balanced salt solution and cut into small fragments. These pieces were washed in cold saline and then homogenized in a mechanical homogenizer for 15 min. at 50,000 r.p.m. The homogenizer was kept cool by means of circulating cold tap-water. The homogenate was then placed in a sonic disintegrator for 10 minutes with vibrations of about 8 kc./sec. A smear was then made and examined microscopically to ensure that all the cells had been ruptured. Finally, all the homogenates were pooled and sterility tests performed, and standardization was achieved by simple viscosity measurements. All the homogenates were diluted with Hanks balanced salt solution up to the point where it was possible to pass them through a No. 19-gauge injection needle. Two renal allotransplanted groups were given SKCF 0·2 or 2·0 ml. on alternate days postoperatively until death. An additional group of intact non-operated animals were injected with 2·0 ml. SKCF for 10 - 14 days, during which time specimens for erythropoietin assay were obtained; no change in haematocrit or renal function was noted in the group during this period.

Erythropoietin (ESF) assays were completed, as previously described, by injection of plasma samples into polycythaemic adult Ha/ICR Swiss mice.[10] Each test mouse received subcutaneous injections of 0·5 ml. of plasma daily for 3 successive days. On the fourth day they were injected intravenously with 1 μc of ^{59}Fe in 0·5 ml. saline. Twenty-four hours later they were bled from the dorsal aorta, and the radioactivity of the blood sample was measured in a well-type scintillation counter. The results were expressed as the percentage of the 24-hour incorporation of radioactive iron into the circulating red cells. Results were also expressed as the ratio of % ^{59}Fe 24-hour uptake (test plasma)/% ^{59}Fe 24-hour uptake (saline-injected controls). Assays from mice with haematocrit levels less than 60% at the end of the experiment were discarded. Over 5 animals were assayed with each test plasma.

RESULTS

Survival

The average survival time following renal allotransplantation of the 6 untreated control animals from non-inbred groups was 9·7 ± 5·6 days before uraemic death from graft rejection ensued (Table I). There were no animals in any of the groups that died from technical problems such as thrombosis of the vascular anastomoses. All animals died as a result of renal allograft rejection as was confirmed at postmortem examination. Subsequent microscopic studies demonstrated characteristic vascular and cellular features associated with baboon renal allograft rejection, such as interstitial and perivascular lymphocytic collections and obliterations of small arterioles.[11] The second group of 9 animals that received SKCF 2·0 ml. intramuscularly on alternate days survived

TABLE I. SURVIVAL RATES IN TREATED AND UNTREATED BABOON RENAL ALLOGRAFTS

Group	*Days survival (± 1 SD)*	*Total No. animals*
Untreated	9·7 ± 5·6	6
Subcellular kidney cell fraction (SKCF)* (2·0 ml. intramuscularly)	2) ± 21	9
Subcellular kidney cell fraction (SKCF) (0·2 ml. intramuscularly)	16 ± 9	6
Total		21

* An additional 5 animals were given 2·0 ml. SKCF intramuscularly for 10–14 days without operation.

*Date received: 10 March 1969.

20 ± 21 days and had diminution of characteristic renal allograft microscopic rejection features (Table I).[31] One animal from this group lived 89 days postoperatively. The third group of 6 animals receiving 0·2 ml. SKCF intramuscularly on alternate days survived 16 ± 9 days (Table I). The decrease in the degree of renal morphological rejection was less marked in this group than in the preceding group. None of the 5 unoperated animals that received SKCF injections died during the period that they were receiving these injections.

Renal Function

The dose-related increased survival in the animals which received SKCF was also associated with improved levels of postoperative renal plasma flow (RPF) and creatinine clearance (GFR) (Table II). This increase was most apparent in the group receiving 2·0 ml. SKCF on alternate days. In the group receiving 0·2 ml. SKCF renal function was more similar to the untreated allotransplanted controls, except that some animals from this group were still living 3 weeks postoperatively, although with reduced levels of RPF and GFR. Only in the untreated animals at 2 weeks after renal allografting was significant anaemia (Hct<20%) detected (Table II). All other animals were non-anaemic (Hct>25 vols.%) during their total postoperative survival times. In our experience in the intact baboon, with intact kidneys, ESF is released in response to acute or intermittent bleeding only when the haematocrit is below 20 vols.%.[32]

Erythropoietin (ESF)

Table III summarizes the erythropoietic (ESF) activity seen in the untreated and SKCF-treated baboons. Untreated animals had a progressive postoperative rise in ESF associated with a progressive decline in RPF and GFR due to graft rejection. At 2 weeks after renal transplantation the ESF levels were 16·2 times greater than in saline controls (Table III). Pre-operative values in the

TABLE II. RENAL FUNCTION AND HAEMATOLOGICAL STATUS IN TREATED AND UNTREATED BABOON RENAL ALLOGRAFTS *

Values			*Untreated*	*SKCF (2·0 ml.)*	*SKCF (0·2 ml.)*
Pre-operative values					
RPF†		(ml./min.)	175 ± 28	140·5 ± 40·6	151·6 ± 42·5
Ccr‡		(ml./min.)	38·3 ± 9·8	46·8 ± 11·7	34·5 ± 15·9
Hct		(vols.%)	36·6 ± 6·6	41·8 ± 3·4	38·6 ± 3·6
Body-wt		(kg.)	12·5 ± 5·8	13·8 ± 1·4	12·9 ± 4·8
Postoperative values					
1 week:	RPF	(ml./min.)	59 ± 11·7	93·7 ± 6·7	50·0 ± 3·1
	Ccr	(ml./min.)	17·3 ± 7·9	19·8 ± 11·6	13·4 ± 6·4
	Hct	(vols.%)	33·6 ± 5·1	32·5 ± 5·7	30·5 ± 2·1
2 weeks:	RPF	(ml./min.)	60 ± 10·2	65 ± 2·5	46·5 ± 4·1
	Ccr	(ml./min.)	10·6 ± 3·8	13·5 ± 2	5·4 ± 4·6
	Hct	(vols.%)	18·0 ± 1·0	25·4 ± 8·5	32·5 ± 2·1
3 weeks:	RPF	(ml./min.)		65 ± 21	22·5 ± 12
	Ccr	(ml./min.)		4·7 ± 4·4	5·0 ± 0·4
	Hct	(vols.%)		35·3 ± 3·0	42·7 ± 5·7

* All values are mean (± 1 SD).
† RPF = renal plasma flow.
‡ Ccr = endogenous creatinine clearance.

TABLE III. ERYTHROPOIETIN (ESF) RESPONSES IN TREATED AND UNTREATED BABOON RENAL ALLOGRAFTS *

Treatment group	*Pre-operative levels*		*Postoperative levels*					
			1 week		*2 weeks*		*3 weeks*	
	24-hr ^{59}Fe % uptake†	*Ratio*‡	*24-hr ^{59}Fe % uptake*	*Ratio*	*24-hr ^{59}Fe % uptake*	*Ratio*	*24-hr ^{59}Fe % uptake*	*Ratio*
Untreated	0·86 ± 0·27	1·42	1·74 ± 0·40	2·90	9·75 ± 0·75	16·2	—	—
SKCF (2·0 ml.)	0·57 ± 0·07	0·99	14·47 ± 1·48	25·4	17·44 ± 1·30	30·6	16·98 ± 2·87	29·8
SKCF (0·2 ml.)	0·61 ± 0·06	1·01	1·50 ± 0·13	2·46	1·34 ± 0·28	2·19	4·05 ± 0·16	6·64
SKCF (2·0 ml.)**	1·53 ± 0·25	2·55						

* Values are mean ± SEM unless otherwise indicated.
† 24-hour % ^{59}Fe uptake in polycythaemic Ha/ICR Swiss mice.
‡ Ratio of $\frac{\text{24-hour \% } ^{59}\text{Fe uptake (test plasma)}}{\text{24-hour \% } ^{59}\text{Fe uptake (saline control)}}$ Saline controls averaged 0·60 ± 0·15% 24-hour ^{59}Fe uptake.
** Unoperated, SKCF-injected (10–14 days) group.

same animals were only 1·42 times greater than the saline-injected mice. In contrast, animals treated with 2·0 ml. SKCF had values at all postoperative periods which were 25 times or more greater than those of plethoric saline-injected mice (Table III). These sustained ESF elevations did not correlate with renal functional (Table II) or morphological evidence of graft rejection. The values were increased at the time of death, but were similarly elevated at 1 and 2 weeks after allotransplantation.

ESF levels in the animals treated with 0·2 ml. SKCF were not elevated in the early postoperative period. The levels at 1 week and 2 weeks were less than those of the untreated controls. Elevated ESF levels were, however, present in those animals surviving 3 weeks after renal transplantation, before death from rejection. Unoperated, SKCF-injected (2·0 ml. SKCF) baboons had elevations in ESF activity above other pre-operative specimens obtained from baboons not treated with SKCF. The SKCF-injected animals (Table III) had values over 2·5 times above those of control or non-SKCF-injected pre-operative baboons.

DISCUSSION

Erythropoietin (ESF) release is associated with a measured decline of renal function, and progressive histological evidence of renal graft rejection. The present results are similar to other untreated baboon allografts in this regard.[6] This series of animals (Table I) had no detectable renal anastomotic vascular obstructions present. The ESF elevations are therefore in part relatable to the intrarenal alterations or to the treatment *per se*. The measured kidney functions (Table II) confirm the renal ischaemia which was progressive and relatable to the typical microscopic appearance of rejection.[11] It is not apparent, however, on the basis of the aforementioned evidence, why, on a dose-related basis, SKCF (subcellular kidney fraction) treatment should be associated with sustained ESF elevations when administered to renal-allografted baboons. These ESF elevations were marked (Table III) and apparent both early and late in the postoperative period. The higher dose does not correlate with the level of renal function observed or diminution in histological evidence of renal rejection in this same group.[11] SKCF injections into intact rabbits do not induce a plethoric state.[12] Doses above 2·0 ml. SKCF are associated with intravascular agglutinations due to the apparent properties of the preparation.[13,14] This limits the further testing of the dose relationship of SKCF administration and ESF in baboon renal allografts.

However, noticeable erythropoietic effect is seen when SKCF is given to unoperated, intact baboons. A renal erythropoietic factor (REF)[15] has been characterized by Gordon and associates. REF is found in the renal tissue, and has been further characterized by additional biochemical techniques.[15] It appears that the present SKCF preparation, in sufficient amounts, may contain REF or other stimulants to ESF which, when administered to intact non-operated or renal allografted baboons, results in erythropoietin release or activation. This is not directly relatable to the degree of renal rejection or level of kidney function. Other unmonitored hormonal alterations may be operative and must be further characterized.

SUMMARY

Erythropoietin (ESF) is released in increased amounts from untreated baboon renal allografts. ESF levels correlate well with the decline in renal plasma flow, and glomerular filtration rate. Treatment with subcellular kidney cell fractions (SKCF) prolongs survival, improves renal function, and diminishes morphological graft rejection features on a dose-related basis. ESF is, however, always 25 times or greater above control levels in high-dose, renal-transplanted, SKCF-treated animals. SKCF injection to unoperated baboons also augments ESF responses. The ESF elevations are believed to be due to release or stimulation of renal erythropoietic factor (REF), or erythropoietin precursors in the baboon allografts by SKCF.

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