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Antibodies in alloimmunized uraemic patients treated with recombinant erythropoietin

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Abstract We have retrospectively analysed sera from 52 already sensitized uraemic patients collected over 1 year and compared erythropoietin (EPO)-treated with non-EPO-treated patients. Significantly fewer ($P<0.01$) patients (33%) on dialysis because of the rejection of their kidney grafts received EPO than patients on dialysis because of underlying kidney disease (71%). EPO treatment reduced the number of additional blood transfusions, since 3/28 EPO-treated but 12/24 non-EPO-treated patients were given blood ($P<0.05$). Among the EPO-treated patients, 64% showed a loss of panel-reactive antibodies (PRA), as measured by the microlymphocytotoxic technique, while only 12.5% of the non-treated patients showed a loss of PRA ($P<0.01$). In the subgroup of transplanted patients, PRA loss was only found among the EPO-treated patients, but their number was small ($P<0.05$). The class, subclass and specificities of the antibodies, as determined by FACS (flow cytometry) analyses, showed no distinct differences between EPO- and non-EPO-treated patients. The differences were significant between transfused and previously transplanted patients.

Key words Erythropoietin
Antibodies · Flow cytometry
Kidney transplantation · Blood transfusions · Immunoglobulins
HLA antibodies

Introduction

HLA immunization constitutes a major clinical problem in the field of transplantation. Blood transfusion, pregnancy and rejection of previous grafts cause sensitization against HLA antigens, and the HLA antibodies produced may persist for years [1]. It is nowadays possible to avoid blood transfusions in renal anaemia by giving recombinant erythropoietin (EPO) instead [2]. This results in a smaller percentage of sensitized patients. However, in patients who are already sensitized, the effect of EPO and avoidance of blood transfusions is not clear. While some report a decrease in the titres of HLA antibodies and lowered panel-reactive antibody (PRA) levels after the initiation of EPO [3], others report no effect [4, 5].

We have studied this question by analysing the PRA levels, the immunoglobulin classes and the specificities of the antibodies in 52 sensitized patients awaiting kidney transplantation. All had been given blood transfusions. Twenty-four of them had also lost previous kidney grafts. Sera for antibody analyses were taken over a period of 1 year.

Patients and methods

Patients

The study group consisted of 28 patients with end-stage kidney disease (ESKD) who were given EPO in recommended dosages to prevent anaemia. Twenty-seven had undergone haemodialysis and one patient, CAPD. Eight patients had previously received kidney grafts. The control group consisted of 24 patients with ESKD who had not been given EPO for various reasons. Of these, 18 had undergone haemodialysis and 6, CAPD; 16 of these had previously received kidney grafts.

Methods

In general, a serum sample was collected from each patient every 3rd month. If the patient had received blood transfusions during the study period, another serum sample was drawn 2 weeks after the transfusion. The sera were stored frozen and analysed retrospectively.

All sera were tested for PRA using the NIH micro lymphocytotoxic technique against a panel of spleen lymphocytes from 17 donors. This covered most of the HLA specificities described earlier [6]. The spleen cells were separated into T and B lymphocytes, using the immunomagnetic separation method with Dynabeads [7]. The flow cytometric (FACS) analysis previously described [6] was used for Ig class and IgG subclass analyses and determination of HLA specificity of the antibodies.

Statistics

For comparison of groups, the non-parametric chi-square analysis with Yates correction was used.

Table 1 Characteristics of EPO-treated versus non-EPO-treated uraemic patients. All were transfused earlier

	EPO treatment		*P*
	+	−	
No. of patients	28	24	
No. of grafts previously lost	8	16	< 0.01
CAPD/HD treatment	1/27	6/18	NS
PRA reduction	18	3	< 0.01
No. of patients requiring blood	3	12	< 0.01

Results

Significantly fewer patients (8/24, 33%) on dialysis because of chronic rejection/loss of kidney graft function than those on dialysis because of underlying kidney disease (20/28, 71%) had received EPO treatment (Table 1). EPO treatment reduced the need for blood, since only 11% of the EPO-treated patients were given blood, but 50% of the control group was given transfusion ($P < 0.01$). While 64% of the EPO group showed a loss of PRA as measured by the NIH technique, only 12.5% of the control group showed this finding ($P < 0.01$).

The HLA specificity and class of antibody, determined by the FACS analysis, evidenced no significant differences between EPO- and non-EPO-treated patients. Instead, the variations were found between previously transfused and previously kidney-grafted patients. Thus, patients with previous transfusions had antibodies against class I antigens or non-HLA antibodies, while patients with previous transplantations had antibodies against class I, class II and non-HLA antibodies and often a mixture (Table 2). IgG1 as the sole subgroup was found significantly more often among transfused patients (13/28) than among patients with previous graft losses

Table 2 Specificity of antibodies in sera from patients immunized by blood transfusions or by allogenic kidney grafts

	Previous transfusions	Previous transplantations	*P*
Class I	13	2	< 0.01
Class II	–	3	NS
Class I + non-HLA	15	4	< 0.01
Class I/II + non-HLA	–	12	< 0.001
Non-HLA	–	3	NS

Table 3 Immunoglobulin class and subclasses of HLA class I/II-specific antibodies in sera from patients immunized by blood transfusions or previous graft loss

	Previous transfusions	Previous transplantations	*P*
IgG1	13	2	< 0.01
IgG2	–	1	
IgG3	3	2	
IgG4	1	–	
Mixture of IgG classes	11	20	< 0.01
IgA	7	10	
IgM	5	5	

(2/24, $P<0.01$). The latter were more likely to have a combination of two or three IgG subclasses (20/24) than the former (12/28, $P<0.01$).

IgA was found in 17 patients and IgM, in 13 patients. These were patients who had had previous transfusions or transplantations. The original kidney disease in these patients showed no clear pattern (Table 3).

Discussion

In this study of 52 sensitized uraemic patients, we have investigated whether EPO treatment has any impact at the antibody level during an observation time of 1 year. We found that 2/3 of the EPO-treated patients showed a decrease in PRA levels compared with 1/8 of the untreated patients. One reason for this was probably that EPO significantly reduced the need for further blood transfusions, as has been noticed by others [7]. Another reason may be that the EPO-treated patients were significantly more often patients who had received blood only and not kidney transplants before.

Immunization with a kidney transplant gives quite a different antibody response from that of the leucocyte-poor transfusions which we use for our patients. Our analysis of antibody characteristics with the FACS technique showed that antibodies following immunization with blood transfusions are of HLA class I antigen or non-HLA origin. Often, IgG1 subclass or IgG3 is found. After allogeneic transplantation there is usually a mixture of antibodies against both HLA class I and II and non-HLA antibodies.

Why did so few patients on dialysis due to the rejection of previous grafts receive EPO treatment? Many of those with chronic rejection had uncontrolled hypertension, and it was thought dangerous to start EPO in such patients because of the risk of aggravating the hypertension. Moreover, there was no experience of using EPO together with immunosuppression, and the risk of rejection was not known. However, today (5 years later) we usually use lower EPO dosages, and hypertension is not longer a clinical problem. Moreover, we initiate treatment with EPO earlier, before patients are started on dialysis, and we have noted no acute rejection episodes (personal observations). A similar experience is reported by Ettenger et al. [8]. It may be added that the human growth hormone given to our transplanted children has been associated with acute rejection [9].

In conclusion, EPO treatment results in a lowering and finally lack of detectable PRA by the avoidance of transfusions. Blood-transfused patients have different types of antibodies from those in previously grafted patients, and it seems easier to lose the former. Whether EPO per se can cause the lymphocytes to produce antibodies or not could not be determined. In vitro experiments have shown a stimulatory effect on human B cells [10], and contradictory changes in the lymphocyte population in EPO-treated patients have been described [11, 12]. A study on healthy humans has shown significant lymphopaenia after 6 weeks of EPO treatment, which is an exciting finding [13]. Thus, the immunological effect of EPO remains an interesting question. For clinical purposes a new study of the patients with previous transplants who were given EPO seems important.

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