ORIGINAL ARTICLE

The FcγRIIIA-158 VV genotype increased the risk of post-transplant lymphoproliferative disorder in T-cell-depleted kidney transplant recipients – a retrospective study

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ABSTRACT

Post-transplantation lymphoproliferative disorder (PTLD) is a severe complication in organ transplant recipients. The use of T lymphocyte-depleting antibodies (TLDAb), especially rabbit TLDAb, contributes to PTLD, and the V158F polymorphism of Fc gamma receptor IIIA (FcγRIIIA) also named CD16A could affect the concentration–effect relationship of TLDAb. We therefore investigated the association of this polymorphism with PTLD in kidney transplant recipients. We characterized the V158F polymorphism in two case–control cohorts (discovery, $n = 196$; validation, $n = 222$). Then, we evaluated the binding of rabbit IgG to human FcγRIIIA-158V and FcγRIIIA-158F. The V158F polymorphism was not linked to PTLD in the overall cohorts, but risk of PTLD was increased in VV homozygous recipients receiving TLDAb compared with F carriers in both cohorts, especially in recipients receiving TLDAb without muromonab (discovery: HR = 2.22 [1.03–4.76], $P = 0.043$, validation: HR = 1.75 [1.01–3.13], $P = 0.049$). *In vitro*, we found that the binding of rabbit IgG to human NK-cell FcγRIIIA was increased when cells expressed the 158-V versus the 158-F allotype. While the 158-V allotype of human FcγRIIIA binds rabbit immunoglobulin-G with higher affinity, the risk of PTLD was increased in homozygous VV kidney transplant recipients receiving polyclonal TLDAb.

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Introduction

Post-transplant lymphoproliferative disorder (PTLD) is the second most frequent malignancy in organ transplant recipients. The incidence of PTLD is increased in the first post-transplant year, but the risk remains beyond the first year [1-4]. Indeed, the 10-year cumulative incidence is close to 2%, about 10 to 20 times higher than in the general population [1,4,5]. The mortality and the risk of graft loss remain high after PTLD [5-7]. To prevent PTLD, we must identify individual pretransplantation PTLD risk factors. By now, the best described risk factors of PTLD are the seronegative EBV status at the time of transplantation and the immunosuppression that can affect the antiviral T-cell response [8,9]. However, their clinical significance remains relatively limited because a large majority of adult recipients is infected by EBV before the transplantation and the contribution of each immunosuppressive drug is difficult to determine.

Induction therapy using T lymphocyte-depleting antibodies (TLDAb) was found associated with increased incidence of PTLD [1,4,9,10]. The mechanisms underlying this risk remain unknown, although an impaired antiviral response after T-cell depletion is usually hypothesized from the following findings: (i) the risk of PTLD was found reduced after treatment with polyclonal TLDAb (pTLDAb) like rabbit antithymocyte globulin (rATG) or horse antilymphocyte globulin (ALG) versus mouse muromonab-CD3 antibodies, which induces greater T-cell depletion [11]; and (ii) a dose reduction in TLDAb in modern induction regimens has been associated with reduced PTLD incidence [4,9]. pTLDAb induces lymphocyte depletion in the peripheral blood primarily by complement-dependent cell lysis, activation-induced cell death, and antibody-dependent cell-mediated cytotoxicity (ADCC) [12], involving the Fc gamma receptor IIIA (FcγRIIIA). In humans, this low-affinity receptor for the Fc fragment of immunoglobulin G (IgG) links IgG-sensitized target cells to FcγRIIIA-bearing cytotoxic cells, such as natural killer (NK) cells and monocytes/macrophages leading to target cell lysis.

The *FCGR3A* gene, encoding the FcγRIIIA, features a functional allelic polymorphism (rs 396991) generating allotypes with a phenylalanine (F) or a valine (V) at amino acid position 176 [13,14]. The FcγRIIIA-158V allotype has a higher affinity for human IgG (especially IgG1 and IgG3), associated with enhanced clinical response to several therapeutic human IgG1 monoclonal antibodies [15-18]. Interestingly, Ternant *et al.* [19,20] recently reported that kidney transplant recipients with homozygous VV genotype exhibited higher T-cell depletion upon treatment with horse and rabbit pTLDAb. While it has been reported that the V158F polymorphism is not associated with altered risk of developing PTLD in organ transplant recipients without T-cell depletion [21], this has not been evaluated in recipients receiving pTLDAb, whose proportion is increasing [22]. The aim of the present study was therefore to assess the effect of the V158F polymorphism on the risk of PTLD in kidney transplant recipients, with a focus on those who have received pTLDAb.

Material and methods

Patients

Discovery cohort

Among kidney recipients who underwent renal transplantation in our unit between 1985 and 2015

($n = 2107$), we identified 52 patients with PTLD and DNA samples were available for 49 of them. Ethics committee approval was granted for the collection of DNA samples, and the study was approved by the Comité de Protection des Personnes Tours-Région Centre Ouest 1 (DC 2013-1780). Then, we selected three recipients without PTLD for each recipient with PTLD with the nearest date of transplantation because dosing strategies of ATG and ALG have greatly changed over time. Date of death, graft loss, or last visit was considered as the censoring date. Among the 196 matched recipients, 147 had received pTLDAb.

Validation cohort

We obtained DNA samples extracted from peripheral blood or tumoral tissue in 278 matched recipients (1:1) from a French multicentric case–control study limited to recipients receiving transplants since 1990 (Figure 1). Cases and controls were matched one-to-one on transplant centre, sex, age at transplantation (+/- 5 years), year of transplantation (+/− 1 year), graft order (1st, 2nd, or 3rd transplant), and pretransplant EBV status. To approximate time-to-event analysis, controls were selected to have the same time length of immunosuppression as the transplant-to-PTLD period in cases. We excluded 16 matched recipients already included in our discovery cohort, and genotyping was not feasible in 19 recipients (38 matched recipients were therefore excluded). Finally, we excluded 2 matched recipients because PTLD was recently diagnosed in a control recipient. Among the 222 matched recipients, 129 had received pTLDAb.

FCGR3A genotyping

Single-step multiplex allele-specific PCR assays were performed as previously described [23] with minor modification. The 25 µl reaction mixture contained 10 ng genomic DNA, 400 nM forward primer (5′-TCCA AAAGCCACACTCAAAGTC-3′), 400 nM reverse V allele primer (5′-AGACACATTTTTACTCCCATC-3′), and 200 nM reverse F allele primer (5′-GCGGGCAGGGC GGCGGGGGCGGGGCCGGTGATGTTCACAGTCTCT-GATCACACATTTTTACTCCCATA-3′), 400 µM of each dNTP, 2 mM $MgCl_2$, and 0.5 U Taq DNA polymerase in its buffer (Promega, Madison, WI, USA). PCR conditions were 3.5 min at 95°C followed by 35 cycles, each at 95°C for 20 s, 56°C for 20 s, and 72°C for 30 s. Next, PCR products (137- and 81-bp fragments for F and V alleles) were resolved on 8% acrylamide gel (Invitrogen) and visualized after ethidium bromide staining.

Effect of the V158F polymorphism on rabbit polyclonal IgG binding to human FcγRIIIA

CD16-transduced NK-92 cell lines (FcγRIIIA-158V or FcγRIIIA-158F) used to evaluate binding to FcγRIIIA were kindly provided by Dr. B. Clémenceau (INSERM CR 1232 CRCINA, Nantes, France). Cells were cultured in RPMI-1640 medium (Sigma, Albuquerque, NM, USA) supplemented with 10% fetal bovine serum (PAA Laboratories, Velizy-Villacoublay, France), 100 UI/ml interleukin 2 (Proleukin, Chiron Corp., Emeryville, CA, USA), 2 mM L-glutamine (Sigma), penicillin (100 UI/ml), and streptomycin (0.1 µg/ml) (Sigma) at 37°C under a humidified 5% CO_2 atmosphere. FITC-conjugated anti-CD16 monoclonal antibody (mAb) clone 3G8 and rabbit polyclonal IgG (pIgG) were from Beckman Coulter and Sigma, respectively. Tegeline® (human pIgG) was kindly provided by LFB.

The mAb 3G8 (mouse IgG1) recognizes an epitope within the binding site of human IgG. Therefore, it inhibits binding to FcγRIIIA and was previously used to evaluate the binding of rituximab to FcγRIIIA by flow
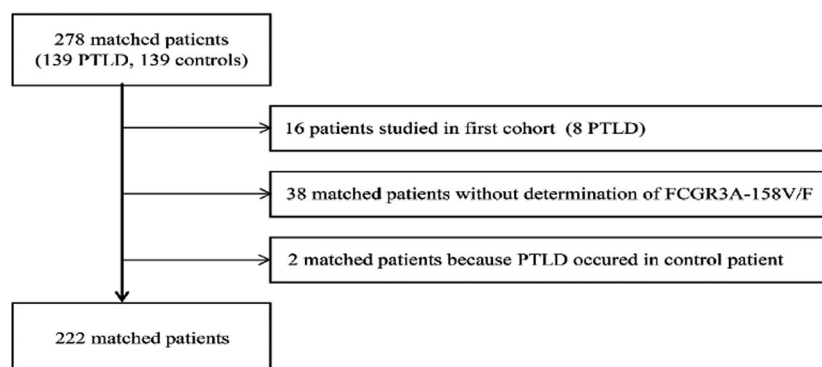
Figure 1 Flow chart (validation cohort). From 139 paired of kidney recipients enrolled into a French multicentric matched case–control study, 111 were finally eligible to build our validation cohort.

cytometry [24]. We evaluated the ability of rabbit or human pIgG to inhibit the binding of FITC-conjugated 3G8 to the FcγRIIIA-expressing NK-92 cell line. Briefly, CD16-transduced NK-92 cells (FcγRIIIA-158V or FcγRIIIA-158F) (2×10^5 in 100 μl) were incubated with FITC-conjugated 3G8 (dilution 1:100) for 30 min at 4°C. Cells were then incubated for 30 min at 4°C with 0.01 to 10 mg/ml of rabbit or human pIgG and analyzed by flow cytometry. All flow cytometry analyses were performed with 10 000 events with a Gallios flow cytometer and Kaluza 1·3 software (Beckman Coulter, Brea, CA, USA). The results are expressed as percentage inhibition of 3G8 binding: (% of 3G8-positive NK-92 FcγRIIIA-158V or FcγRIIIA-158F cells in the absence of pIgG − % of 3G8-positive NK-92 FcγRIIIA-158V or FcγRIIIA-158F cells in the presence of pIgG) × 100 ÷ % of 3G8-positive NK-92 FcγRIIIA-158V or FcγRIIIA-158F cells in the absence of pIgG.

Statistical analysis

Data are expressed as percentage or mean ± SD for normally distributed variables and median (range) for nonnormally distributed variables. Qualitative data were compared by chi-square test. Quantitative data were compared by Mann–Whitney U-test. A Cox regression was performed to identify the risk factors of PTLD in the discovery cohort. We successively assessed all recipients ($n = 196$), recipients receiving pTLDAb with ($n = 147$) or without ($n = 141$) muromonab-CD3 antibodies, which is no longer used but was known to represent a strong risk factor of PTLD. Second, we adjusted the association between V158F polymorphism and PTLD for recipient age, type of pTLDAb (rATG or ALG), and recipient EBV serology. We carried out a similar analysis with three successive groups in the validation cohort with an adjustment for recipient age and recipient EBV serology (but not duration and type of pTLDAb because this information was not available in the validation cohort). Results are expressed as hazard ratios (HRs) and 95% confidence intervals (95% CIs). We additionally generated Kaplan–Meier curves to compare actuarial PTLD-free survival (censored at 20 years) in VV, VF, and FF recipients from both cohorts receiving pTLAD without muromonab-CD3, in order to assess a potential dose effect of V15F polymorphism on risk of PTLD. Curves were compared using log-rank test. To evaluate the effect of the V158F polymorphism on rabbit pIgG binding to FcγRIIIA, simple linear regression was calculated by the method of least squares for each inhibition to compare the half-maximal inhibitory concentration (EC_{50}). Statistical analyses

involved the use of SAS v9.1 (SAS Inst., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the discovery and validation cohorts

The characteristics of cases (PTLD) and controls (no PTLD) in both cohorts are presented in Table 1. Cases and controls did not differ in age and had a similar proportion younger than 18 years in the discovery cohort (cases: 3/49, controls: 8/147, $P = 1$) and the validation cohort (cases: 1/111, controls:0/111, $P = 1$). Otherwise, cases and controls did not differ in demographic data, incidence of acute rejection, and maintenance immunosuppressive regimen, except for a slightly higher proportion of cases than controls receiving azathioprine in the validation cohort. Importantly, the proportion of patients seronegative for EBV before transplantation was not different in VV and F-carriers in discovery cohort (7.2% vs. 10.4%, $P = 1$) and validation cohort (13.5% vs. 12.4%, $P = 0.857$).

In the discovery cohort, 147 (75%) recipients received pTLDAb (rATG and/or ALG). Except for two recipients with steroid-resistant rejection, pTLDAb were used as induction therapy. Most recipients received rATG ($n = 137$, 93.2%), combined with ($n = 42$: cases = 11, controls = 31) or without ALG ($n = 95$, cases = 23, controls = 72). Ten recipients received ALG without rATG (cases = 2, controls = 8). Overall, cases and controls did not differ in the use of pTLDAb. In the validation cohort, 129 (58%) recipients received pTLDAb (rATG: 114), with a higher proportion in cases than in controls.

Increased risk of PTLD in homozygous VV kidney transplant recipients receiving pTLDAb

Discovery cohort

Among the 196 recipients, 67, 34, and 95 were homozygous for FcγRIIIA-158F (FF), homozygous for FcγRIIIA-158V (VV), and heterozygous (VF), respectively. Thus, the frequency of the V allele was 41.3%, respecting the Hardy–Weinberg equilibrium ($P = 0.997$). The median delay between transplantation and PTLD diagnosis was 4.9 years (range 0.1 to 20.5). On univariate analysis in the overall cohort, the V158F polymorphism was not significantly linked with PTLD and muromonab-CD3 antibody treatment was the sole

Table 1. Kidney transplant recipient characteristics.

	Discovery cohort (n = 196)			Validation cohort (n = 222)		
	PTLD (n = 49)	No PTLD (n = 147)	P-value	PTLD (n = 111)	No PTLD (n = 111)	P-value
Age at transplantation, years	44.5 [4.5–74.6]	44.9 [3.0–74.1]	0.492	49 [15–76]	49 [20–79]	0.477
Male recipient, number (%)	26 (53.1)	86 (58.5)	0.505	69 (62.2)	69 (62.2)	1
First transplantation, number (%)	45 (91.8)	137 (93.2)	0.753	99 (89.2)	98 (88.3)	0.832
Deceased donor, number (%)	48 (98.0)	145 (98.6)	1			
Acute rejection, number (%)	15 (30.6)	53 (36.1)	0.488	29 (26.1)	30 (27.1)	0.879
CMV mismatch (D + R-)	14 (28.6)	44 (29.9)	0.857			
Pretransplant recipients EBV seronegative*	5 (13.9)	4 (7.3)	0.474	15 (13.5)	13 (11.7)	0.686
HLA A, B, DR mismatches, number (%)†			0.333			0.263
0–1	3 (6.1)	8 (5.4)		9 (8.1)	9 (8.1)	
2–4	28 (57.1)	100 (68.0)		74 (66.7)	83 (74.8)	
5–6	18 (36.7)	39 (26.5)		24 (21.6)	15 (13.5)	
Year of transplantation			0.908			0.717
1985–1994	23 (46.9)	68 (46.3)		28 (25.2)	25 (22.5)	
1995–2004	17 (34.7)	53 (36.1)		60 (54.1)	66 (59.5)	
2005–2014	9 (18.4)	26 (17.7)		23 (20.7)	20 (18.0))	
Basiliximab (%)	6 (12.2)	29 (19.7)	0.236	24 (21.6)	30 (27.0)	0.879
Lymphocyte-depleting agents, number (%)						
Polyclonal (rATG and/or ALG)‡	36 (73.5)	111 (75.5)	0.775	73 (69.6%)	56 (54.9)	**0.030**
Muromonab	5 (10.2)	2 (1.4)	**0.011**	2 (1.8%)	0 (0.0)	0.498
Initial maintenance immunosuppressive treatments, number (%)						
Ciclosporine	38 (77.6)	122 (83.0)	0.394	78 (70.3)	80 (72.1)	0.767
Tacrolimus	10 (20.4)	23 (15.7)	0.440	29 (26.1)	26 (23.4)	0.641
Azathioprine	27 (55.1)	88 (59.9)	0.558	50 (45.0)	35 (31.5)	**0.038**
Mycophenolate mofetil	21 (42.9)	60 (40.8)	0.802	57 (51.3)	71 (64.0)	**0.021**
mTOR inhibitors	0 (0.0)	1 (0.7)	1	3 (2.7)	5 (4.5)	1
Steroids	49 (100)	147 (100)	1	108 (97.3)	109 (98.2)	0.722
Homozygous VV patients, number (%)	11 (22.4)	23 (15.6)	0.276	22 (19.8)	15 (13.5)	0.207

D+, cytomegalovirus-seropositive donor; R-, cytomegalovirus-seronegative recipient; EBV, Epstein–Barr virus; HLA: human leukocyte antigen; mTOR, mammalian target of ramamycin.

*Data missing in 105 patients from discovery cohort (PTLD: 13, No PTLD: 92).

†Data missing in 8 patients from validation cohort (PTLD: 4, No PTLD: 4).

‡38 patients (PTLD: 13, No PTLD: 25) did not receive induction in validation cohort. Bold is used for significant differences (p-value < 0.05)

Table 2. Risk factors of PTLD in discovery cohort according to the induction therapy

	HR	95% CI	*P*-value
All patients (*n* = 196)			
FCGRIIIA-158 VV (vs. F-carrier)	1.61	0.82–3.13	0.169
T lymphocyte-depleting agents (vs. no)	0.75	0.40–1.41	0.368
Muromonab-CD3 (vs. no)	**5.22**	**2.07–13.19**	**0.0005**
Recipient age (per year)	1.00	0.98–1.02	0.884
Patients treated by T lymphocyte-depleting agents (rATG and/or ALG, *n* = 147)			
FCGRIIIA-158 VV (vs. F-carrier)	**2.08**	**1.01–4.35**	**0.046**
Type of T lymphocyte-depleting agents (vs. ALG)	0.97	0.67–1.39	0.853
Duration of T lymphocyte-depleting agents (per day)	0.97	0.88–1.06	0.453
Muromonab-CD3 (vs. no)	**4.61**	**1.62–13.06**	**0.004**
Recipient age (per year)	1.00	0.98–1.02	0.973
Patients treated by T lymphocyte-depleting agents (rATG and/or ALG) without muromonab-CD3 (*n* = 141)			
FCGRIIIA-158 VV (vs. F-carrier)	**2.22**	**1.03–4.76**	**0.043**
Type of T lymphocyte-depleting agents (vs. ALG)	1.00	0.68–1.47	0.992
Duration of T lymphocyte-depleting agents (per year)	0.94	0.85–1.03	0.199
Recipient age (per year)	1.00	0.98–1.02	0.897

Bold is used for significant associations (p-value < 0.05)

risk factor of PTLD (Table 2). In 147 recipients receiving pTLDAb, we found that VV homozygous recipients had a higher risk of PTLD (HR = 2.08 [95% CI 1.01–4.35], *P* = 0.046), whereas muromonab-CD3 antibody treatment remained strongly associated with PTLD (HR = 4.61 [1.62–13.06], *P* = 0·004). Finally, the VV genotype was still associated with PTLD in the 141 recipients receiving pTLDAb without muromonab-CD3 antibodies as shown in Table 2 (HR = 2.22 [1.03–4.76], *P* = 0.043). This greater risk remained significant after adjustments on recipient age, type of pTLDAb, and recipient EBV serology (HR = 3.44 [1.19–11.11], *P* = 0.023).

Table 3. Risk of PTLD in homozygous VV kidney transplant recipients receiving T lymphocyte-depleting agents

	Validation cohort		
	HR	95% CI	*P*-value
Patients treated by T lymphocyte-depleting agents (rATG and/or ALG) (*n* = 129)			
FCGRIIIA-158 VV (vs. F-carrier)	1.69	0.97–2.94	0.0653
Muromonab-CD3 (vs. no)	**5.77**	**1.38–24.1**	**0.016**
Recipient age (per year)	1.01	0.99–1.02	0.593
Patients treated by T lymphocyte-depleting agents (rATG and/or ALG) without muromonab-CD3 (*n* = 127)			
FCGRIIIA-158 VV (vs. F-carrier)	**1.75**	**1.01–3.13**	**0.049**
Recipient age (per year)	1.00	0.99–1.02	0.562

Bold is used for significant associations (p-value < 0.05)

Validation cohort

Among the 222 recipients, 72, 37, and 113 were FF, VV, and VF, respectively. Thus, the frequency of the V allele was 42.1%, respecting Hardy–Weinberg equilibrium (*P* = 0.898). The time between transplantation and PTLD occurrence (6.49 [0.11–25.28] years) did not differ from that in the discovery cohort (*P* = 0.186). We confirmed on univariate analysis that the V158F polymorphism was not significantly linked with PTLD in the overall cohort (HR = 1.37 [0.78–2.56], *P* = 0.268), but was associated with PTLD in 129 recipients receiving pTLDAb (HR = 1.69 [0.97-2.94], P = 0.065), especially in 127 recipients who did not receive muromonab-CD3 antibodies (Table 3, HR = 1.75 [1.01–3.13], *P* = 0.049). This association remained significant after adjustments for recipient age and recipient EBV serology (HR = 1.92 [1.02–3.71], *P* = 0.044).

We additionally raised the question of V158F polymorphism dose effect in all patients receiving pTLDAb but not muromonab-CD3 antibodies (discovery and validation cohorts were pooled for this analysis, while we failed to show a difference between VV, VF, and FF in the discovery cohort). We assessed 48 VV (25 from discovery cohort/23 from validation cohort), 134 VF (67/67), and 86 FF (49/37) (distribution of patient in cases and controls is provided in supplementary data; Table S1). As shown in Figure 2, we observed that risk of PTLD was different in three groups (*P* = 0.026), with the highest risk observed in VV recipients (VV vs. VF: *P* = 0.029; VV vs. FF: *P* = 0.011; VV vs. F-carrier:
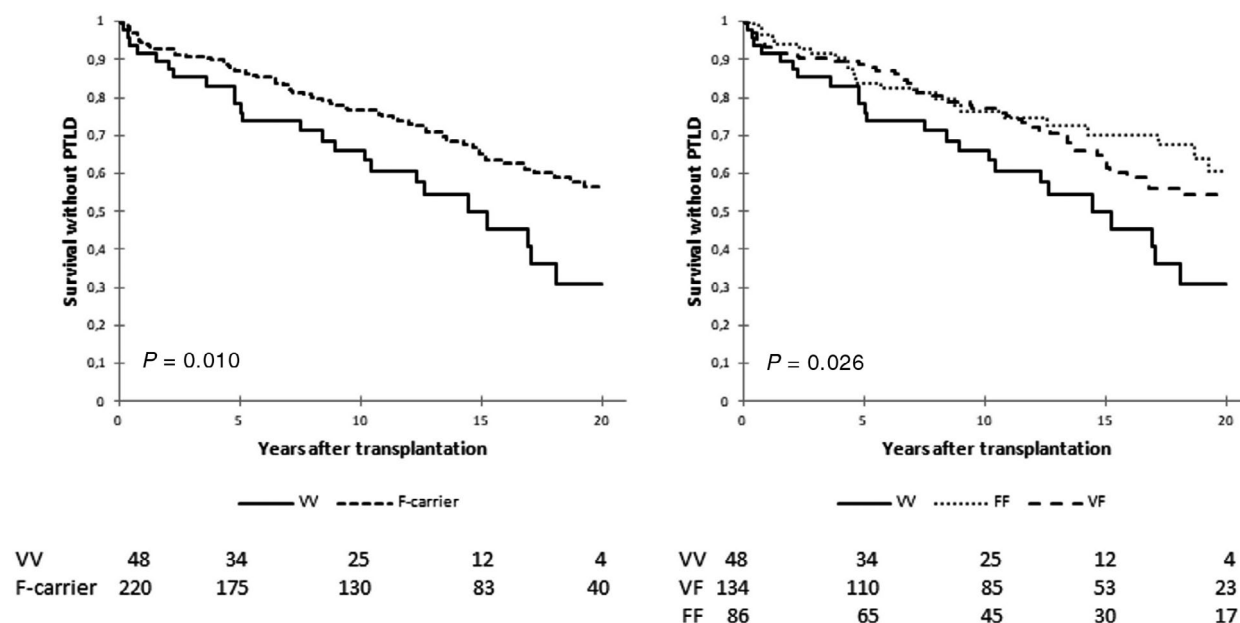
Figure 2 PTLD-free survival by V158F polymorphism in patients from both cohorts who received T lymphocyte-depleting antibodies but no muromonab. Patients were classified in two groups (VV and F-carriers, left panel) or three groups (VV, VF, and FF recipients, right panel). Kaplan–Meier plots were compared with log-rank test.

$P = 0.010$). Risk of PTLD in FF and VF recipients was not different ($P = 0.460$).

Characteristics of PTLD in discovery and validation cohorts

PTLD characteristics were quite similar in the two cohorts (Table 4A). The only difference concerned the localization of the tumor with a lower proportion of primary central nervous in the validation cohort PTLD, balanced by a higher percentage of PTLD within the kidney graft. We then assessed the PTLD characteristics by V158F polymorphism (Table 4B). We did not observe any differences in two cohorts with regard to the localization, the tumor EBV status and the cell type involved, that was almost exclusively B cells irrespective of the genotype. Additionally, the proportion of early-onset PTLD did not differ between VV homozygous and F-carrier recipients. Finally, PTLD characteristics did not differ by V158F polymorphism in recipients treated with pTLDAb (data not shown).

Effect of the V158F polymorphism on binding of rabbit pIgG to human FcγRIIIA

We wondered whether the association between the V158F polymorphism and the susceptibility to rATG in kidney transplant recipients may be related to a difference in rATG binding to human FcγRIIIA. To address

this question, we used our previously described flow cytometry assay [23] to compare the *in vitro* ability of rabbit pIgG to inhibit the binding of FITC-conjugated 3G8 mAb on the human NK-92 cell line expressing the V or F allotype of FcγRIIIA. rATG could not be used in this assay because it contains antibodies for human NK-cell membrane antigens. As expected and previously reported with rituximab and purified NK cells [23], we found a higher ability of human pIgG to inhibit the binding of 3G8 mAb to FcγRIIIA on NK-92 cells expressing the 158-V versus the 158-F allotype: 50% inhibition of 3G8 mAb binding (EC50) was achieved on average with 0.09 and 0.29 mg/ml pIgG ($n = 3$), respectively (Figure 3). Results obtained with rabbit pIgG were similar: 50% inhibition of 3G8 mAb binding to FcγRIIIA-158V- and FcγRIIIA-158F-expressing NK-92 cells was achieved with 0.04 and 0.2 mg/mL pIgG, respectively. Thus, these findings unambiguously demonstrated higher binding of rabbit IgG on NK-92 cells expressing the FcγRIIIA-158V compared with cells expressing the FcγRIIIA-158F allotype.

Discussion

In this study, we report for the first time that the V158F polymorphism of FcγRIIIA is an independent risk factor of PTLD in kidney transplant recipients after polyclonal T lymphocyte depletion therapy. Risk of PTLD was

Table 4. Comparison of post-transplant lymphoproliferative disorder (PTLD) characteristics between discovery and validation cohorts (A) and according to V158F polymorphism (B)

A

	Discovery cohort (n = 49)*	Validation cohort (n = 111)†	P-value
Early-onset PTLD, number (%)	6 (12.3)	24 (21.6)	0.161
Localization, number (%)			
Gastrointestinal tract	12 (24.5)	21 (21.4)	0.675
Lymph nodes	21 (42.9)	27 (27.6)	0.062
Primary central nervous	11 (22.4)	9 (9.2)	**0.027**
Graft	3 (6.1)	18 (18.4)	**0.049**
Hematopoietic organs	5 (10.2)	11 (11.2)	0.851
Other	12 (24.5)	23 (23.4)	0.891
Single site PTLD, number (%)	38 (77.6)	86 (73.0)	0.310
EBV-related, number (%)	26 (66.7)	41 (53.9)	0.190
Histologic data			
B-cell PTLD	44 (93.6)	94 (95.9)	0.682
T-cell PTLD	3 (6.1)	4 (4.1)	0.682

B

	Discovery cohort (n = 49)*			Validation cohort (n = 111)†			All patients (n = 160)		
	F-carrier n = 38	VV n = 11	P-value	F-carrier n = 89	VV n = 22	P-value	F-carrier n = 127	VV n = 33	P-value
Early-onset, number (%)	5 (13.2)	1 (9.1)	1	22 (24.7)	2 (9.1)	0.151	27 (21.3)	3 (9.1)	0.137
Localization, number (%)									
Gastrointestinal tract	10 (26.3)	2 (18.2)	0.708	19 (23.5)	2 (11.8)	0.352	29 (24.4)	4 (14.3)	0.319
Lymph nodes	17 (44.7)	4 (36.4)	0.737	21 (25.9)	7 (41.1)	0.377	38 (31.9)	11 (39.3)	0.458
Primary central nervous	9 (25.0)	2 (18.2)	1	9 (11.1)	0 (0.0)	0.352	18 (15.1)	2 (7.1)	0.367
Graft	2 (5.3)	1 (9.1)	0.542	15 (18.5)	3 (17.6)	1	17 (14.3)	4 (14.3)	1
Hematopoietic organs	3 (7.9)	2 (18.2)	0.311	9 (11.1)	2 (11.8)	1	12 (10.1)	4 (14.3)	0.508
Others	10 (27.8)	2 (18.2)	0.708	21 (25.9)	4 (23.5)	0.938	31 (26.1)	6 (21.4)	0.612
Single-site PTLD, number (%)	28 (73.7)	10 (90.9)	0.415	69 (83.1)	17 (89.5)	0.730	97 (80.2)	27 (90.0)	0.208
EBV-related, number (%)	21 (65.6)	5 (71.4)	1	35 (56.5)	6 (42.9)	0.389	56 (59.6)	11 (52.4)	0.546
B-cell PTLD†	35 (97.2)	9 (81.8)	0.132	76 (95.0)	18 (100.0)	1	111 (95.7)	27 (93.1)	0.561

Early-onset: PTLD occurring during the first post-transplant year.
*Missing data: tumoral EBV status (n = 10), histologic data (n = 2).
†Missing data: localization (n = 13), number of sites (n = 13), tumoral EBV status (n = 35), histologic data (n = 13).

Figure 3 Effect of the V158F polymorphism on rabbit pIgG binding to FcγRIIIA expressed on NK-92 cells. NK-92 cells transduced to express the FcγRIIIA-158V or the FcγRIIIA-158F allotype were incubated with a fixed concentration of FITC-conjugated anti-CD16 3G8 mAb, then varying concentrations of rabbit or human pIgG antibody for 30 min at 4°C for flow cytometry analysis.

increased twofold in homozygous VV recipients from our monocentric cohort and was confirmed in a larger and more recent multicentric cohort designed differently. [20] Accordingly, we demonstrated *in vitro* experiments that the FcγRIIIA 158-V allotype binds rabbit pIgG more efficiently than the 158-F allotype.

So far, studies that assessed the role of the V158F polymorphism on the PTLD course have focused on the outcome following the diagnosis and shown either an improved survival in VV patients treated or not with rituximab or the absence of influence of the V158F polymorphism on the response to rituximab [21,25]. Stern et al. failed to observe an association between the V158F polymorphism and the risk of PTLD, which might be considered as conflicting with our results at first glance [21]. However, we obtained similar results in our two cohorts when all patients (*i.e.,* who received or not pTLDAb) were analyzed. It is also of note that only 61 recipients received pTLDAb in the previous study. We therefore consider that results of Stern study are not conflicting with ours that restrict the effect of the V158F polymorphism on PTLD to patients with lymphocyte depletion therapy. As well, heart and liver-kidney transplant recipients were included in the study of Stern et al. The association between the V158F polymorphism and PTLD should be specifically assessed in such patients, who have a greater risk of PTLD than kidney transplant recipients [26]. Owing to the fact that the increased risk of PTLD in VV recipients is restricted to patients receiving pTLDAb, overwhelmingly rATG in the present study, our results suggest a link between T lymphocyte depletion and the occurrence of PTLD. We definitely demonstrated *in vitro* that the membrane FcγRIIIA-158V allotype bound rabbit IgG with a higher affinity than the FcγRIIIA-158F allotype, as was also

indirectly suggested in pharmacological population studies reporting that the depletion of CD3^{+} and CD4^{+} lymphocytes was extended with the number of V alleles [19,20]. This biological evidence of the functionality of the V158 polymorphism with rabbit IgG and the replication of our clinical results in a validation cohort are equally important to consider our results as reliable and compelling. Indeed, both steps missed in studies that established an association between the risk of PTLD and the presence of a polymorphism in HLA and cytokines (TNF-α, IL-28, IL-10, TGF-β) genes that have not been confirmed thereafter [27-30]. *FCGR3A* gene polymorphism has been associated with clinical responses to different cytolytic mAbs [14-16]. These pharmacogenetic studies and the present one demonstrate that FcγRIIIA-expressing cells are involved in the mechanism of action of these mAbs and of pTLDAb. In addition, an *in vitro* genotype–phenotype association has been observed: The *FCGR3A* polymorphism affects the affinity of human mAbs [23] and that of pTLDAb (present study) for FcγRIIIA and accordingly the level of ADCC mediated by NK cells *in vitro* [16,23]. Finally, kidney transplant recipients with homozygous VV genotype exhibited higher T-cell depletion upon treatment with horse and rabbit pTLDAb [19,20]. Taken together, these results suggest that the increased risk of PTLD observed in VV recipients may result from a deeper T-cell depletion related to enhanced pTLDAb-mediated ADCC favoring the emergence of lymphomatous cells. It can be highlighted that we did not observe a dose effect of polymorphism, suggested by pharmacologic study that showed an increase in sensitivity of patients to r-ATG treatment with the number of V alleles [20]. A similar behavior of VF and FF patients is classically observed with monoclonal therapeutic antibodies.

PTLD is very heterogeneous, and classically two types are considered based on the role of oncogenic EBV. The EBV-positive PTLDs represent 50–80% of cases. They can occur in EBV-positive recipients but are particularly frequent in EBV-negative recipients (mostly pediatric recipients) infected after the transplantation, especially by a graft harvested from a EBV-positive donor. Monitoring the EBV load is only recommended in EBV-negative recipients [31], while its usefulness in EBV-positive recipients remains debated [32,33]. As compared with EBV-driven PTLD, EBV-negative PTLDs occur more often later and feature some histological and genetic characteristics, while their proportion increases these last years [33–35]. However, the impact of PTLDs on graft and patient survivals did not seem to be affected by the EBV status of the tumor [34]. In our study, the association between the V158F polymorphism and the risk of PTLD was not affected after adjustment for the recipient EBV serology and the proportion of EBV-negative tumor was similar in VV and F-carrier recipients. Furthermore, we did not observe any differences regarding PTLD characteristics that could have suggested a predominant effect of the SNP on EBV-driven PTLD, in particular the proportion of early-onset disease. Determining the FcγRIIIA-V158F polymorphism before the transplantation may therefore be useful to assess the risk of both EBV-driven and EBV-negative PTLDs. Additionally, our study suggests that a deep T-cell depletion fosters the trigger of EBV-negative PTLDs that remained largely unknown [8]. Finally, because our cohort consisted mainly in EBV-seropositive adult patients, we conclude that the impact of genotyping in EBV-seronegative recipients remains to elucidate.

Importantly, we consider FcγRIIIA-V158F polymorphism characterization as clinically pertinent because VV recipients represented about 15% of our recipients, in agreement with frequencies in Caucasian individuals reported in previous studies but also because the minor allele frequency does not greatly change around the world, in Asian, Hispanic, or African populations [36,37]. It is known that pTLDAb are more efficient than interleukin-2 receptor antagonists to prevent rejection in recipients at high immunologic risk [31]. Therefore, we do not suggest to contraindicate use of pTLDAb in all VV recipients. However, genotyping may provide an important information to clinicians for recipients at lower immunologic risk for whom induction treatment and nondepleting interleukin-2 receptor antagonists could be used alternatively in F-carriers, whereas the former should be avoided in VV patients.

Our study has several limitations. First, the number of patients in the discovery cohort was small. However, results were confirmed in the second larger independent and differently designed cohort. Second, the analyses were performed retrospectively and some data were missing regarding the histology and the EBV status of the tumor. This weakness is nevertheless observed in the vast majority of studies regarding PTLD because it remains a rare disease (especially in kidney transplantation) occurring over a long period after the transplantation. Third, transplantation occurred over a long time, during which medical practices, particularly immunosuppressive strategies like dose and duration of TLDAb, have greatly changed. Therefore, we matched cases and controls on the date of transplantation in both cohorts. In addition, we observed that the V158F polymorphism increased the risk of PTLD in recipients with pTLDAb but not muromonab-CD3 treatment, which is no longer used. Fourth, the link between PTLD and the V158F polymorphism, which additionally affected the affinity of rabbit pIgG to FcγRIIIA, supports that the substantial post-transplant T-cell depletion may participate in subsequent development of PTLD. Nevertheless, a retrospective case-control study can never definitively establish a causal relationship. Unfortunately, the evaluation of T-cell depletion was not possible because CD3+, CD4+, and CD8 + T lymphocytes counts before and after transplantation were missing in a large proportion of patients.

In summary, we report an increased risk of post-transplantation lymphoproliferative disorder in VV homozygous *FCGR3A* kidney transplant recipients receiving pTLDAb, especially thymoglobulin, in two independent cohorts. These results may lead to a better evaluation of the benefit/risk balance of the use of depleting polyclonal antibodies in solid-organ transplantation and possibly reduce the incidence of PTLD.

Authorship

PG: performed literature search, figures, study design, data collection, data analysis, data interpretation, and writing. LL: involved in figures, data collection, data analysis, data interpretation, and writing. JS and DM: collected data. J-MH: carried out data analysis, data interpretation, and writing. SC: performed data interpretation and writing. SM: involved in data collection and data analysis. ML, EM, PM, ME, CV, NK, NB, P-FW, CM, MH, AT, IE, and MB: performed data collection and writing. PM: involved in study design, data collection, data analysis, and writing. VG-G: involved in study design, data analysis, and writing. GT: involved in

study design, data collection, data analysis, data interpretation, and writing.

Conflicts of interest

The authors of this manuscript have no conflicts of interest to disclose as described by *Transplant International*. The results presented in this paper have not been published previously in whole or part.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Distributon of patients receiving polyclonal T lymphocytedepleting antibodies without muromonab-CD3 by status (PTLD or niPTLD) in discovery and validaton cohorts.

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