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Role of excretory graft function for erythropoietin formation after renal transplantation

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Abstract. To examine the role of renal excretory function for erythropoietin (EPO) formation we have determined the kinetics of plasma immunoreactive EPO (irEPO) in patients with end-stage renal disease undergoing renal allotransplantation (RTX).

In 13 patients with immediate excretory graft function (imGF) and stable haemoglobin (Hb) concentrations (median Hb 9·5 g dl^{-1} and median irEPO 18 mU ml^{-1} before RTX) irEPO increased significantly on day 4 after RTX to a median value of 29 mU ml^{-1} and 2 days later reached a plateau of 34·4 ± 3·3 mU ml^{-1} (mean ± SD of daily median values during days 6–20). In patients with imGF having acute blood loss and subsequently receiving transfusions, irEPO responded in an inverse fashion to changes in Hb concentrations.

In 12 patients with delayed graft function (dGF) (median Hb 8·8 g dl^{-1} and median irEPO 15 mU ml^{-1} before RTX) irEPO levels during the period of excretory failure remained either unchanged or displayed marked variations with peak values greatly exceeding those of patients with imGF. These variations were not related to changes in Hb concentrations and irEPO levels did not change following alterations in Hb concentrations. Upon recovery of excretory function irEPO approached the values found in patients with imGF.

The results suggest that an intact excretory renal function is not a prerequisite for the capability to produce EPO, but correlates with the oxygen-dependent regulation of EPO formation.

Keywords. Delayed graft function, immediate graft function, radioimmunoassay, renal anaemia.

Preliminary results were presented in abstract form at the 19th Annual Meeting of the German Society of Nephrology, Göttingen 1988.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; dGF, delayed graft function; EPO, erythropoietin; imGF, immediate graft function; irEPO, immunoreactive erythropoietin; RTX, renal transplantation; VUR, vesicoureteric reflux.

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Introduction

Patients suffering from end-stage renal disease (ESRD) generally develop an anaemia that results predominantly from inappropriately low erythropoietin (EPO) production by the damaged kidneys [1,2]. The physiological mechanisms underlying EPO formation and their possible alterations during kidney disease are not yet determined. In particular, it is not known whether the inappropriate EPO formation during ESRD results from destruction of the EPO-producing cells or functional alterations accompanying excretory renal failure. Recent observations in acute renal failure suggest that EPO formation may be impaired when there is functional failure but no chronic structural damage to the kidney [3,4]. To further elucidate the role of excretory renal function for the biosynthesis of EPO, we decided to study the relationship between EPO formation and renal excretory function in renal allograft recipients, in whom functioning grafts displayed a temporary delay in the onset of excretory function.

Previous studies addressing the correction of anaemia after renal transplantation (RTX), have shown that increases in EPO levels precede the reticulocytosis and rise in haematocrit following RTX [5,6,7] and in some cases EPO rose as early as the first to third post-transplant day [5,7]. An investigation by Besarab *et al.* [6] includes some data on EPO formation in a subgroup of patients with delayed graft function. While EPO did generally not increase until excretory graft function commenced, increments in some patients appeared to be related to differences in immunosuppressive regimens. However, in these patients, EPO values were not related to haemoglobin concentrations and sampling intervals were at least 3 days. Furthermore, a very recent study reports on a biphasic increase in EPO, with a first increase that was independent of graft function [8].

In the present investigation we determined plasma immunoreactive EPO (irEPO), haemoglobin and serum creatinine concentrations daily for at least 3 weeks following RTX in patients under standardized immunosuppressive therapy, and compared the time

course and magnitude of increases in irEPO in patients with immediate graft (imGF) function with that in patients with delayed graft function (dGF).

Patients and methods

Patients

A total of 27 consenting patients who underwent renal transplantation during two study periods were included in this investigation. Patient characteristics and the underlying renal disease are given in Table 1. None of the patients had received transfusions at least 3 months before RTX or had been treated with recombinant EPO. According to excretory graft function, patients were divided into two groups with immediate and delayed graft function, respectively. ImGF was considered present if (1) serum creatinine values declined continuously and approximately exponentially after RTX, and (2) reached values less than 300 μmol l^{-1} within, maximally, 7 days ($n=15$).

Table 1. Clinical details of grafted patients studied

Patient	Cause of renal failure	Sex	Age (years)	Months on HD
(a) *Immediate graft function*				
1	glomerulonephritis	m	56	11
2	glomerulonephritis	m	48	81
3	pyelonephritis	m	20	8
4	ADPKD	f	51	21
5	interstitial nephr.	f	56	41
6	pyelonephritis and unilat. renal hypoplasia	m	23	16
7	pyelonephritis, VUR	m	48	171
8	glomerulonephritis	m	54	49
9	nephrosclerosis and urate nephropathy	m	63	23
10	IgA nephropathy	m	31	11
11	pyelonephritis	m	17	18
12	urate nephropathy	m	62	79
13	glomerulonephritis	m	67	34
14	nephrosclerosis	m	62	12
15	glomerulonephritis	m	48	54
(b) *Delayed graft function*				
16	unknown	f	26	12
17	glomerulonephritis	m	51	106
18	focal sclerosis	m	49	95
19	glomerulonephritis	m	27	64
20	renal dysplasia	m	23	34
21	diabetic nephropathy	m	48	11
22	diabetic nephropathy	m	51	34
23	glomerulonephritis	f	30	100
24	focal sclerosis	m	42	103
25	pyelonephritis, VUR	m	32	108
26	glomerulonephritis	m	41	39
27	nephrocalcinosis (urogen. tuberculosis)	f	44	84

HD, hemodialysis; ADPKD, autosomal dominant polycystic kidney disease; VUR vesicoureteric reflux.

All patients received the first transplant except patients no. 16, 18 20 (second transplant) and 23 (fourth transplant). Patients no 3 and 11 received live-related grafts.

Patients were grouped as having dGF when serum creatinine values (1) either did not decline spontaneously after RTX so that temporary continuation of haemodialysis was required ($n8$) or the decline in creatinine levels was protracted ($n=4$), and (2) serum creatinine values less than 300 μmol l^{-1} were reached within 30 days post-RTX. Patients who did not meet the criteria of one of the two groups either because graft failure was prolonged or irreversible ($n=5$) or worsening of graft function occurred after serum creatinine levels of 300 μmol l^{-1} had been reached ($n=7$), were not included.

In all patients included, warm ischaemia time of grafts was less than 7 min; cold ischaemia time was 1420 (1252–1533) min and 1718 (1476–2145) min (median and interquartile range) in patients with imGF and dGF, respectively.

Immunosuppressive therapy

Standardized immunosuppressive therapy consisted of cyclosporine A (10 mg kg^{-1} day^{-1} started immediately post-transplantation and dose later adjusted to achieve serum levels of 200–300 ng ml^{-1}) and steroids (500 mg, 250 mg and 100 mg methylprednisolone on days 0, 1 and 2, and 1 mg prednisolone kg^{-1} day^{-1} started on post-transplant day 3 and reduced daily by 0·1 mg kg^{-1} day^{-1} during the initial 7 days, and then by 2·5 mg every 14 days to a maintenance dose of 7·5 mg day^{-1}). Additionally, azathioprine (2 mg kg^{-1}) was given to patients with acute graft rejection or those on high immunological risk, i.e. with preformed cytotoxic antibodies or rejection of previous grafts.

Additional drug treatment

All patients were on antacids and local antimycotic agents. Other drugs were calcium antagonists, beta-blocking agents or converting enzyme inhibitors for treatment of hypertension and loop diuretics if required.

Peripheral venous blood was withdrawn from all patients before RTX and daily between 08.00 and 10·00 for at least 3 weeks and up to 56 days following RTX, for determinations of plasma irEPO concentrations, serum creatinine and haemoglobin concentrations.

Measurement of erythropoietin

EPO was measured in heparinized plasma samples by radioimmunoassay as described previously [9]. In brief, 100-μl samples plus 20 μl of 30% bovine serum albumin were incubated for 24 h with 100 μl rabbit antiserum raised against pure recombinant human EPO. One-hundred μl of (^{125}I) iodotyrosyl-EPO (8×10^{-11} mol l^{-1}; Amersham International, Amersham, UK) were then added and after an additional incubation period of 24 h separation of free and bound

ligand was carried out using a second antibody technique. The second International Reference Preparation of human urinary EPO (WHO) was used as standard. The lower detection limit of the assay is 5 mu ml^{-1}, the interassay coefficient of variation is 6·7% for a sample containing 44·2 ± 3·0 mu irEPO ml^{-1} (mean ± SD). Geometric mean EPO level for non-anaemic adults is 17·9 mu ml^{-1} (95% range from 11–31 mu ml^{-1}; $n = 84$).

Serum creatinine levels and haemaglobin concentrations

These were determined by routine laboratory methods.

Statistics

Unless otherwise indicated, data are presented as median and interquartile range (in brackets). Mann–Whitney test and Wilcoxon signed-rank test were used to compare median values of unpaired and paired observations, respectively. Student's unpaired *t*-test was used to compare mean values. A P value $< 0{\cdot}05$ was considered significant.

Results

Patients with imGF

EPO formation was analysed separately in two patients experiencing marked changes in haemoglobin concentrations in the post-transplant phase and in the remaining patients with stable haemoglobin ($n = 13$). Figure 1 shows the time-course of plasma irEPO levels following RTX in these latter 13 patients with imGF in relation to serum creatinine and haemoglobin concentrations. Parallel to the decline in serum creatinine

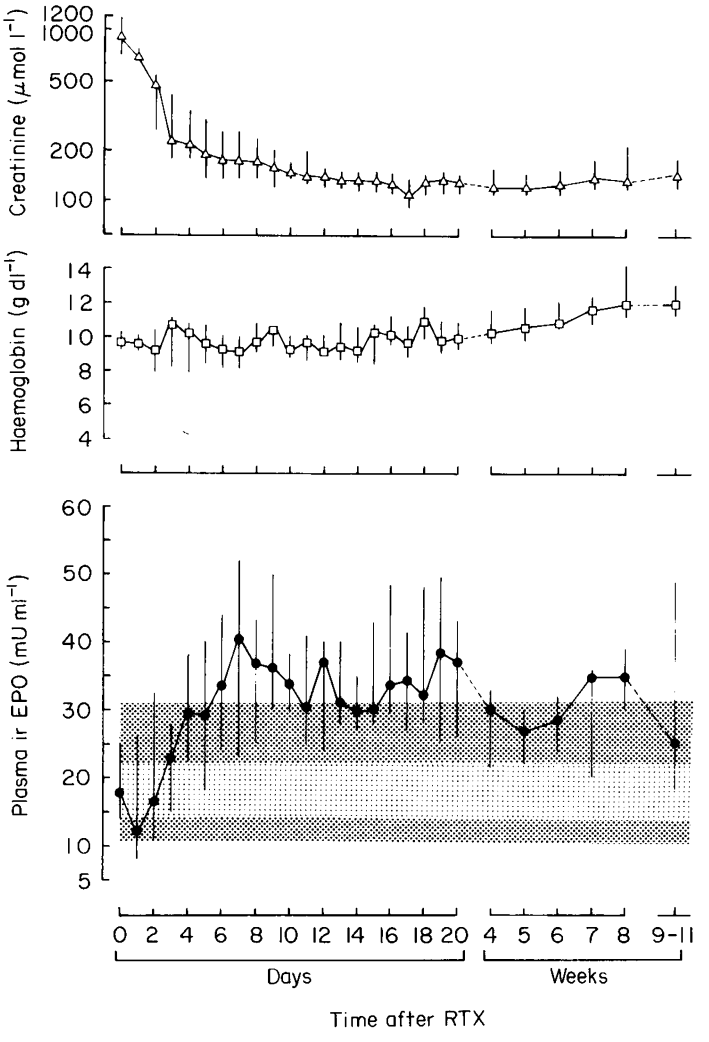

Figure 1. Temporal pattern of serum creatinine (upper), haemoglobin (middle) and plasma irEPO (lower) concentrations in patients with imGF after RTX. Data are median values and interquartile range in 13 patients studied from days 0 to 20 and in seven of these patients additionaly studied between weeks 4 and 11. For comparison, interquartile range and 95% range of serum irEPO in healthy adults is included (stippled). Following RTX EPO levels were first significantly elevated on post-transplant day 4 ($P < 0{\cdot}02$).

values, median irEPO level increased significantly from 18 (14–25) mU ml^{-1} to 29 (22–38) mU ml^{-1} on post-transplant day 4 ($P < 0.02$) and on day 6 reached a plateau of 34.4 ± 3.3 mU ml (mean $\pm$ SD of daily median values on days 6–20). During the first three post-transplant weeks none of these patients exhibited any variation in haemoglobin concentrations greater than 2·2 g dl^{-1} or received transfusions. In seven of these patients irEPO and haemoglobin concentrations were further determined weekly up to week 8 following RTX, and between weeks 9 and 11. irEPO values were 31.3 ± 3.7 mU ml^{-1} (mean $\pm$ SD of weekly median values) between weeks 4 and 8, and median irEPO level was 25 (18–49) mU ml^{-1} between weeks 9 and 11. Haemoglobin concentrations increased continuously from 10·3 (9·3–11·2) g dl^{-1} in week 4 to 12·1 (11·6–12·7) g dl^{-1} between weeks 9 and 11 (Fig. 1).

Two additional patients with imGF (no., 14, 15; Table 1) experienced a temporal marked decline in haemoglobin concentrations between post-transplant days 29 and 35, and 14 and 33, respectively. Both patients showed significant increases in their irEPO levels during these periods. In one patient (no. 14) in whom the haemoglobin concentration fell from 9 to 5·6 g dl^{-1} due to wound bleeding, and subsequently rose to 10·2 g dl^{-1} following surgical revision and transfusion, irEPO values increased from a median value of 31·5 (28–38) mU ml^{-1} before blood loss (days

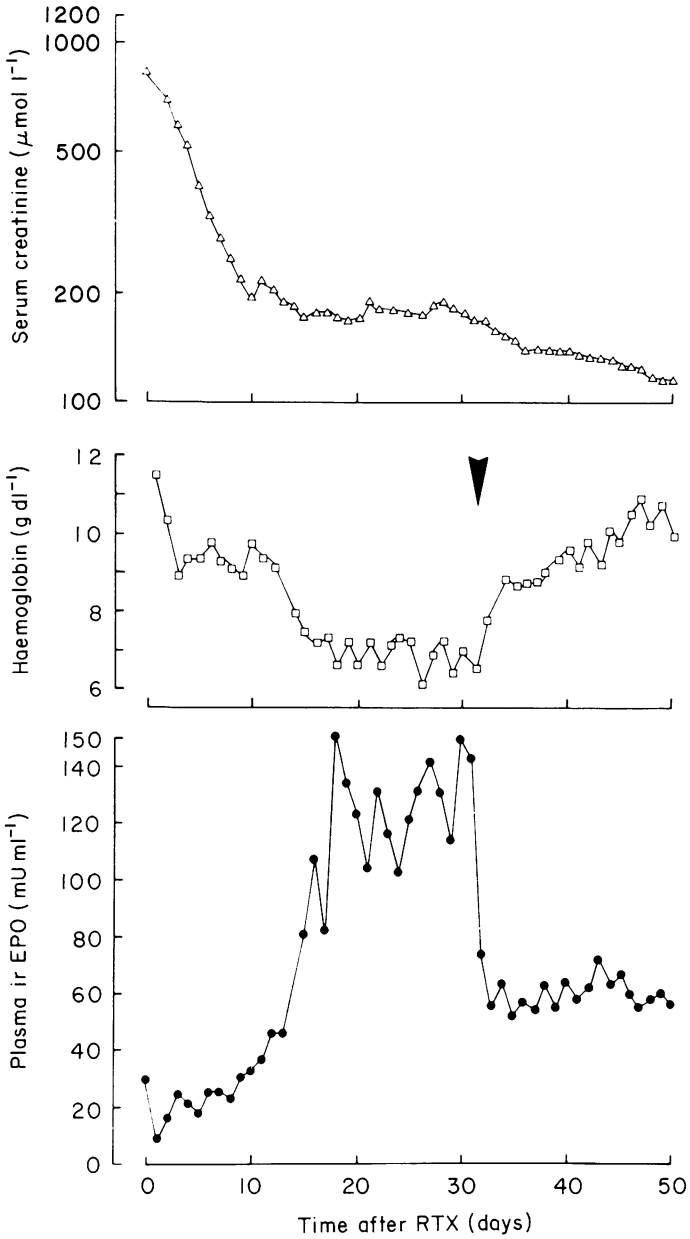

Figure 2. Time-course of serum creatinine (upper), haemoglobin (middle) and plasma irEPO (lower) concentrations in a patient experiencing acute blood loss due to gastrointestinal bleeding and subsequently receiving transfusion of two units of erythrocytes on day 31 following RTX (arrow).

4–29) to a median value of 88 (71–114) mU ml^{-1} during the reduction in haemoglobin concentrations, and after transfusion declined again to a median value of 15·5 (11–17) mU ml^{-1}. The time-course of EPO levels in relation to haemoglobin for the other patient (no. 15) is depicted in Fig. 2. Parallel to the reduction in haemoglobin concentrations due to gastrointestinal bleeding, irEPO increased to a median value of 130 (107–133) mU ml^{-1}.

Patients with dGF

In patients with delayed graft function, EPO formation following RTX was much more variable. The temporal pattern of plasma irEPO levels and haemoglobin concentrations in the whole group of 12 patients with dGF, during the time-period when serum creatinine concentrations were higher than 300 μmol l^{-1}, is illustrated in Fig. 3. For comparison, interquartile range (dark stippled) and absolute range (light stippled) of values in patients with imGF is included. Pre-transplant irEPO was not significantly different in both groups ($P > 0{\cdot}15$). During the phase of excretory failure, median EPO values in patients with dGF increased only slightly from 15 (12–18) mu ml^{-1} to $22{\cdot}7 \pm 5$ mU ml^{-1} (mean $\pm$ SD of daily median values between days 4 and 24; $P < 0{\cdot}001$ vs. patients with imGF) and were generally below the lower quartile of those in patients with imGF. However, irrespective of the lower median irEPO levels, the absolute range of irEPO levels in patients with dGF was considerably higher, and maximum values in these patients markedly exceeded those during imGF. The lower median EPO values were obviously not due to higher erythrocyte mass, since haemoglobin concentrations were even slightly below those in patients with imGF ($8{\cdot}7 \pm 0{\cdot}7$ g dl^{-1} vs. $9{\cdot}7 \pm 0{\cdot}6$ g dl^{-1}; mean $\pm$ SD of daily median values; $P < 0{\cdot}001$). Furthermore, except patient no. 16 (see below) none of the patients with dGF was transfused.

Analysis of the time-course of irEPO levels in individual patients with dGF revealed two typical

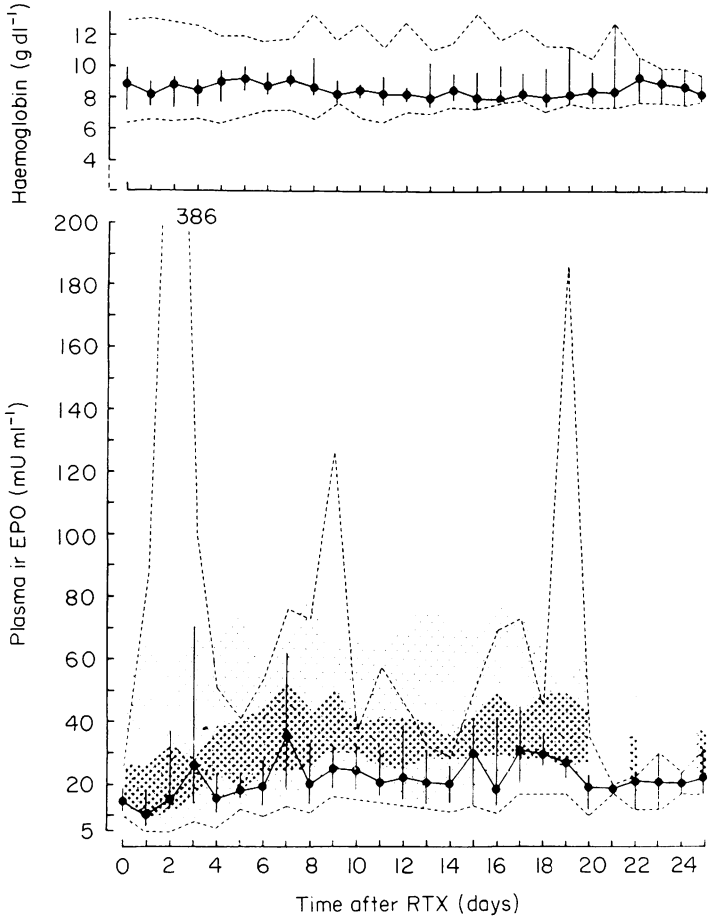

Figure 3. Temporal pattern of haemoglobin (upper) and plasma irEPO (lower) concentrations in 12 patients with dGF, including only values during the phase of excretory graft failure (serum creatinine > 300 μmol l^{-1}). Values are median (filled circles) with interquartile range (solid bars) and absolute range (broken line). For comparison, interquartile range (dark stippled) and absolute range (light stippled) of plasma irEPO in patients with imGF is included. Except on days 7–9 and 11, median irEPO in patients with dGF was not significantly different from pre-transplant values. On days, 4–6, 8–11, 14 and 20, median irEPO in patients with dGF was significantly lower than in patients with imGF.

Table 2. Graft function and irEPO levels in patients with delayed graft function

Patient no.	Duration of excretory graft failure (days)*	Haemodialysis After RTX	Graft biopsy (days post RTX)	Plasma irEPO (mU ml^{-1}) Before RTX	Excretory graft failure (*min*-median-*max*) During	After†
(a) *Patients with little change in irEPO during excretory graft failure*						
16	40	yes	slight REJ (9)	18	*13*—19—*31*	*26*—39—*45* (15)
20	34	yes	ATN (4); slight REJ, vasculopathy (25)	15	*9*—19—*27*	not determined
22	6	no	not performed	20	*20*—22—*30*	*20*—29—*49* (18)
23	20	yes	ATN, slight REJ (4)	9	*8*—12—*25*	*11*—20—*28* (31)
25	17	yes	slight REJ (8)	25	*8*—14—*27*	*27*—36—*45* (15)
(b) *Patients with transient elevations in irEPO during excretory graft failure*						
17	20	yes	ATN (12)	12	*18*—35—*213*	*43*—53—*66* (6)
18	28	yes	slight REJ, tubular necrosis, vasculopathy (10); REJ, vasculopathy (19)	14	*11*—22—*386*	*29*—38—*61* (22)
19	17	yes	REJ	14	*10*—33—*127*	*21*—31—*43* (6)
21	7	no	not performed	17	*8*—12—*85*	*15*—22—*50* (16)
24	8	yes	not performed	12	*7*—22—*68*	*20*—31—*40* (17)
26	11	no	not performed	5	*5*—15—*57*	*12*—19—*32* (11)
27	19	no	not performed	34	*16*—30—*168*	*31*—51—*100* (23)

*Days post RTX, during which serum creatinine values were above 300 μmol l^{-1}.
†Numbers in parentheses are number of observations after excretory graft failure.
REJ rejection; ATN, acute tubular necrosis.
In patient no. 20 EPO determinations could not be continued because he was transferred to another hospital.

patterns, according to which patients could be grouped (Table 2). Five patients (Nos 16, 20, 22, 23 and 25) did not show any significant increase in irEPO levels during the phase of excretory failure and no single value exceeded 31 mU ml^{-1}, while the median pre-transplant value was 18 (15–20) mU ml^{-1}. An increase in irEPO into the range of values in patients with imGF occurred, however, after excretory function had resumed (Table 2). Daily irEPO values for one of these patients (no. 16) are illustrated in Fig. 4. During the first 40 post-transplant days there were only very slight changes in irEPO concentrations and an obvious increase of irEPO was not observed until excretory graft function commenced. At that time irEPO increased, even though haemoglobin concentrations due to blood transfusion were approximately 3 g dl^{-1} higher than during the early phase.

In the remaining patients with dGF (nos 17–19, 21, 24, 26 & 27) irregular transient increases in irEPO levels were observed during the period of excretory graft failure with peak levels up to 368 mU ml^{-1} and baseline values that were not different from pre-transplant levels (group B, Table 2). In these patients a sustained elevation in EPO was also found after the end of excretory graft failure and at the same time intra-individual variations became less pronounced (Table 2). Figure 5 demonstrates a typical example for this pattern of irEPO levels (no. 18). It is apparent that the variations in irEPO concentrations were not related to changes in haemoglobin concentration. With the onset of excretory function irEPO levels approached the values found in patients with imGF.

Individual duration of excretory graft failure and histological diagnosis in those patients biopsied are included in Table 2. Median cold ischemia time of grafts in patients with little change in irEPO (group A) was 2030 min (range: 1528–2450 min) and slightly higher than in patients with transient elevations in irEPO (group B) (median: 1645 min; range: 1390–2259 min) ($P < 0{\cdot}05$). Analysis of medication in patients with imGF and dGF revealed no single drug or therapeutic scheme that was consistently associated with a specific pattern of EPO levels following RTX. As expected, additional immunosuppression with azathioprine had been mainly given to patients in the group with dGF (nos 16–18, 20–23, 25 and 27). However, application of azathioprine did not correlate with different time-courses of EPO levels in these patients and was also given to two graft recipients with imGF (nos 1 and 2). Furosemide and calcium antagonists were also given to patients in both groups with different graft function (nos 4, 5, 7, 9, 11, 13, 16, 18, 19, 21–24, 26, and 27 and nos 4, 6, 7, 8, 11, 12, 14, 15, 18, 21 and 22, respectively). Some patients received addi-

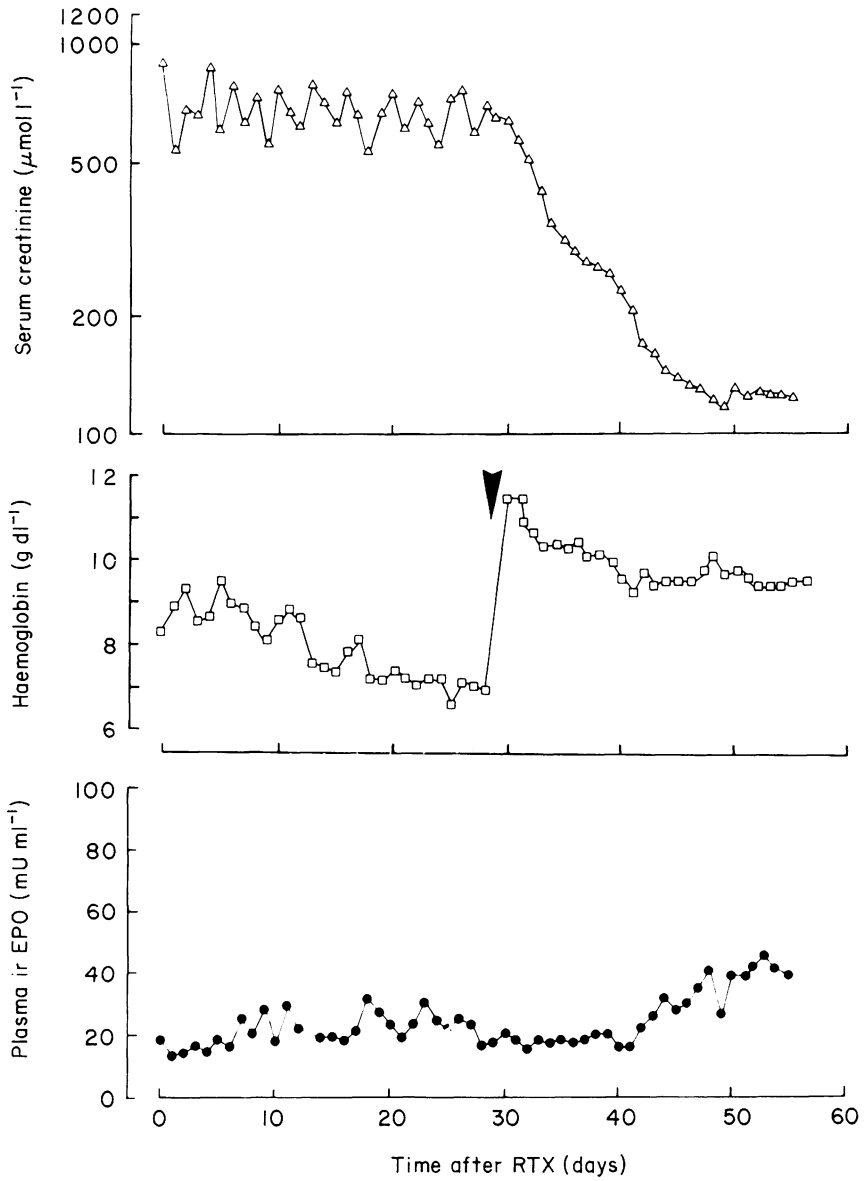

Figure 4. Time-course of serum creatinine (upper), haemoglobin (middle) and plasma irEPO (lower) concentrations in a patient with dGF (no. 16). During the first 40 days post-transplantation there was only very slight change in irEPO levels despite continuously declining haemoglobin concentrations until day 28, and a subsequent rise in haemoglobin levels following transfusion of two units erythrocytes on day 29 (arrow). An obvious increase in irEPO occured only after day 40, when excretory graft function had commenced.

tional antihypertensive medication with β-blocking agents (nos 11, 18 and 23), clonidine (nos 7, 17, 23 and 26) or a converting enzyme inhibitor (no. 11).

Discussion

Before the availability of recombinant human EPO for replacement therapy, renal transplantation was virtually the only way to correct the anaemia of end-stage renal disease. Early studies employing bioassays for EPO demonstrated that this correction of anaemia after RTX was frequently associated with an increase of EPO in graft recipients [10–12]. However, the low sensitivity of the bioassays available at that time made it difficult to precisely assess the pattern of EPO formation after RTX. Subsequent studies have shown that a rise in EPO may be detectable within 1 to 3 days following RTX when radioimmunoassays are being used [5–7]. The sensitivity of these immunoassays is about tenfold higher than that of bioassays and the validity of immunoassay determinations is confirmed by a close correlation of bioactivity and immunoreactivity at higher concentrations of the hormone [9,13].

The present study indicates that the kinetics of irEPO formation following RTX are variable, depending on the excretory function of the graft. In patients with imGF, EPO levels increased continuously after RTX and reached a plateau after about 1 week (Fig. 1).

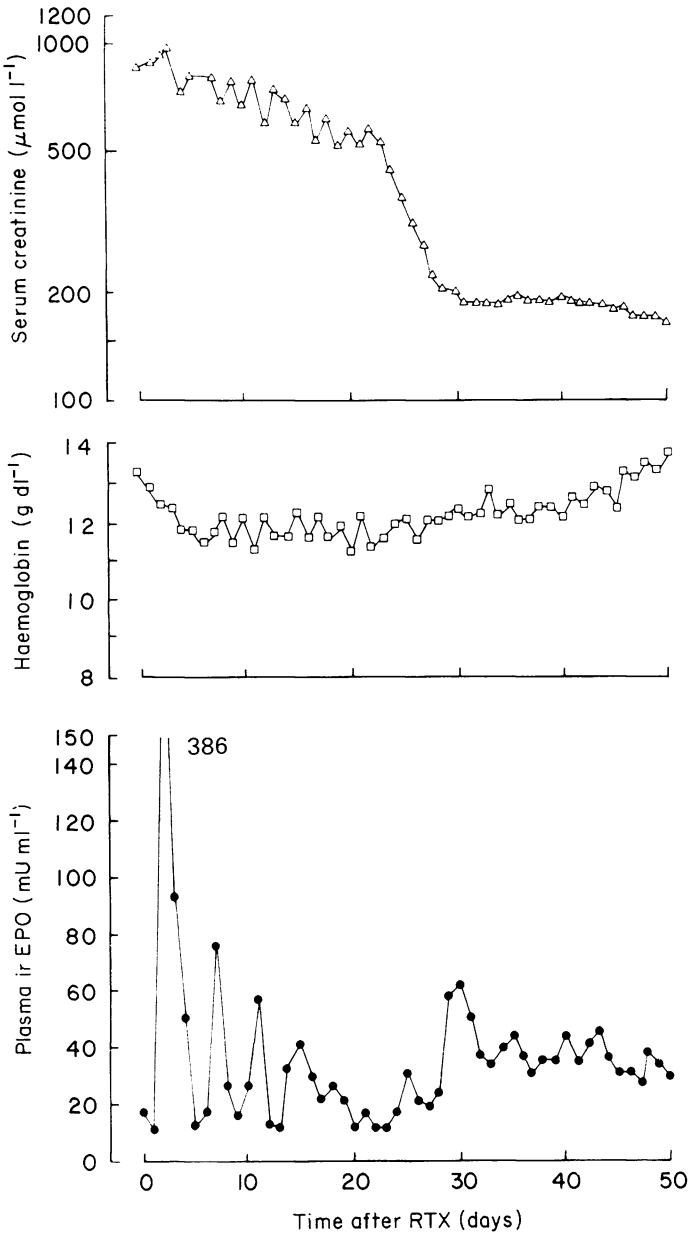

Figure 5. Time course of serum creatinine (upper), haemoglobin (middle) and plasma irEPO (lower) concentrations in a patient, in whom irEPO levels showed marked variations during the phase of excretory graft failure (no. 18). A continuous increase in irEPO alike in patients with imGF, occured after day 28 when excretory graft function had commenced.

This temporal pattern confirms qualitatively and extends two previous studies, also employing radioimmunoassays, that demonstrated early increases of irEPO after RTX [5,7]. In contrast to Besarab *et al.* [7], who found a typically a fourfold increase in EPO within the first 2 post-transplant weeks, increases were less pronounced, and only about two- to threefold in our patients. This difference may be related to various factors, including individual haemoglobin concentrations, immunosuppression and additional drug therapy. More importantly, the questions arise if EPO formation following RTX is subject to oxygen-dependent regulation and if the moderate increase in irEPO of about 16 mU ml^{-1}, observed in our patients, is appropriate in comparison with non-renal failure patients. According to the relationship between EPO levels and haematocrits or haemoglobin concentrations established by Erslev *et al.* [14] and Cotes [13] for non-renal anaemias, the median EPO concentration of 34 mU ml^{-1} during the plateau phase would fit into the confidence limits for the actual haemoglobin concentration, even though only one intact kidney contributes to the rise in EPO. Also the increase in EPO in two patients having acute blood loss (Fig. 2) is well in accordance with the normal inverse exponential relationship between EPO and haemoglobin concentra-

tions. In addition, the EPO formation in grafted kidneys tends to decline again once anaemia ameliorates [5,7,8,15] (Fig. 1), also indicating an intact regulation. Regarding the erythropoietic responsiveness to EPO, it remains nevertheless noteworthy, that after restoration of renal function a relatively slight increase in EPO levels is sufficient for the correction of renal anaemia.

Taken together, the findings in patients with imGF suggest a regulated EPO formation in grafted kidneys that parallels the onset of excretory function. Since transplants lack innervation and lymph drainage, two factors that have been implicated in EPO formation and secretion [16,17], neither appear to be essential for a regular increase of EPO levels.

To assess the importance of excretory graft function for EPO formation, kinetics of EPO levels in patients with dGF (Figs 3–5; Table 2) can be compared with that in patients with imGF. During the phase of excretory failure we found no EPO formation in these patients that appeared to be regulated by haemoglobin concentrations. In some patients (group A, Table 2) EPO levels did not change at all in response to the pre-existing anaemia or additional blood loss (Fig. 4). In others (group B, Table 2) marked variations of EPO were found that exceeded the values in patients with imGF and were not related to changes in haemoglobin concentrations (Fig. 5). A comparatively high increase in EPO within the first week after RTX was recently also observed by Sun *et al.* [8], who averaged EPO levels in 31 transplant recipients. Although these investigators did not analyse their data separately according to different degrees of excretory graft function, the mean serum creatinine level in the whole group did not decline until day 10, suggesting that many of their patients would have met our criteria of dGF.

Although we cannot definitely exclude the possibility that the native kidneys or the liver are responsible for some of the acute variations of circulating irEPO in patients with dGF, several lines of indirect evidence suggest that these increased amounts of EPO are mainly derived from the grafted kidneys. First, in patients with ESRD, serum EPO values do not generally exceed the normal range of non-anaemic individuals [1,2], as was also obvious from the pre-transplant values recorded in this investigation. Some variations in serum EPO in untransplanted patients have been observed in response to hypoxia [18,19], but these increases have not been of the extent seen in the present investigation. Second, the occurence of these spiking elevations was confined to periods of excretory graft failure and, third, was not associated with any specific type of underlying renal disease. In particular none of our patients in this group had polycystic kidneys, whereas Besarab *et al.* [7] found an early EPO peak in association with this disease. Experimental results in animals [20] and indirect evidence in humans [21] indicate that kidneys contain no stores for EPO, which could have been released, and it appears, therefore, that *de novo* synthesis of EPO in the grafted kidney was stimulated, apparently independent of blood oxygen-carrying capacity. From the data recorded in this study we were unable to define the nature of these stimuli. Both groups of patients, those without increase in EPO during excretory graft failure as well as those with transient elevations, comprised cases with different causes of graft failure and we found no association with the duration of graft failure or haemodialysis and drug treatment. The observation that in the group of patients with little increase in irEPO the cold ischaemia time of the grafts was higher than in patients with temporary elevations in irEPO, might indicate that EPO formation depends partially on the initial quality of the graft. Furthermore, among the possible determinants of EPO synthesis, direct drug effects on EPO-producing cells, e.g. effects of cyclosporine, as well as renal haemodynamic alterations, tissue injury and release of inflammatory cytokines, have to be considered. Although in the intact kidney reduction in arterial renal blood flow is only a minor stimulus for EPO formation [22], abnormal perfusion in kidneys with minimal excretory function or regional vasoconstriction may be of more significance.

Irrespective of the cause, the transient high EPO concentrations in some patients indicate that the failure to adequately respond to the anaemia during excretory graft failure was not due to lack of ability to synthesize the hormone. Consequently it appears, rather that the oxygen dependent regulation of EPO is disturbed during excretory graft failure. Since the mechanisms underlying the physiologic regulation of EPO are poorly understood, the reason for this disturbance remains speculative as well. Thus it is possible that a local factor associated with the pathophysiological derangements in the non-functioning graft inhibits the regulatory processes. Alternatively, the failure to adequately and continuously regulate EPO formation may indicate that ongoing excretory renal function is a prerequisite for the adaptation of EPO to changes in blood oxygen content. Although the cells producing EPO are probably not part of the nephron, but located in the cortical interstitium [23,24], experimental evidence indicates that the regulation of EPO is related to tubular function [25] and this may account for the temporal concurrence between onset of excretory graft function and regulated EPO formation in both groups of patients with dGF as well as in patients with imGF.

In conclusion, we have shown that EPO formation after renal transplantation is related to excretory graft function. The onset of excretory graft function turned out not to be an absolute requirement for EPO production, but the oxygen-dependent regulation of EPO seems to operate only in the presence of excretory function. Regarding untransplanted patients with ESRD, this suggests that the lack of excretory function in their diseased kidneys may contribute to the

inappropriately low EPO formation that accounts for the development of renal anaemia.

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