

Interindividual variability in the concentration–effect relationship of antilymphocyte globulins – a possible influence of FcγRIIIa genetic polymorphism

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- There is interindividual variability in the antilymphocyte globulin (ALG) effect, but there is no pharmacokinetic–pharmacodynamic study of this subject.
- In addition, a time dependence of the pharmacokinetics of some therapeutic antibodies has been described.
- ALGs may partly act by antibody-dependent cellular cytotoxicity (ADCC), but their mechanism of action in humans is not known.

WHAT THIS STUDY ADDS

- Horse ALG pharmacokinetics can be described using a two-compartment model with time-dependent central volume of distribution.
- After an initial concentration-independent lymphocyte depletion, the concentration–effect relationship can be described using a physiological indirect response model.
- The genetic polymorphism of FcγRIIIa at position 158 may influence the ALG concentration–effect relationship and these polyclonal antibodies may therefore act by ADCC.

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AIMS

Polyclonal antilymphocyte globulins (ALGs) are currently used in transplantation, but the sources of interindividual variability of their effect are poorly understood. No pharmacokinetic–pharmacodynamic (PK–PD) study of ALG is available. Moreover, the genetic polymorphism of FcγRIIIa, a receptor for the Fc portion of immunoglobulins involved in antibody-dependent cellular cytotoxicity (ADCC), may influence their concentration–effect relationship.

METHODS

Fourteen kidney transplant patients treated by horse ALG were included in a prospective, noncomparative study. A population two-compartment PK model including a time dependence of the central volume of distribution was developed. Total lymphocyte count was used as biomarker of effect. Concentration–effect data were described using a physiological indirect response model, combining concentration-dependent and -independent inhibitions of lymphocyte input into the circulation. In addition, six kidney transplant patients in whom ALG concentrations were not available were included retrospectively. All patients were genotyped for FCGR3A.

RESULTS

Both the PK and the PK–PD model described the data satisfactorily and showed high interindividual variability. Asymptotic $T_{1/2-\alpha}$ and $T_{1/2-\beta}$ -values were 1.3 and 25 days, respectively. The concentration of ALG leading to a 50% inhibition of lymphocyte input (IC_{50}) was lower in FCGR3A-V carriers than in FCGR3A-F/F patients (383 ± 199 vs. 593 ± 209 mg l⁻¹, $P = 0.008$).

CONCLUSIONS

This is the first description of the ALG effect on lymphocyte count using PK–PD modelling. Our results show that part of the variability in their concentration–effect relationship may be explained by FcγRIIIa genetic polymorphism and therefore that horse ALG may deplete lymphocytes by ADCC.

Introduction

Antilymphocyte globulins (ALGs) are polyclonal antibodies used in organ transplantation to prevent or treat acute allograft rejection. ALGs are obtained by immunizing animals (rabbits or horses) with human lymphoid cells (lymphoblasts, lymphocytes or thymocytes). Although ALGs have been in use for several decades, the interindividual variability in patient response remains poorly understood [1, 2]. Monitoring of drug effect is based on lymphocyte numeration, because ALG doses may be decreased when lymphopenia is obtained or the drug readministered to maintain it [3]. Although lymphocyte count is an established biomarker of ALG therapeutic effect, the study of its quantitative relationship with ALG concentrations has not been reported.

The mechanism of action of ALG is probably complex, but it eventually results in target cell destruction. Several mechanisms have been observed *in vitro*, including apoptosis, complement-dependent cytotoxicity (CDC) and also antibody-dependent cellular cytotoxicity (ADCC) in the presence of cytotoxic cells expressing receptors for the Fc portion of IgG (FcγR) [4]. The *FCGR3A* gene, which encodes FcγRIIIa, a FcγR expressed by macrophages and natural killer cells, displays a single nucleotide polymorphism at position 559 of the cDNA (G or T, rs number 17857127), which generates two FcγRIIIa allotypes, with a valine (V) or a phenylalanine (F) at position 158, respectively [5, 6]. Non-Hodgkin's lymphoma patients with the 158V allotype have a better response to rituximab, an anti-CD20 cytolytic therapeutic antibody, than patients with the 158F allotype [7–11].

FCGR3A polymorphism may therefore be a genetic factor influencing the effect of cytolytic therapeutic antibodies, although it remains to be demonstrated that this is also true for antibodies of animal origin (with an animal Fc portion). The aim of this study was to analyse the concentration–effect relationship of horse ALG in renal transplant patients, with the hypothesis that *FCGR3A* polymorphism may influence the ALG effect, as measured by lymphocyte depletion, and to explain part of the interindividual variability of its effect.

Methods

Patients

This study was based on both a prospective and a retrospective group of patients. The prospective group consisted of 14 kidney transplant patients included in a noncomparative study of horse ALG pharmacokinetics (PK) and pharmacodynamics (PD), recruited in four renal transplant centres (Caen, Poitiers, Reims and Tours, France). They had received a first cadaveric renal transplantation with a cold ischaemia time <48 h. The patients were not hyperimmunized (panel-reactive IgG antibodies <75%) and had

never been treated with horse ALG. This prospective study was approved by the local ethics committee (CCPPRB of Tours) and all patients gave written informed consent, both for the clinical study and for *FCGR3A* genotyping.

The retrospective group was also studied to increase the number of patients and to validate our models. It consisted of six renal transplant recipients treated with the same horse ALG. They had been transplanted in the centre of Tours and gave written informed consent for *FCGR3A* genotyping. Patients' characteristics are shown in Table 1.

Treatment

Antilymphocyte globulin dosing regimen In the prospective group, ALG (Lymphoglobuline®; Genzyme Imitix-SangStat, Lyon, France) was administered through a central venous catheter or an arteriovenous fistula. The first infusion was started within the first hour after patient entry in the recovery room and lasted for 24 h. The second infusion was started at least 12 h after the end of the first infusion and subsequent infusions over a period of 8–12 h, separated by a 24-h period. The initial dose was 10 mg kg⁻¹ day⁻¹ without exceeding 600 mg per infusion. After the first two infusions, the dose was halved if T-cell lymphopenia was achieved, with a minimum of 100 mg per infusion. T-cell lymphopenia was defined by one of the two following criteria: a CD3+ lymphocyte count of <20 cells mm⁻³ (measured using flow cytometry) or a total lymphocyte count of <200 cells mm⁻³. The conditions leading to ALG discontinuation were a platelet count of <50 000 cells mm⁻³ or a leucocyte count of <1500 mm⁻³. When severe adverse effects possibly related to the studied ALG were observed, they were replaced by Thymoglobuline® (Genzyme Imitix-SangStat), an ALG of rabbit origin. Patients in the retrospective group had been administered an initial dose of 10 mg kg⁻¹. Subsequent doses had been adjusted to maintain a CD3+ count of <20 cells mm⁻³.

Concomitant immunosuppressive treatment Concomitant immunosuppressive treatment consisted of ciclosporin (Neoral®; Novartis Pharmaceuticals, Basel, Switzerland), mycophenolate mofetil (Cellcept®; Roche, Neuilly sur-Seine, France) and corticosteroids. Ciclosporin was given at an initial dose of 8 mg kg⁻¹ day⁻¹, starting after the sixth infusion of ALG, if serum creatinine was <250 μmol l⁻¹. A 48-h overlap between ciclosporin introduction and ALG discontinuation was maintained, in order to ensure adequate immunosuppression. The dose of ciclosporin was individually adjusted to reach trough concentrations of 150–250 ng ml⁻¹. Mycophenolate mofetil was administered orally prior to transplantation, at an initial dose of 2 g day⁻¹, subsequently adjusted according to clinical and haematological parameters. On the day of transplantation, 250 mg of methylprednisolone were infused before and after surgery, in the latter case before the start of ALG infusion. During the first week, 1 mg kg⁻¹ day⁻¹ of methylprednisolone or prednisolone were given before each ALG infusion.

Table 1

Characteristics of the patients

Patient	Gender	Age (years)	Weight (kg)	Number of infusions	Total dose (mg)	Initial disease
Prospective group						
P1	F	37	50	4	1600	Vesico-ureteral reflux
P2	M	23	65	7	3300	IgA nephropathy
P3	M	32	76	8	3900	Tubulointerstitial nephropathy
P4	M	45	57	6	2700	Nephroangiosclerosis
P5	M	52	67	7	3100	Glomerulonephritis
P6	M	37	75	8	3800	Focal and segmental hyalinosis
P7	F	41	71	5	2200	Glomerulonephritis
P8	M	56	78	7	3400	Congenital renal hypoplasia
P9	M	47	64	7	4200	Type II diabetes
P10	M	53	66	9	4500	Polycystic kidney disease
P11	F	41	52	12	4200	Alport syndrome
P12	F	33	50	7	2600	Alport's syndrome
P13	M	43	75	7	2400	Glomerulonephritis
P14	F	52	67	7	3600	Polycystic kidney disease
Mean (SD)		42 (9)	65 (10)	7.2 (1.8)	3250 (850)	
Retrospective group						
R1	F	66	54	8	3100	Nephroangiosclerosis
R2	M	27	69	5	1900	Tubulointerstitial nephropathy
R3	F	55	43	7	2400	Polycystic kidney disease
R4	M	65	71	8	2700	Type II diabetes
R5	F	41	59	5	1500	Glomerulonephritis
R6	F	31	53	4	1900	Type I diabetes
Mean (SD)		48 (17)	58 (10)	6.2 (1.7)	2250 (600)	
P value*	NS	NS	NS	NS	P = 0.026	

*Comparison between prospective and retrospective groups.

After day 7, the dose was progressively reduced. Before each ALG infusion, 5 mg of dexchlorpheniramine was administered.

Biological measurements

Antilymphocyte globulin serum concentrations At the time of the first infusion, ALG serum concentrations were measured before and 12 h and 18 h after the start of the first infusion, on days 4, 6, 8, 10 and 14 before the beginning of each ALG infusion and on days 30, 60 and 90 after the end of the first infusion. The number of available serum samples was reduced if horse ALG had to be discontinued because of side-effects. The day 30 sample was missing for patients P1, P4 and P7. Days 60 and 90 samples were missing for patients P1, P4, P6 and P7. Serum concentrations of horse ALG were measured by Imtix-SangStat (Marcy l'Etoile, France) using an enzyme-linked immunosorbant assay (ELISA) method. Briefly, the ELISA plates were coated with a goat antibody directed against equine IgG and incubated with diluted patient's serum (1 : 500, 1 : 1500 and 1 : 4500). The presence of ALG was revealed by a goat antihorse IgG labelled with peroxidase. The lower limit of quantification was 0.3 ng ml⁻¹.

To detect possible immunization against horse ALG, blood samples were drawn before the first infusion and on days 10, 14 and 30. Measurements of human IgG and IgM directed against ALG were done by an ELISA using goat antihuman IgG or IgM labelled with peroxidase.

Lymphocyte count In both the prospective and retrospective groups, the total lymphocyte count was measured before transplantation, at the end of the first infusion and every day from day 2 to day 14. For patients P1, P4, P7 and P14, who were switched to another ALG during the course of the study, lymphocyte data were truncated, the first data point to be removed being the one measured after the first infusion of the other ALG.

FCGR3A genotyping FCGR3A genotyping was performed using a real time allele-specific polymerase chain reaction assay based on SYBR Green fluorescence [12].

Pharmacokinetic and pharmacokinetic-pharmacodynamic modelling

Pharmacokinetic modelling Models with one, two or three compartments and first-order elimination from the central compartment were tested. Compartmental PK parameters, i.e. volumes and clearances of central (V_c and CL_c) and peripheral compartments (V_p and CL_p), and half-lives (distribution and elimination, $T_{1/2-\alpha}$ and $T_{1/2-\beta}$) were estimated. Both individual and population approaches were used. Separate modelling of the infusion period (i.e. from the beginning of the first to the end of the last infusion) and the postinfusion period showed that some estimated PK parameters were time dependent: notably, V_c was smaller during the infusion period than after. A population PK

model was therefore developed to take into account the decrease of V_c with time.

The interindividual variability in CL_c , V_p and CL_p was described using an exponential model as follows:

$$\theta_i = \theta \cdot \exp(\eta_i) \quad (1)$$

where θ_i is an individual parameter, θ is the mean (typical) parameter for the population and η_i is a random-effect parameter describing the interindividual variability. Volume of distribution of the central compartment at time t ($V_{c,t}$) was described as:

$$V_{c,t} = \left[V_{c,0} + \left(\frac{V_{c,1}}{t} \right) \right] \cdot \exp(\eta_i) \quad (t > 0) \quad (2)$$

where $V_{c,0}$ and $V_{c,1}$ are baseline and time-varying components of V_c , respectively. This equation describes a hyperbolic decrease in V_c with time. Other functions were tested (including exponential decrease), but they led to less satisfactory results.

In the long run, the parameters $V_{c,0}$, $T_{1/2} \cdot \alpha$ and $T_{1/2} \cdot \beta$ reach an asymptotic value. The expression $V_{c,0} + (V_{c,1}/t)$ tends to $V_{c,0}$, and $T_{1/2} \cdot \alpha$ and $T_{1/2} \cdot \beta$ tend to their 'real' values.

Pharmacokinetic–pharmacodynamic modelling PK–PD modelling of patients from the prospective and the retrospective groups was performed using indirect-response models. These models are suitable when drugs act indirectly by inhibiting or stimulating the production or the loss of endogenous elements that are biomarkers of drug response [13]. Stimulation or inhibition functions commonly used in indirect-response modelling are driven by drug blood concentrations. Since ALG leads to lymphocyte depletion, its effect may be described either by inhibition of lymphocyte input in the blood or by stimulation of lymphocyte elimination from the blood. The PD data were better described using indirect-response models with inhibition of lymphocyte input.

At the beginning of the infusion period, especially in the 48 h following the initiation of the first infusion, lymphocyte depletion was massive and seemed independent of ALG serum concentration. This may be explained by a nonlinear relationship between the amount of ALG bound to target cells (i.e. the 'effect site' concentration) and ALG serum concentration. Therefore, an inhibition function independent of ALG plasma concentration but dependent on time was added during the infusion period. The final indirect-response model with inhibition of lymphocyte input was:

$$\frac{dR}{dt} = k_{in} \cdot I_0(t) \cdot I(t) - k_{out} \cdot R \quad (3)$$

where R is blood lymphocyte count, k_{in} is the zero-order rate constant of the input of lymphocytes into the blood, k_{out} is the first-order rate constant of their elimination.

$I_0(t)$ and $I(t)$ are, respectively, time- and concentration-dependent inhibition functions as follows:

$$I_0(t) = 0 \quad (t = 0)$$

$$I_0(t) = \left(\frac{t}{T_{inf}} \right)^2 \quad (0 < t < T_{inf})$$

$$I_0(t) = 1 \quad (t = T_{inf})$$

$$I(t) = 1 - \frac{C_p^\gamma}{IC_{50}^\gamma + C_p^\gamma}$$

where IC_{50} is ALG blood concentration leading to 50% of maximum ALG-induced inhibition of input, γ is a shape factor and T_{inf} is the duration of the infusion period for each patient. At the beginning of the infusion period, $I_0(t)$ takes the value of 0, assuming a concentration-independent total lymphocyte depletion. During the infusion period, $I_0(t)$ increases towards a value of 1, following a quadratic function and, at the end of the infusion period, lymphocyte depletion is fully concentration dependent.

For the patients of the prospective group, the indirect response models used their own PK parameters. For the patients of the retrospective group, for whom no PK data were available, typical values of population PK parameters of the prospective group were used.

Modelling methods The comparisons of the different PK and PK–PD models tested were made by visual inspection of plots of observed and predicted dependent variable (either concentration or effect) vs. time and of plots of residuals vs. predicted variable and vs. time, and analysis of coefficients of variation (CV) of parameter estimations, interindividual CV of the estimated parameters, residuals sums of squares and Akaike's information criteria (AIC). Population PK analysis was based on the FOCE method, using WinNonMix 2.0.1 (Pharsight Corp., Mountain View, CA, USA). Individual PK–PD modelling was performed with WinNonLin professional 4.1 (Pharsight Corp.).

Statistical analysis

Characteristics of the patients of the retrospective and the prospective groups were compared using Mann–Whitney test. The PD parameters estimated in F/F patients were compared with those estimated in V carriers (V/V + V/F) using Mann–Whitney test. The PD parameters estimated in the prospective and in the retrospective groups, in F/F patients as well as in V carriers, were compared with the same test. Statistical analyses were carried out using Instat (GraphPad Software, San Diego, CA, USA).

Results

A total of 155 ALG blood concentrations were available in the 14 patients of the prospective group. None of the

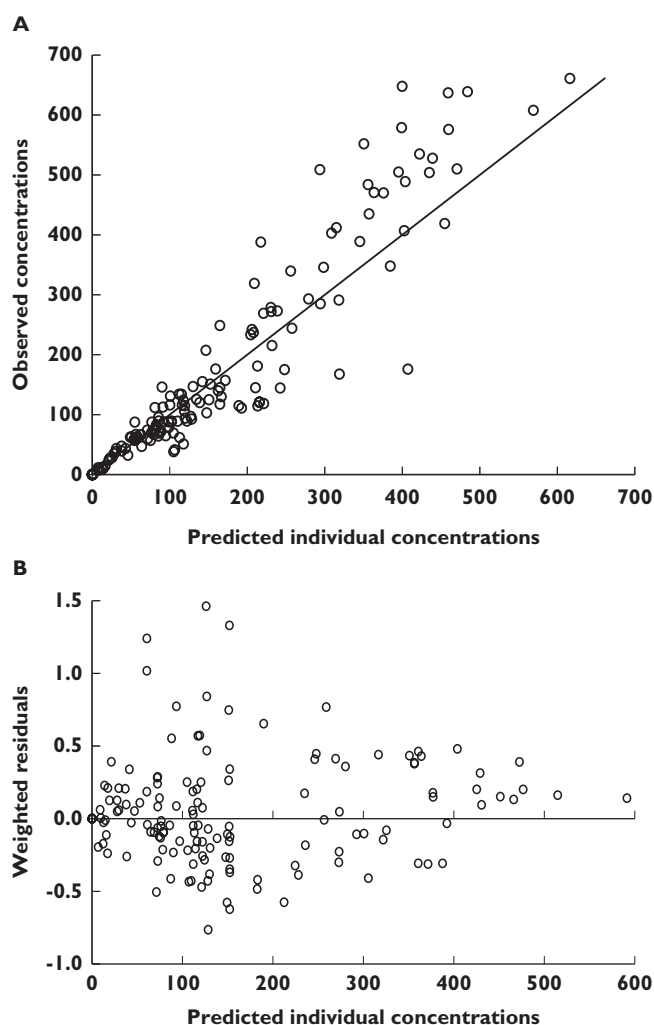


Figure 1

Individual model-predicted vs. observed concentrations (A) and weighted residuals vs. individual model-predicted concentrations (B)

patients developed IgG or IgM against horse ALG. The two-compartment PK model with a time-dependent V_c allowed a satisfactory description of ALG concentrations (Figures 1 and 2, Table 2). Asymptotic values of V_c (2.0 l) and $T_{1/2-\alpha}$ (1.3 days) were reached in approximately 6 days, whereas asymptotic value of $T_{1/2-\beta}$ (25 days) was reached in 4 days. An important interindividual variability was observed (Table 2). No influence of demographic characteristics or *FCGR3A* genotype on PK parameters was observed (data not shown).

The indirect response model with inhibition of lymphocyte input gave a satisfactory description of lymphocyte count over time (Figure 1) in 10 patients, showing high interindividual variability. One patient was *FCGR3A-V/V*, two were F/F and seven were heterozygous (Table 3, Figure 3). The F/F patients had a significantly higher IC_{50} than V carriers ($n = 10, P = 0.04$, Table 3). In four patients, the

PD parameters could not be estimated because of an insufficient number of lymphocyte counts; all were heterozygous. Patients in the retrospective group were similar to those in the prospective group, except for total dose, which was lower in the retrospective group (Table 1). In this group, three patients were F/F and three were V/F. No difference in estimated PD parameters was observed between the prospective and retrospective groups. A comparison of F/F and V carriers was therefore performed after pooling these two groups. The higher value of IC_{50} of F/F subjects compared with that of V carriers was confirmed ($n = 17, P = 0.008$, Table 3, Figure 3).

Discussion

A limited number of studies on ALG pharmacokinetics are available in the literature and only a few of them have studied horse ALG. Most studies of rabbit ALG PK have used log-linear regression and reported median $T_{1/2}$ values of around 14 days, with high interindividual variability [14–16]. Waller *et al.* reported mean $T_{1/2-\alpha}$ and $T_{1/2-\beta}$ of 14 days and 30 days, respectively [17]. Compartmental PK analysis was applied in only one study [18], reporting mean $T_{1/2-\alpha}$ and $T_{1/2-\beta}$ of 3 days and 18 days, respectively. In horse ALG, using log-linear regression, Schmidtke *et al.* [19] reported a median $T_{1/2}$ of 12 days, whereas Filo *et al.* [20] reported a $T_{1/2}$ ranging from 5 to 10 days. No compartmental modelling of horse ALG has been reported. In the present study, a satisfactory description of horse ALG concentrations was obtained using a population two-compartment model with first-order elimination from the central compartment and a time-varying central volume of distribution. Estimated asymptotic values of $T_{1/2-\alpha}$ and $T_{1/2-\beta}$ were 1.3 and 25.5 days, with interindividual coefficients of variation of 35 and 29%, respectively. Estimated $T_{1/2-\beta}$ is therefore longer than that previously reported [19, 20], but these studies used log-linear regression. The estimated typical asymptotic value of V_c was 2.0 l, which is half of the plasma volume.

The time-varying V_c observed in the present study may be explained by changes in the binding of ALG to their targets, i.e. lymphocytes but also other cells [21], with time. In the initial part of the infusion period, target antigens are in excess and ALGs seem therefore to ‘disappear’ from the serum, leading to an apparent very high volume of distribution. Later, when target cells are lysed and/or target antigens are coated by ALGs, the apparent volume of distribution of ALG decreases towards its value in the absence of target antigen. Such a time dependence of PK parameters of therapeutic antibodies has not been reported before using a compartmental approach, even if changes in elimination $T_{1/2}$ with time have been described. Studying multiple doses rabbit ALG, Guttman *et al.* [16] indeed reported a $T_{1/2}$ of 44 h during the first 24 h and a terminal $T_{1/2}$ of 14 days. However, when infusions are frequent

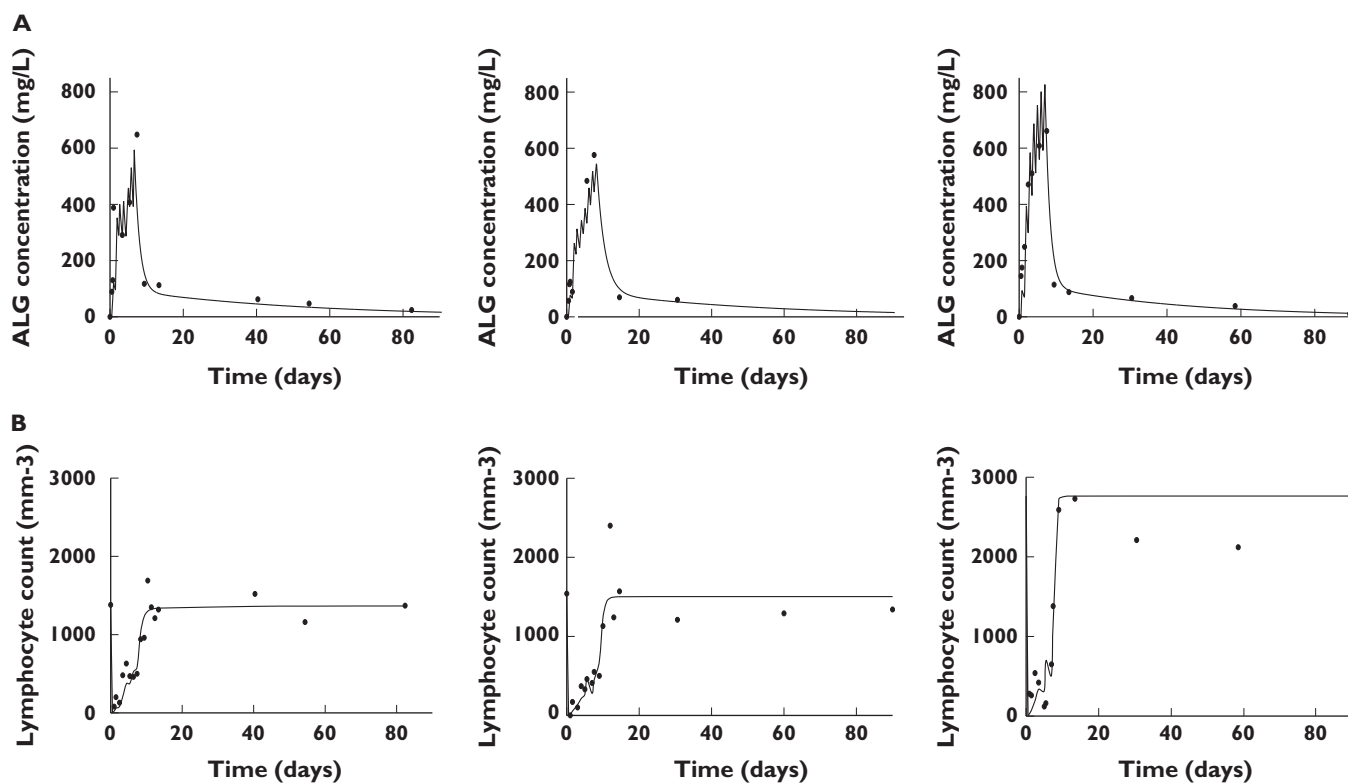


Figure 2

Observed (●) and model-predicted (lines) (A) antilymphocyte globulin (ALG) concentrations and (B) lymphocyte count, from left to right, in a V/V (P5), a V/F (P6) and a F/F (P9) patient. P5, P6 and P9 were administered 3100, 3800 and 4200 mg of horse ALG, respectively. The models used were: (A) a two-compartment model with first-order elimination and a time-varying central volume of distribution and (B) a physiological indirect response model with inhibition of input of lymphocytes in the circulation. This model combined concentration-dependent and -independent functions of inhibition of input

Table 2

Estimated antilymphocyte globulin pharmacokinetic parameters using a two-compartment model with a time-varying component for V_c

$n = 14$	Parameter	Typical value	Precision of estimation (%)*	Interindividual CV (%)
	$V_{c,t}$ (L.h)	22.4	5.6	40
	V_c (L)	2.0	3.5	40
	CL_c (L.h ⁻¹)	0.017	5.4	21
	V_p (L)	8.0	7.4	28
	CL_p (L.h ⁻¹)	0.022	5.9	18
	$T_{1/2-\alpha}$ (days)	1.3	6.0	35
	$T_{1/2-\beta}$ (days)	25.5	6.0	29

V_c and CL_c are volume of distribution and clearance of the central compartment, respectively, $V_{c,t}$ is the time-varying component of V_c , V_p and CL_p are volume of distribution and clearance of the peripheral compartment, respectively, $T_{1/2-\alpha}$ and $T_{1/2-\beta}$ are asymptotic distribution and elimination half-lives, respectively.

*CV percentage, Standard error of estimate/mean × 100.

compared with drug $T_{1/2}$, it may not be possible to differentiate the distribution phase from the terminal elimination phase without compartmental modelling.

The concentration–effect relationship was analysed using blood lymphocyte count, the biomarker used routinely to monitor the ALG effect. Using a physiological indirect response model, the best description was given by a

model describing an inhibition of lymphocyte input into the circulation. This could appear paradoxical, since ALGs act by inducing lymphocyte lysis. However, cytolysis may not be intravascular: if it takes place in tissues (notably in secondary lymphoid organs), inhibition of production is plausible, since circulating lymphocytes are in transit from one tissue or organ to another. In this case, it would not be

Table 3

Estimated antilymphocyte globulin pharmacodynamic parameters using an indirect response model with inhibition of lymphocyte input

Patient	Génotype <i>FCGR3A</i>	$K_{in}(\text{mm}^{-3}\cdot\text{h}^{-1})$	$K_{out}(\text{h}^{-1})$	$IC_{50}(\text{mg}\cdot\text{L}^{-1})$	gamma ($\text{mg}\cdot\text{L}^{-1}$)
Prospective group (n = 10)					
P2	V/F	8 476	3.94	302	2.9
P3	F/F	6 912	3.04	412	6.0
P5	V/V	6 902	5.05	359	2.8
P6	V/F	6 941	4.62	409	4.9
P9	F/F	9 924	3.59	674	4.3
P10	V/F	12 146	10.64	225	0.5
P11	V/F	3 415	3.03	276	2.9
P12	V/F	4 796	2.46	332	5.9
P13	V/F	5 251	3.75	140	1.1
P14	V/F	10 013	11.11	371	3.6
All genotypes		7 478 (2683)	5.12 (3.13)	350 (142)	3.5 (1.8)
F/F patients		8 418 (2130)	3.32 (0.39)	543 (186)	5.1 (1.2)
V-carriers		7 243 (2880)	5.57 (3.37)	302 (87)	3.1 (1.8)
p value*		0.71	0.40	0.04	0.18
Retrospective group (n = 6)					
R1	F/F	8 237	8.05	331	5.0
R2	F/F	2 622	6.42	748	0.3
R3	F/F	6 346	10.49	799	2.8
R4	V/F	8 041	9.94	143	1.0
R5	V/F	7 869	7.70	191	0.4
R6	V/F	4 318	6.02	410	1.8
All genotypes		6 239 (2310)	8.10 (1.81)	437 (278)	1.9 (1.8)
F/F patients		5 735 (2857)	8.32 (2.05)	626 (257)	2.7 (2.4)
V-carriers		6 743 (2102)	7.89 (1.97)	248 (143)	1.0 (0.7)
All patients (n = 16)					
All genotypes		7 013 (2546)	6.24 (3.03)	383 (199)	2.9 (1.9)
F/F patients		6 808 (2716)	6.32 (3.10)	593 (209)	3.7 (2.2)
V-carriers		7 106 (2597)	6.21 (3.15)	287 (100)	2.5 (1.8)
p value*		0.91	0.99	0.008	0.39

Results are expressed as mean (SD). K_{in} and k_{out} are constants of input and output, respectively. IC_{50} is the concentration leading to 50% of lymphocyte input inhibition. Values of IC_{50} estimated were not different between prospective and retrospective groups ($p = 0.38$).

*Comparison between F/F patients and V carriers.

actual inhibition of input, but rather destruction taking place upstream from the circulation.

Our PK–PD model took into account two phases of lymphocyte depletion, one not concentration dependent and taking place at the beginning of the infusion period, and the other concentration dependent, taking place later. Because of the rapid and massive capture of ALGs by their targets, serum concentrations measured during the initial part of the infusion period cannot reflect the concentration in the biophase, i.e. at the site of action. Therefore, the rapid lymphocyte depletion observed at the beginning of the treatment cannot be described as a function of measured ALG concentrations. When an equilibrium between serum concentration and concentration in the biophase is obtained, a concentration–effect relationship can be recovered.

Rapid lymphocyte depletion was observed by Preville *et al.* [4, 22, 23] in the cynomolgus monkey after administration of rabbit ALG and it was found to take place primarily in peripheral lymphoid tissues. Apoptosis and CDC seem to occur at high ALG concentrations, whereas ADCC would occur at low ALG concentrations [4, 22, 23].

Our results with horse ALG in kidney transplant patients confirm this rapid lymphocyte depletion.

Our PK–PD model allowed estimation of IC_{50} , a PD parameter which is an index of individual patient susceptibility to ALG. In the prospective group, the IC_{50} of *FCGR3A*-V carrier patients was significantly lower than that of F/F patients (Table 3, Figure 3), reflecting a higher potency of ALG in V carriers. To increase the number of patients and to validate the models, we chose to study also a retrospective group in whom ALG concentrations were simulated. The pooling of the two populations of patients confirmed the lower value of IC_{50} in V carriers compared with F/F patients (Table 3, Figure 3). In the representative examples displayed in Figure 2, one can observe that the F/F patient had a rapid recovery of his lymphocyte count despite higher dose and concentrations of ALG.

The better response of patients carrying the V allotype may be explained by the fact that IgG1 binds with higher affinity to the 158V allotype of $Fc\gamma RIIIa$ than to the 158F allotype, as shown for rituximab [24, 25]. In our study, the significant difference in IC_{50} may therefore be explained by a higher affinity of horse IgG for the human $Fc\gamma RIIIa$ -V allo-

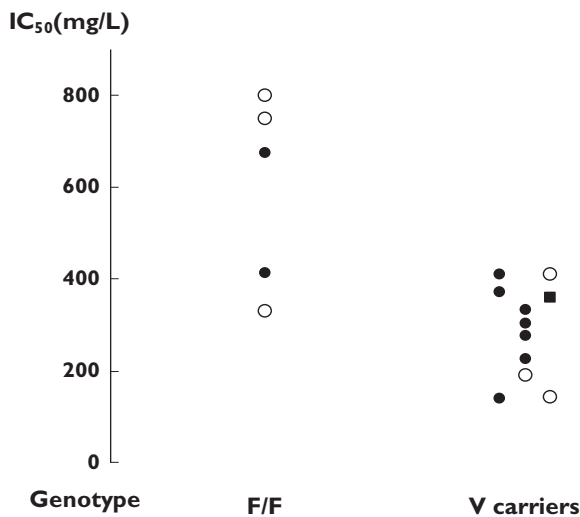


Figure 3

Estimated IC₅₀ of 10 patients from the prospective group (F/F and V/F patients are represented by ● and the V/V patient by ■) and six patients from the retrospective group (○) according to their FCGR3A-158V genotype

type than for the FcγRIIIa-F allotype. Affinities of human IgG1, IgG3 and IgG4 for human FcγRIIIa 158V are higher than those for FcγRIIIa-158F. Such an influence is plausible in horse, but would be difficult to show *in vitro*, because of the existence of several (at least seven) IgG subclasses in the horse [26]. An influence of FCGR3A genotype on the clinical response of non-Hodgkin's lymphoma patients to rituximab is well known [27]. This genetic polymorphism may influence the effect of other monoclonal antibodies, such as infliximab [28] and anti-rhesus D monoclonal antibodies [29], which act, at least partly, by cytotoxicity. The quantitative influence of FCGR3A polymorphism on the concentration-effect relationship of rituximab was studied using an *in vitro* ADCC model [25]. Maximal lysis, which could be obtained by increasing antibody concentrations, did not differ between V/V and F/F donors, but the concentration leading to 50% of maximal lysis (EC₅₀) was ≈ 4 times lower for V/V than for F/F donors. In the present study, we estimated an IC₅₀ of ALG effect on lymphocyte count twice lower in V-carriers than in F/F patients. This may be explained by the fact that V-carriers were mostly heterozygotes. Overall, our results suggest that the horse ALG mechanism of action on depletion of lymphocytes may depend on ADCC, especially at distance from the treatment, and that V-carriers may be more susceptible to the effect of this polyclonal antibody.

In conclusion, we have studied the concentration-effect relationship of ALG in kidney transplant patients using lymphocyte count as a biomarker, and a physiological indirect response model. The estimated value of IC₅₀, the parameter describing ALG potency, was found to be twice lower in FCGR3A-158V-carrier patients than in F/F

patients. FCGR3A-158V carriers may therefore be more susceptible to ALG than FCGR3A-158 F/F patients. Larger studies are needed to confirm these results.

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