ORIGINAL ARTICLE

IL-6 production by monocytes is associated with graft function decline in patients with borderline changes suspicious for acute T-cell-mediated rejection: a pilot study

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SUMMARY

Although borderline changes (BL) suspicious for acute T-cell-mediated rejection represent a diagnostic category, its clinical relevance is questioned leading to heterogeneous therapeutic management. We hypothesized that measuring IL-6 secretion by peripheral blood mononuclear cells identifies patients with ongoing graft damage. We examined the association between secreted IL-6 and the change in estimated glomerular filtration rate at 6 months after the biopsy (ΔeGFR). We then conducted phenotypic and functional studies on patient and mouse innate immune cells in the blood and the kidney. In a training set, ΔeGFR was strongly associated with IL-6 levels, showing a clinically meaningful decline of 4.6 ± 1.5 ml/min per increase in log10 IL-6 ($P = 0.001$). These results were consistent after adjustment and were reproduced in a validation cohort. Phenotyping of peripheral blood cells revealed that the main source of IL-6 was $CD14^{+}CD16^{-}CCR2^{+}HLA\text{-}DR^{+}CD86^{+}CD11c^{+}$ inflammatory monocytes. There was a significant correlation between IL-6 secretion and interstitial dendritic cell density in the biopsy. Finally, characterization of mouse kidney dendritic cells revealed that they share features with macrophages and function as effector cells secreting IL-6. In conclusion, measuring IL-6 secreted by peripheral blood cells can be useful in the management of patients with BL in the absence of a concurrent inflammatory condition.

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Introduction

Biopsies with borderline changes suspicious for acute T-cell-mediated rejection (BL) present a challenge for transplant physicians [1]. One of the mandates of the Banff renal working group on T-cell-mediated rejection (TCMR) is to determine whether the BL category should be modified to identify lesions specific for active TCMR [1]. From a clinical management standpoint, the BL category is of undetermined clinical significance [2]. Since 2005, the range of histological lesions that are classified as BL has been broadened and now varies from tubulitis (t) with minor interstitial inflammation (i), scored as t1-3 with i0-1, to mild t with variable i, scored as t1 with i2-3 [3,4]. More recently, the score of total inflammation in the allograft cortex, including scarred and nonscarred areas (ti score), has been shown to be a better predictor of graft outcomes than the "i" score, but as of yet has not replaced "i" score in the Banff diagnostic scheme [1,5–7]. Molecular phenotyping suggests that most cases of BL probably do not represent rejection [8]. However, as molecular and histological studies were performed on different samples (formalin-fixed samples for histological assessment versus frozen samples for molecular assessment), this issue is still not fully resolved [9].

Although it might prove difficult to identify a tissue-specific lesion for active TCMR, the characterization of other nonhistological signs of immunological activity in patients with BL could potentially be helpful. We have previously shown in different cohorts of kidney recipients that patients with rejection of any kind have systemic activation of the innate immune system, as witnessed by high levels of proinflammatory cytokines, such as IL-6, IL-1β, TNF-α, and MCP-1 (also referred to as CCL2) secreted *ex vivo* by peripheral blood mononuclear cells (PBMCs) [10–12]. In a validation set, IL-6 was shown to be the best predictor of acute rejection [10]. More recently, we demonstrated that dendritic cells (DC) cluster with T cells infiltrating the human allograft and observed direct contact between DCs and T cells by electron microscopy [13]. Furthermore, we found that high DC density in kidney allografts was associated with greater T-cell proliferation and with poor outcomes in patients with high total inflammation scores [13]. Whether or not there is a link between peripheral activation of the innate immune cells in the blood and those within the kidney is still not well defined.

In this study, we tested and validated whether measuring PBMC IL-6 secretion was prognostically useful to predict decline in graft function in patients once BL is confirmed by histology. We further characterized the subset of PBMCs responsible for IL-6 secretion, and then explored the association of IL-6 with DC and macrophage density in allograft biopsies with BL. Finally, we characterized mouse kidney DCs to examine their phenotypes and functions, in particular, their production of IL-6.

Materials and methods

Study design and population

This was an observational study with prospective collection of biological samples and clinical data in a testing set and an independent validation set. The testing set was obtained from a single-center cohort of 105 participants enrolled between January 2012 and June 2015. Patients were invited either to provide blood and urine sample at the time of an indication graft biopsy and follow-up samples at 3 months ($n = 55$) or to participate in a longitudinal sample collection at several time points post-transplant ($n = 50$). Of these, fifty-nine patients provided biological samples at the time of an indication biopsy (Fig. 1). Among them, 32 patients diagnosed with BL were included [7] using the threshold of t1i0, in line with the current classification. We excluded patients with complete criteria for antibody-mediated rejection (ABMR), infection or glomerulonephritis by light and electron microscopy. Twenty-six patients had BL without any sign of ABMR, whereas six had nondiagnostic components of ABMR. The validation set was assembled from patients enrolled at the Brigham and Women's Hospital and Quebec University Health Center ($n = 15$). No patients were lost to follow-up. A smaller independent study cohort of nine patients with BL was used for phenotypic characterization of cells in the biopsy [13]. The study was approved by the institutional ethics committees and is consistent with the Principles of the Declaration of Istanbul.

Pathologic classification

The biopsies were graded by the local attending pathologists according to the Banff 1997 criteria, which were updated in 2003, 2008, and 2013 [1,3,14]. Pathologists were blinded to the results of the cytokine measurements.

Definition of exposures and outcomes

The primary outcome was the change in estimated glomerular filtration rate (eGFR) at 6 months compared with eGFR at the time of the biopsy. ΔeGFR was calculated as $eGFR_{6\ month} - eGFR_{biopsy}$. Exposure was

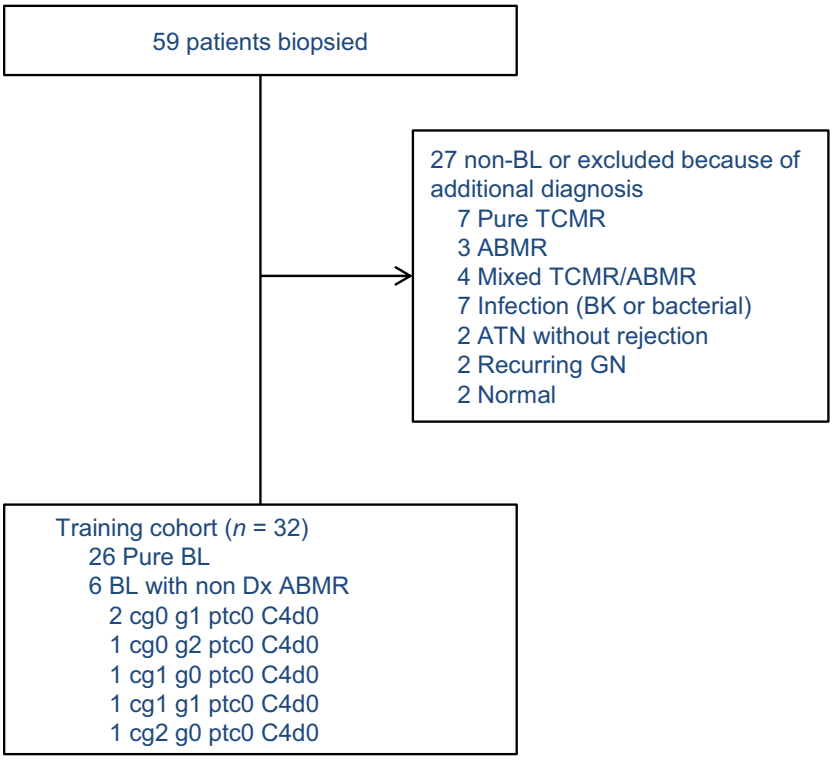


Figure 1 Study flowchart.

defined as the IL-6 levels at the time of the biopsy. Because IL-6 levels were not normally distributed, log10 transformation was used.

Cell isolation and cytokine assay

PBMC samples were isolated, frozen, stored, and thawed as described previously [10], then incubated for 24 h without stimulation in X-vivo 15 serum-free medium (Lonza, Walkersville, MD, USA) in triplicates in a 96-well plate, at a concentration of 5×10^6 cells/ml. IL-6 in cell supernatants was measured using a single ELISA (BioLegend, San Diego, CA, USA). In a second experiment, MCP-1, IL-1β, TNF-α, and INF-γ were measured using a multiplex ELISA (Aushon Biosystems, Billerica, MA, USA).

Flow cytometry of human PBMCs

Reconstituted cells were incubated and then stained as detailed in the Appendix S1.

Characterization of mouse DCs

Dendritic cell features and function were examined as detailed in the Appendix S1.

Statistical analyses

The association between ΔeGFR and IL-6 was assessed using Spearman correlation test and by linear regression models. The association between the primary outcome and MCP-1, IL-1β, and TNF-α was performed using multivariable linear regression models. Proportions of positive cell subsets were compared using Chi-square and Fisher's exact tests. Percentages of IL-6-positive versus IL-6-negative cells were compared by paired *t*-tests for each subset. Mice data were compared using *t*-test. Statistical analyses were performed using STATA version 11.0 (StataCorp, College Station, TX, USA) and graphs using SPSS STATISTICS v.23 (IBM, Armonk, NY, USA) and GRAPHPAD PRISM 5 (GraphPad Software, San Diego, CA, USA). All tests were two tailed, and a $P < 0.05$ was considered statistically significant.

Results

Training cohort and graft dysfunction at 6-month postbiopsy

Baseline demographic, clinical, and histological characteristics of the 32 patients studied in the training cohort are displayed in Table 1. Four patients received antirejection treatment following their biopsy: three patients

Table 1. Clinical characteristics of patients with borderline changes for T-cell-mediated rejection (TCMR).

	BL (n = 32)
Recipient	
Age (year)	47 ± 13
Male gender	12 (38)
First transplant	28 (88)
Time post-transplant (month)	37 [11,114]
Time on dialysis (month)	35 ± 28
eGFR at biopsy (ml/min)	52 ± 21
HLA A-B-DR mismatch	3.0 ± 1.4
HLA A mismatch	1.3 ± 0.8
HLA B mismatch	1.1 ± 0.6
HLA-DR mismatch	0.6 ± 0.6
Donor	
Age (year)	47 ± 15
Male gender	18 (56)
Donor type	
Deceased	26 (81)
Living—unrelated	2 (6)
Living—related	4 (13)
Immunosuppression at biopsy	
Induction	21 (66)
Number of maintenance agents	2.8 ± 0.5
Corticosteroids	32 (100)
Prednisone dose (mg)	7.4 ± 1.9
Calcineurin inhibitor	30 (94)
Cyclosporine	2 (6)
Tacrolimus	28 (88)
Tacrolimus T0 level (ng/dl)	4.7 ± 1.8
Antimetabolite	26 (81)
Mycophenolate	23 (72)
Mycophenolate dose (ng/dl)*	1326 ± 323
Azathioprine	3 (9)
Azathioprine dose (mg)	83 ± 29
Sirolimus	1 (3)
Biopsy scores	
T	1.2 ± 0.5
I	0.2 ± 0.4
V	0.0 ± 0.0
Ct	1.5 ± 0.7
Ci	1.6 ± 0.9
Cv	2.0 ± 1.0
Ah	1.9 ± 1.1
Mm	0.7 ± 0.9
Ti	0.7 ± 0.7
G	0.1 ± 0.4
Ptc	0.0 ± 0.0
Cg	0.1 ± 0.4
C4d	0.1 ± 0.2
t and i scores	
t1i0	25 (78)
t1i1	4 (13)
t2i1	1 (3)
t3i0	1 (3)
t3i1	1 (3)

Data are provided as mean ± standard deviation, n (%), or median [25th, 75th percentiles]. Comparisons were performed using unpaired t-test or Fisher's exact test. eGFR, estimated glomerular filtration rate. Biopsy scores are provided according to the Banff classification (scores 0–3): t, tubulitis; i, interstitial; v, intimal arteritis; ct, tubular atrophy; ci, interstitial fibrosis; cv, fibrous intimal thickening; mm, mesangial increase; inflammation; ah, arteriolar hyaline thickening; mm, mesangial increase; ti, interstitial inflammation in total parenchyma; g, glomerulitis; ptc, peritubular capillaritis; cg, allograft glomerulopathy; C4d, staining for the C4d component of the complement.

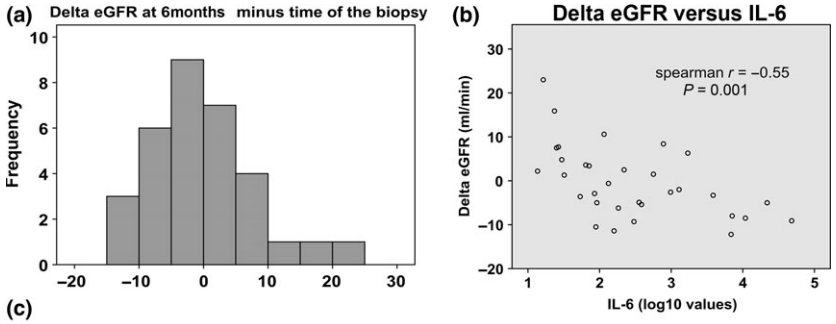
*In mycophenolate mofetil equivalent.

had an increase in serum creatinine level above 25% of their baseline value and received solumedrol (total dose 600 mg) and one patient who had a low mycophenolate mofetil dose at the time of diagnosis was increased from 1250 to 1750 mg daily. In all cases, urinary tract infection was ruled out by urine culture and SV40 staining was negative for polyomavirus nephropathy.

There was no graft loss during the follow-up period. The eGFR at 6 months compared with baseline (ΔeGFR) varied substantially between patients, from −12 to +23 ml/min (Fig. 2a). Seventeen patients (53%) suffered from a decline in eGFR. We conducted simple linear regression analyses to examine potential relationships between baseline characteristics (listed in Table 1) and ΔeGFR. The only finding was a nearly significant association with tacrolimus through level (P = 0.069). There was no evidence of a correlation between ΔeGFR and any other clinical or histological characteristic, in particular, neither with "t", "i" nor with "ti" scores (P = 0.86, P = 0.63, and P = 0.54, respectively, Table S1).

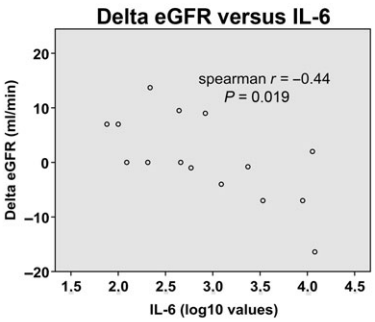
Association between ΔeGFR and IL-6 levels in cell culture supernatants

PBMCs collected at biopsy were cultured under resting conditions, and IL-6 levels were measured in supernatants. Because of the skewed distribution of the cytokine levels, we used log10 IL-6 values to examine their association with ΔeGFR. There was a significant raw negative correlation between ΔeGFR and IL-6 levels (σ = −0.55, P = 0.001; Fig. 2b). Consistent with these findings, the unadjusted linear regression model showed a significant relationship, in which, each increase in one log10 IL-6 was associated with a clinically meaningful decline in eGFR of 4.6 ± 1.5 ml/min (P = 0.001; Fig. 2c). This association was robust following progressive adjustment for initial eGFR at biopsy, antirejection



(c)

	β	SE	*P*
Log10 IL-6	–4.6	1.5	0.001
Adjusted model 1	–4.5	1.5	0.002
Adjusted model 2	–5.6	1.6	0.002
Adjusted model 3	–5.0	1.4	0.002



(e)

	β	SE	*P*
Log10 IL-6	–6.8	2.1	0.007
Adjusted model 1	–5.9	2.2	0.019
Adjusted model 2	–5.9	1.7	0.005
Adjusted model 3	–6.0	1.8	0.007

Figure 2 Estimated glomerular filtration rate (eGFR) and its relationship with IL-6 and other proinflammatory cytokines. (a) Distribution of the changes in eGFR at 6 months compared with eGFR at biopsy (ΔeGFR, eGFR at 6 month—eGFR at biopsy). (b) Dot plot of ΔeGFR versus log10 IL-6 values measured in cell culture supernatants in the training cohort. (c) Linear regression models for the prediction of ΔeGFR by log10 IL-6. The β coefficient quantifies the ΔeGFR per each increase in 1 log10 IL-6. Model 1 is adjusted for eGFR at the time of biopsy. Model 2 is adjusted for eGFR at the time of biopsy and for antirejection treatment following biopsy. Model 3 is adjusted for eGFR at the time of biopsy, antirejection treatment following biopsy, and time post-transplant. (d) Dot plot of the validation cohort. (e) Linear regression models of the validation cohort. β, beta coefficient; SE, standard error of the beta coefficient; *r*, correlation coefficient.

treatment, and time post-transplant (Fig. 2c and Table S2). We next sought to determine if there was an association between IL-6 levels and t, i, t + i, or ti scores and found no such evidence. In all, these findings indicate a strong link between a proinflammatory IL-6 state in the circulation and a decline in graft function at 6 months. They further suggest that IL-6 levels provide different, additional clinical information to the histological t and i scores.

To better assess the cellular activation in the clinical setting, we next compared IL-6 levels of BL patients to those of the non-BL patients and classified them under their clinical diagnosis and also under the following categories: overt rejection (TCMR, ABMR, or mixed rejection), infection, and noninflamed biopsies (acute tubular necrosis and normal biopsies; Fig. S1). Similar to our previous report [10], we found that patients with BL had a range of IL-6 levels encompassing both inflamed and noninflamed conditions (Fig. S1a,b). The mean (±SEM) Log10-IL6 was 2.4±0.2 vs. 2.8±0.4 for pure BL and non-diagnostic ABMR, respectively (p=0.30). These observations were consistent with the hypothesis that, whereas some BL have ongoing immune activation, others are immunologically quiescent.

Association between ΔeGFR and other proinflammatory cytokines or IFN-γ

Because we previously found an association, albeit of lower magnitude, between acute rejection and other cytokines, we examined these cytokines in the cell culture. We found a significant, but less robust, association between ΔeGFR and each of MCP-1 and IL-1β but not with TNF-α (Table S3). Because TCMR has been classically associated with IFNγ, we also quantified this cytokine in the supernatant. We found no signal for an association between IFNγ levels and ΔeGFR, neither by simple linear regression nor by multivariable linear regression ($P = 0.52$ and $P = 0.56$, respectively; data not shown). Taken together, these results suggest that the signal observed with IL-6 is not seen or only detected to a lower degree with other proinflammatory markers.

Stability of IL-6 production over time

Three-month follow-up blood samples were available for 19 patients. To determine the variability in IL-6 production over time, we studied these samples under the same conditions. During this time, all the patients kept a stable immunosuppression maintenance regimen and did not suffer from serious concurrent medical conditions, except two patients who had acute pyelonephritis and one who developed BK viremia. These diagnoses were made after the biopsy. These three patients were analyzed separately. The remaining patients were compared with six controls, who underwent protocol biopsies showing no significant allograft inflammation (t0i0) and from whom blood was available at the time of the biopsy and 3 months thereafter. As displayed in Fig. S2a, IL-6 levels in controls were fairly stable over time, with a mean (SD) change of −0.11 (0.40) log10 value between the 3-month and baseline time points. Patients with BL had a large range of between-patient variation in IL-6 levels (from −1.48 to 1.78 log10 value) although the within-patient variations were minimal (Fig. S2b). The three patients with infection at the 3-month time point had a marked increase in IL-6 levels [mean (SE) 1.76 (0.42) log10 value from baseline, $P = 0.002$; Fig. S2c]. In all, these data indicate that the secretion of IL-6 by PBMCs *ex vivo* was relatively stable over time, except for patients who developed an active infection.

External validation

To validate these observations, we assembled an independent, multicenter cohort of 15 patients with BL on indication biopsies. First, the data from this set confirmed that IL-6 in patients with BL varies widely, from 1.9 to 4.1 log values (Fig. 2d). Second, we observed a similar association between ΔeGFR and log IL-6 and found that this relationship was of similar magnitude both in the unadjusted and adjusted models (-6.8 ± 2.1 and -6.0 ± 1.8 ml/min per logIL-6 for unadjusted and fully adjusted models, respectively, $P = 0.007$ and $P = 0.007$, respectively; Fig. 2d,e and Table S4).

Phenotype of IL-6-secreting cells

We next sought to examine the precise cell subset responsible for IL-6 secretion. We first coupled intracellular IL-6 staining to surface staining for T ($CD3^+$) cells, B ($CD19^+$) cells, monocytes ($CD14^+$), and DCs ($CD14^-CD11c^+$) [15]. Whereas only a minimal fraction of T and B cells expressed IL-6, a large proportion of monocytes and DCs expressed the cytokine (Fig. 3a,b). To better characterize the innate cell subset responsible for IL-6 secretion, we used a multicolor panel and found that compared with IL-6-negative cells, IL-6-positive cells were predominantly $CD14^+CD16^-CCR2^+HLA\text{-}DR^+CD86^+CD11c^+$, which can be termed inflammatory monocytes (Fig. 3c,d). Collectively, our data indicate that these cells are the main source of IL-6 in the peripheral blood of kidney recipients with BL.

To further study the relationship between IL-6 secretion of PBMC and innate immune cells within the graft, we analyzed an independent set of patients with BL and investigated the correlation between IL-6 secretion by PBMCs and the frequency of interstitial monocytes/macrophages (CD68) and DCs (CD209) in the biopsy ($n = 9$). Interestingly, we found a strong correlation between IL-6 levels and the frequency of DCs ($\sigma = 0.71$, $P = 0.03$) but not with macrophages ($\sigma = 0.35$, $P = 0.36$; data not shown).

Phenotypic and functional characterization of kidney DCs

This led us to explore the potential link between IL-6-secreting monocytes in the blood with BL and innate immune cells within the kidney. Monocytes are composed of a heterogeneous population of circulating blood cells belonging to the mononuclear phagocyte system [16]. Once migrated in the tissue, they differentiate into effector cells [15]. Although the nomenclature for these cells is still controversial, they can be termed monocyte-derived macrophages or DCs [17,18]. Here, we used the C57Bl/6 and $CX3CR1^{GFP+}$ mice and examined the morphology of these cells and their secretion of IL-6. First, we observed that DCs' resident in the kidney was smaller than splenic DCs and as demonstrated previously [19], had a higher percentage of $CX3CR1^+F4/80^+$ cells, suggesting phenotypic features of macrophages (Fig. 4a,b). Second, kidney DCs demonstrated a higher secretion capacity for IL-6, a lower capacity for proliferation of CFSE-labeled $CD8^+$ and $CD4^+$ T cells, and a lower production of IL-2 in mixed lymphocyte reaction (MLR) supernatants, compared with splenic DCs (Fig. 4c–e). These results were confirmed in an antigen-specific model in which kidney and splenic DCs were incubated with OVA-1 peptide for 3 h and cocultured with $CD8^+$ T cells from OT-I mice (OVA-specific TCR transgenic mice of C57Bl/6 background in which OT-I $CD8^+$ T cells recognize OVA-I peptides) (data not shown). In all, these results indicate that kidney DCs share features with macrophages and function as effector cells secreting IL-6.

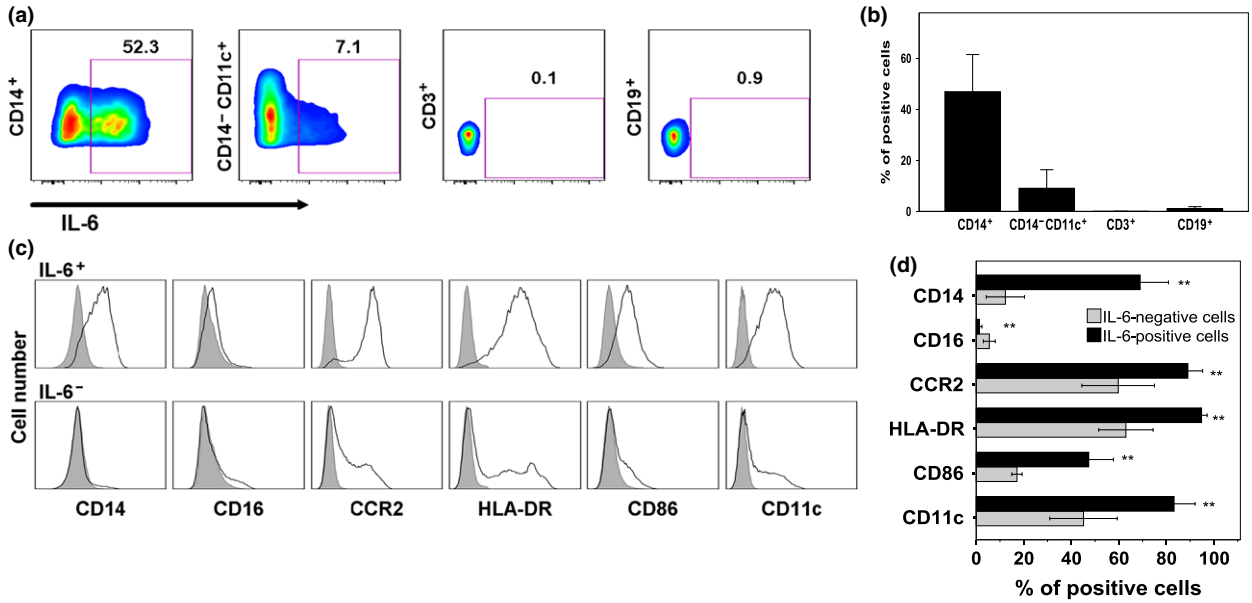


Figure 3 Characterization of peripheral blood mononuclear cells (PBMCs) secreting IL-6. (a) Representative example of the percentages of cells secreting IL-6 in the T cells ($CD3^+$), B cells ($CD19^+$), monocytes ($CD14^+$), and dendritic cells (DCs) ($CD14^-$ $CD11c^+$) subsets. (b) Summarized data of A (mean ± SD). (c) Surface phenotype of IL-6-positive and IL-6-negative PBMCs. Gray shadowed histograms indicate isotype control, and black line histograms indicate markers of interest. (d) Summarized data of c (mean ± SD, $n = 7$; $^{**}P < 0.01$ by paired *t*-test).

Discussion

In this study, we present a cohort of patients with BL for whom we performed a detailed examination of the PBMC inflammatory profile at the time of the biopsy. We analyzed the correlation between IL-6 and progressive graft dysfunction at 6 months. The first relevant finding is that ΔeGFR of these patients varied substantially within this period. Second, there was a strong and clinically relevant association between IL-6 secretion by PBMCs at the time of biopsy and decline in graft function at 6 months. We validated this finding in an external cohort. Although the relevance of the BL category is being questioned, our findings indicate that a marker of PBMC activation, such as IL-6, may provide the missing information to identify patients with ongoing tissue damage among this population. The cells that secreted IL-6 in highest proportion were the classic-type monocytes and DCs [20].

The presence of activated monocytes in the blood of rejectors is reminiscent of the role of these cells following infection, during which there is an increase in circulating immature monocytes, emerging from the bone marrow in a CCR2-dependent fashion [21]. Expression of CCR2 on $CD14^+CD16^-$ circulating monocytes leads to higher migration responsiveness for MCP-1/CCL2 at injured sites, specifically on the endothelial cells [16,21,22]. These observations extend recent findings that we reported on the relationship between high intragraft DC density, high histological inflammation scores, and poor graft prognosis [13]. We observed that, whereas DCs were of donor origin early post-transplant, the proportion progressively decline during the first-year post-transplant [13].

Our findings are also consistent with recent gene expression profile microarray data from renal biopsies of patients with BL. Hruba *et al.* [23] observed and validated that donor age and expression of the macrophage receptor C-type lectin domain family 5 member A (CLEC5A) were independent factors of progression in patients with BL. CLEC5A expression is prominent on recruited blood monocytes that enter the tissue upon inflammation and differentiate into proinflammatory cells [24]. Human CLEC5A+ cells express CD14 and CD11c surface markers and secrete proinflammatory cytokines such as TNF-α and IL-6 [24,25]. These data support the hypothesis that, during BL, recruited blood monocytes differentiate intragraft into inflammatory innate immune cells, which is in continuum with the observations that we made here in the peripheral blood.

It is tempting to speculate that the IL-6 signal observed here is not only a marker of disease activity, but could be a potential therapeutic target. Anti-IL-6 is currently under investigation to prevent damage in inflamed allografts and has been shown to be potentially useful to treat chronic ABMR [26,27]. In an elegant study, Oberbarnscheidt *et al.* [28] demonstrated that

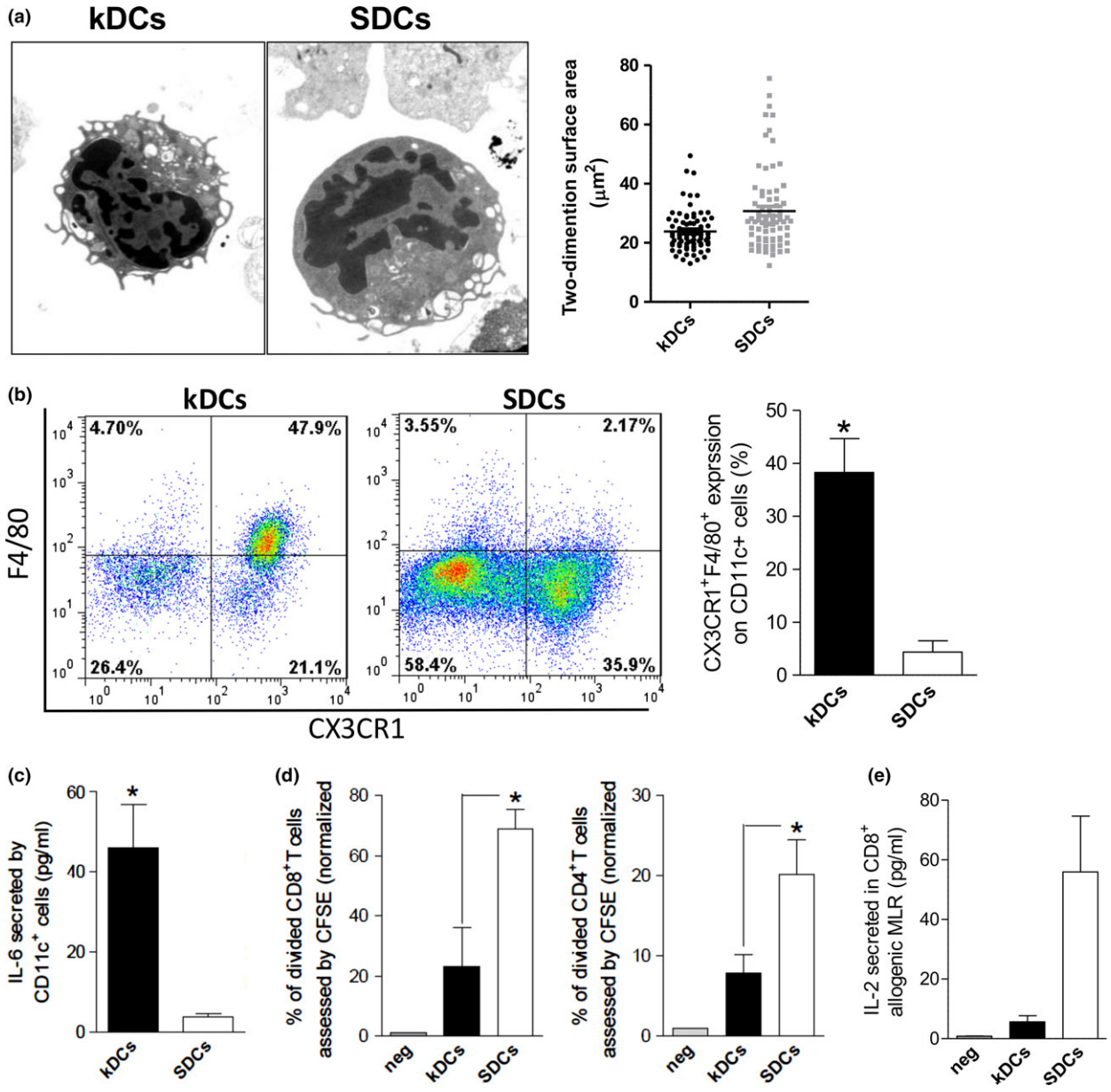


Figure 4 Characterization of kidney dendritic cells (DCs). (a) By morphometric analysis of the two-dimensional surface areas, kidney DCs were found to be smaller than splenic DCs (electron microscopy, original magnification; ×10 000). Notably, the average number of cytoplasmic protrusions and the average number of lysosomes were not significantly different (data not shown). (b) DCs were isolated from the kidneys and spleens of CX3CR1^{GFP+} mice and assessed by flow cytometry. Kidney DCs showed higher percentage of CX3CR1^{+}F4/80^{+} macrophage-like DCs compared with splenic DCs ($P = 0.03$, $n \geq 2$ in each group). (c) IL-6 was assessed in the supernatant of DCs culture using Luminex assay; levels were increased in kidney DCs compared with splenic DCs (*$P = 0.02$, $n = 3$ in each group). (d) Kidney DCs or splenic DCs from C57Bl/6 mice were cocultured with CD8^{+} or CD4^{+} T cells from BALB/c mice. After 72 h, flow cytometry evaluation revealed decreased proliferation of CFSE-labeled CD8^{+} and CD4^{+} T cells cocultured with kidney DCs compared with splenic DCs (*$P = 0.02$, $n = 3$ in each group) (e) A 10-fold lower IL-2 levels were observed when CD8^{+} T cells were cocultured with kidney DCs compared with splenic DCs ($n = 2$ in each group).

recognition of allogeneic grafts can elicit differentiation of monocytes into mature DCs, which, in turn, stimulate T-cell proliferation, underscoring the role of monocytes in initiating rejection. It is conceivable that, under standard immunosuppression, this mechanism leads to a smoldering rejection, which does not produce a full-blown TCMR, but rather BL changes leading to interstitial fibrosis and tubular atrophy. However, the data presented here cannot confirm the causative role of IL-6 in the pathogenesis of rejection.

There are some limitations to this study. First, the sample size is small but comparable to recent studies

examining gene expression profile in patients with BL [23]. Furthermore, the magnitude of the association observed between ΔeGFR and IL-6 levels is clinically relevant. Second, the follow-up period is relatively short. Perhaps the best endpoint to conclusively demonstrate the impact of peripheral inflammation in patients with BL will be a combination of decline in graft function and progressive chronic allograft damage over time. It is plausible that a sizeable proportion of BL cases will not lead to TCMR but contribute to long-term graft damage.

The observations reported in this article suggest that secretion of IL-6 by PBMCs may be a marker of ongoing tissue damage that could indicate, among patients with BL, who would benefit from increased surveillance and perhaps antirejection treatment. The mechanistic data on kidney DCs further suggest that they might originate from an IL-6-secreting blood monocyte precursor, providing the impetus to determine the role of these cells and anti-IL-6 treatment in patients with BL.

Authorship

SADS, IB and OD: designed the study. SADS, IB, MA and NN: enrolled the patients. OD, SB and PV: conducted the experiments supervised by SADS and IB. IB, JR and EL: analyzed the biopsies. SADS, OD and IB: analyzed the data. All authors: discussed and interpreted the results. OD, SADS, IB and AC: wrote the manuscript.

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Conflict of interest

The authors of this manuscript have no conflict of interests to disclose as described by *Transplant International*. The results presented in this paper have not been published previously in whole or part, except in abstract format including an oral communication during the Société Francophone de Transplantation meeting in Liège, December 2016.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Comparison of IL-6 levels between subcategories.

Figure S2. Variation in IL-6 levels over time.

Table S1. Simple linear regression models for the prediction of DeGFR by t, i and ti scores.

Table S2. Detailed linear regression models for the prediction of DeGFR by log10 IL-6.

Table S3. Simple linear regression models for the prediction of DeGFR by MCP-1, IL-1b and TNF-a levels in the culture supernatants.

Table S4. Detailed linear regression models for the prediction of DeGFR by log10 IL-6 in the validation cohort.

Appendix S1. Complete methods.

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