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Elevated Pretransplantation Soluble BAFF Is Associated With an Increased Risk of Acute Antibody-Mediated Rejection

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Abstract

Background—B cells play an important role in renal allograft pathology, particularly in acute and chronic antibody-mediated rejection (AMR). B-cell activating factor belonging to the tumor necrosis factor family (BAFF; also known as BLyS) is a cytokine that enhances B-cell survival and proliferation.

Methods—We analyzed serum BAFF levels in 32 patients undergoing antibody-incompatible (Ai) renal transplantation and 319 antibody-compatible transplant recipients and sought to determine whether there was a correlation with acute rejection and with transplant function and survival.

Results—We demonstrate that, in patients undergoing Ai transplantation, elevated serum BAFF levels at baseline (before both antibody removal/desensitization and transplantation) are associated with an increased risk of subsequent AMR. In antibody-compatible transplant recipients at lower risk of AMR, no statistically significant association was observed between pretransplantation serum BAFF and AMR.

Conclusions—These data raise the possibility that, in high immunologic risk patients undergoing Ai transplantation, the presence of elevated pretransplantation serum BAFF might identify those at increased risk of AMR. BAFF neutralization may be an interesting therapeutic

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strategy to explore in these patients, particularly because such agents are available and have already been used in the treatment of autoimmunity.

Keywords

B cells; BAFF; Antibody-mediated rejection; Antibody-incompatible transplantation

There is increasing interest in B cells in transplantation, with studies indicating a role in acute and chronic antibody-mediated rejection (AMR) and transplant tolerance (1–3). B-cell activating factor belonging to the tumor necrosis factor family (BAFF; also known as BLyS, TALL-1, and THANK) is a cytokine that enhances B-cell survival and proliferation (4). It exists in both a membrane-bound form and as a soluble trimer and is produced by monocytes, macrophages, dendritic cells (DCs), and, to a lesser extent, T cells (4, 5). There are three BAFF receptors: BAFF-R (also known as BR3), TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor), and BCMA (B-cell maturation antigen) (4). These are principally expressed on cells of the B-cell lineage, including plasma cells (6). In addition, BAFF-R is expressed on some activated and regulatory T cells (6, 7). The cytokine APRIL (a proliferation-inducing ligand) also uses TACI and BCMA.

The importance of BAFF in B-cell biology and B-cell-mediated autoimmune diseases has been demonstrated by studies in humans and mice. Transgenic mice that overexpress BAFF have increased B-cell survival, hypergammaglobulinemia, and pathogenic autoantibodies (which arise independently of T-cell help) and spontaneously develop a lupus-like autoimmune disease (8). Furthermore, elevated serum BAFF levels have been described in patients with autoantibody-associated autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjogren's syndrome, and idiopathic thrombocytopenic purpura (9–11).

Studies of BAFF in the context of solid-organ transplantation are less frequent; in a murine cardiac allograft model, BAFF-deficient recipients had extended transplant survival, particularly if a calcineurin inhibitor was administered (7). The deleterious effect of BAFF on allograft survival in this model was dependent on BAFF-R and independent of TACI and BCMA. In a murine islet allograft model, BAFF blockade with a monoclonal antibody (in combination with rapamycin at induction) resulted in long-term survival of major histocompatibility complex-disparate islet allografts (12). In humans, BAFF has been identified by immunohistochemistry in renal transplant biopsies with acute rejection and appeared to correlate with C4d deposition (13). In a small study of long-term renal allograft recipients, the presence of BAFF-positive CD4 T cells in peripheral blood was associated with worse transplant function (14) and elevated BAFF mRNA and protein have been noted in kidneys explanted for chronic rejection, with infiltrating mononuclear cells the principle source (15). BAFF-R-positive B and T cells have also been identified in human renal allografts with chronic rejection (7). More recently, Thibault-Espitia et al. reported that patients undergoing antibody-compatible (Ac) transplantation with higher circulating soluble BAFF had a significantly greater risk of developing donor-specific antibodies (DSA) and that, in patients with stable graft function, high BAFF-R mRNA in peripheral blood mononuclear cells was associated with the development of graft dysfunction (16).

Given the importance of BAFF in B-cell biology and the increasing evidence (outlined above) indicating a role in renal transplantation, we set out to assess serum BAFF levels in patients with end-stage renal failure undergoing renal transplantation and to determine if serum BAFF levels might predict risk of AMR. Here, we demonstrate that, in patients undergoing antibody-incompatible (Ai) renal transplantation, elevated serum BAFF levels at baseline (before both antibody removal/desensitization and transplantation) are associated with a heightened risk of subsequent AMR. Lower BAFF levels did not allow risk stratification. In Ac transplant recipients at lower risk of AMR, the rate of AMR in patients with the highest pretransplantation soluble BAFF was not statistically significantly different from those with lower BAFF levels. These data raise the possibility that, in patients undergoing Ai transplantation, elevated BAFF levels might allow the identification of those at greatest risk of AMR. Given the known roles of BAFF in B-cell biology and the availability of BAFF-blocking agents (17), BAFF neutralization in these individuals may be an interesting therapeutic strategy to explore and might help to determine whether BAFF plays a causative role in rejection.

RESULTS

Patient Demographics

Serum BAFF levels were measured in two patient cohorts: cohort 1 comprised 32 sensitized patients undergoing Ai transplantation, whereas cohort 2 comprised 319 patients undergoing Ac transplantation. The demographics of both cohorts are shown in Table 1. Patients in cohort 1 received basiliximab at induction followed by thymoglobulin in four cases (for details, see Materials and Methods). A higher proportion of patients in cohort 1 had received multiple transplants, in-keeping with their sensitization status. One patient in cohort 1 received a deceased-donor transplant, and the remainder received a living-donor transplant after desensitization. All rejection episodes observed in cohort 1 were antibody mediated.

Elevated Pretransplantation Serum BAFF Is Associated With the Development of Acute AMR in Patients Undergoing Ai Transplantation

In cohort 1 (Ai transplant recipients), serum BAFF levels at baseline, before both antibody removal/desensitization and transplantation, were variable ranging from 0 to 2200 ng/mL (Fig. 1A). By univariate analysis, higher BAFF levels at baseline were associated with an increased frequency of AMR; 35% of patients with a BAFF level less than 10 ng/mL developed AMR compared with 60% with those with a BAFF level of 10 to 100 ng/mL and 100% of those with a BAFF level of more than 100 ng/mL (Fig. 1B; $P=0.01$). Both time to rejection episode and rejection-free survival was significantly different between patients with low (<10 ng/mL), intermediate (10–100 ng/mL), and high (>100 ng/mL) serum BAFF (Fig. 1C; $P=0.0005$). Patients with a pretransplantation serum BAFF more than 100 ng/mL had a likelihood ratio of 1.4 of developing AMR compared with the overall cohort. Multivariate logistic regression confirmed a significant, independent positive association between baseline serum BAFF and risk of AMR ($P=0.007$). Covariates tested were pretransplantation donor-specific alloantibody level, number of previous transplants, total human leukocyte antigen (HLA) mismatch, and mean HLA mismatch.

A receiver operating characteristic curve was generated based on continuous BAFF values (see Figure S1, SDC, <http://links.lww.com/TP/A838>) and confirmed that BAFF levels more than 78 ng/mL had a 100% specificity for predicting AMR (see Table S1, SDC, <http://links.lww.com/TP/A838>).

There was no significant association between pretransplantation serum BAFF levels and pretransplantation DSA level (Fig. 1D,E), suggesting that the high frequency of AMR observed in those with BAFF levels more than 100 ng/mL was not due to higher titres of circulating DSA. Furthermore, the strength of T-cell or B-cell flow cross-match was not significantly associated with higher BAFF levels (see Figure S2A, SDC, <http://links.lww.com/TP/A838>). Elevated serum BAFF levels have been noted after B-cell depletion in both transplant recipients and patients with autoimmune disease (18–20). We do not routinely perform peripheral B lymphocyte counts in the Ai cohort, but we did not observe an inverse relationship between total lymphocyte count and serum BAFF (Fig. 1E). Some investigators have described a positive association between serum BAFF and the concentration of acute-phase response reactants in patients with SLE (21). In the Ai cohort, there was no such correlation between serum BAFF and pretransplantation C-reactive protein (see Figure S2B, SDC, <http://links.lww.com/TP/A838>).

Nonsignificant Association Between Pretransplantation BAFF Levels and the Development of Acute AMR in Patients Undergoing Ac Transplantation

Given the association of high pretransplantation serum BAFF with AMR in cohort 1, we wished to determine whether pretransplantation BAFF levels might predict risk of AMR or of other adverse outcomes after transplantation in a cohort of patients undergoing Ac transplantation (cohort 2). Pretransplantation serum BAFF levels varied from 0 to 539 ng/mL (Fig. 2A). 18.8% of patients with a pretransplantation BAFF level more than 100 ng/mL experienced an episode of AMR compared with 9.9% in those with a pretransplantation BAFF level less than 100 ng/mL ($P=0.35$; Fig. 2B). There was no association between pretransplantation serum BAFF levels and the subsequent development of T-cell-mediated rejection (TCR) (Fig. 2C). Elevated pretransplantation BAFF was not associated with a low lymphocyte count (Fig. 2D; $r^2=0.00025$, $P=0.78$) or with pretransplantation sensitization status (Fig. 2E).

Pretransplantation BAFF and Long-Term Allograft Function and Survival

Given data suggesting that the BAFF axis may be associated with chronic allograft dysfunction (7), we analyzed whether elevated pretransplantation BAFF levels were associated with reduced long-term renal allograft function and survival. In cohort 1 (Ai recipients), serum creatinine at 1 and 2 years after transplantation was similar in those with high, intermediate, and low serum BAFF levels (Fig. 3A). Similarly, in cohort 2 (Ac recipients), there was no significant association between pretransplantation BAFF and serum creatinine at 1 and 2 years after transplantation (Fig. 3B). Allograft survival was not significantly different between patients within BAFF tertiles in either cohort (Fig. 3C, D).

DISCUSSION

In this article, we sought to determine whether serum BAFF levels correlated with transplant outcomes. We chose to measure BAFF in pretransplantation samples, because this might potentially allow pretransplantation patient stratification in future studies. Baseline serum BAFF levels were higher in Ai transplant recipients compared with Ac transplant recipients (mean=229.3 vs. 17.35 ng/mL, respectively) but varied widely in both patient cohorts. This variability may be genetically determined, for example, by single nucleotide polymorphisms (SNPs) or copy number variation. The human *BAFF* gene contains a number of SNPs both within the gene and in its promoter (22, 23). One such promoter polymorphism (rs9514828, -871 C>T) includes a binding site for the transcription factor MZF1 and has been investigated in patients with autoimmune disease; T/T homozygotes at this locus were found to have higher levels of soluble BAFF and BAFF mRNA in peripheral blood mononuclear cells compared with heterozygotes or C/C homozygotes (11, 22). In keeping with these data, the -871T SNP drives higher luciferase expression in a reporter assay (23). However, not all studies have found a significant association between rs9514828 genotype and soluble BAFF (24).

B-cell depletion with rituximab or alemtuzumab may be associated with increased soluble BAFF (18–20). In the current study, we did not observe an inverse relationship between pretransplantation total lymphocyte count and serum BAFF in either patient cohort. However, we cannot be certain that B-cell numbers were normal in patients with the highest BAFF levels. Indeed, a number of studies have described a reduction in circulating B cells in patients with end-stage renal failure (25, 26) and increased soluble BAFF compared with controls (26). Elevated serum BAFF has also been associated with increased acute-phase response reactants in patients with SLE (21), but we observed no such association in the Ai cohort on whom we had pretransplantation C-reactive protein values.

In the present study, we examined circulating soluble BAFF, but it should be noted that this represents only one fraction of the total BAFF, because some remain tethered to the cell membrane (4, 5). One limitation of our study is that we did not have material available to examine BAFF mRNA in peripheral blood mononuclear cells to assess membrane-tethered BAFF. However, Thibault-Espitia et al. reported that BAFF transcripts and soluble BAFF were poorly correlated and that higher soluble BAFF but lower BAFF mRNA were associated with an increased risk of developing DSAs but not with rejection (16).

BAFF transgenic mice develop elevated antibody titres in response to both T-dependent and T-independent antigens and spontaneously develop autoantibodies. However, we did not note any association between the baseline serum BAFF level and the magnitude of the pretransplantation DSA titre in Ai transplant recipients. This may reflect the fact that some alloantibody might be bound to failing graft(s), resulting in an inaccurate estimation of the total alloantibody pool. Alternatively, it may be that alloantibody production in sensitized patients is likely to depend on long-lived plasma cells, whose survival is more dependent on APRIL rather than BAFF (27). In contrast, BAFF selectively enhances plasmablast generation from human memory B cells (28), perhaps explaining the association of pretransplantation serum BAFF with subsequent AMR, presumably driven by recall

response from the memory B-cell pool. Bloom et al. have proposed that the elevated BAFF levels observed post-alemtuzumab may drive the increased rate of AMR observed in some studies where alemtuzumab was used in the absence of calcineurin inhibitors (19). Our data would support this suggestion, although a trial of BAFF neutralization will be required to definitively prove causation.

It is worth noting that, in addition to basiliximab, four patients in the Ai cohort also received antithymocyte globulin after transplantation. It may be that the nature of the biological agent used (a lymphocyte depletion vs. CD25 blockade) could alter the efficacy of high BAFF levels to predict AMR, but the numbers were too small to address this question in the current study.

Given the data implicating BAFF in autoimmunity, a number of therapeutic agents have been developed to target this pathway including a TACI fusion protein (atacept) and an anti-BAFF antibody (belimumab). Clinical trials have demonstrated efficacy in both rheumatoid arthritis and lupus (17). Atacept may be associated with a profound reduction in serum IgG (29), resulting in concerns about excess infection risk; however, belimumab appears relatively safe and has received an Food and Drug Administration approval for its use in SLE. Our data raise the possibility that measurement of pretransplantation serum BAFF might allow the identification of a subset of patients with higher BAFF levels who might benefit from BAFF blockade. Because the safety of belimumab has already been proven in autoimmunity, its application to transplantation should be readily achievable.

In summary, this is the first study examining circulating BAFF in Ai transplant recipients. We have shown that patients with high pretransplantation serum BAFF levels have an increased frequency of AMR after transplantation compared with those with low serum BAFF levels. Further studies are required to determine if measurement of pretransplantation BAFF in sensitized recipients might allow the prediction of those at risk of rejection. Finally, these data raise the possibility that BAFF neutralization may be useful in individuals with high circulating BAFF, particularly those with preformed DSA undergoing antibody reduction therapy who are at increased risk of AMR.

MATERIALS AND METHODS

Patient Cohorts

Serum samples and clinical outcome data were obtained on two cohorts of patients:

Cohort 1—Thirty-two sensitized patients with donor-specific HLA class I and/or II antibodies receiving Ai transplants were studied. Two of the transplants were also ABO incompatible. All but one of these patients had undergone an antibody reduction protocol (consisting of three to six sessions of double-filtration plasmapheresis) followed by two doses of basiliximab 20 mg at days 0 and 4. Antithymocyte globulin was used in patients 029, 039, 051, and 052 due to concerns about early graft dysfunction (for further details, see ref. (30)). Maintenance immunosuppression consisted of mycophenolate mofetil 1000 mg twice a day (started 10 days before transplantation), with dose reduction if white cell count fell below $4.0 \times 10^9/L$. Tacrolimus was started 4 days before transplantation at a dose of 0.15

mg/kg per day in divided doses, with a target trough level of 10 to 15 µg/L in the first month. Prednisolone 20 mg once a day was started at the time of surgery, and methylprednisolone 500 mg was given as a single intravenous dose during the transplant operation.

T-cell and B-cell flow cross-match was performed as follows: for each sample, tested in duplicate, the ratio of the median channel fluorescence of the test sample over that for a negative control AB serum was calculated to give a relative median channel fluorescence. In our laboratory, the threshold for a positive cross-match is set at a relative median channel fluorescence of 4.0 for primary grafts and 2.5 for regrafts.

HLA class I and II specific antibodies were identified using microbead assay. The majority of samples were analyzed using single antigen beads manufactured by One Lambda (Canoga Park, CA), analyzed on the Luminex platform (XMap 200). Some of the transplants were performed early in our series, before single antigen beads were available, using “phenotype” beads (see Table S2, SDC, <http://links.lww.com/TP/A838>). All assays were performed using serum/bead volume ratios according to the manufacturer’s instructions. Raw median fluorescence intensity values were used to follow antibody levels. Values presented are those obtained from undiluted sera. Total reactivity per patient for multiple DSA was obtained by adding individual reactivities together.

Cohort 2—This cohort comprised 319 patients who received an Ac renal transplant at a single UK center between 1999 and 2008. This included 282 consecutive patients transplanted between January 1, 2005 and December 31, 2007, 8 of whom developed biopsy-proven AMR or mixed AMR and TCR (also known as acute cellular rejection). Given the low frequency of the end point of interest (acute rejection), a further 37 patients were identified, transplanted between 1999 and 2008 who subsequently developed biopsy-proven AMR (n=25) or TCR (n=12) giving a total of 319 patients/samples for analysis. The immunosuppression protocol varied during this period. From 1999 to 2001, patients received methylprednisolone at induction followed by cyclosporine (5 mg/kg), azathioprine (1.5 mg/kg), and prednisolone (20 mg, weaned to 5 mg over 3–6 months). From 2002, patients received basiliximab 20 mg at induction (days 0 and 4) followed by maintenance therapy with tacrolimus (0.075 mg/kg twice a day, target trough levels of 8–15 nmol/L) or cyclosporine (5 mg/kg), azathioprine (1.5 mg/kg), or mycophenolate mofetil (500 mg twice a day) and prednisolone (20 mg, weaned to 5 mg over 3–6 months).

Sensitization status of patients was determined by testing patient sera against an HLA-typed lymphocyte panel obtained from healthy volunteer blood donors and patients with B-cell chronic lymphocytic leukemia using dithiothreitol-modified complement-dependent lymphocytotoxicity. HLA antibodies were further characterized using Luminex-based HLA class I and II specific antibody detection kits and Luminex single antigen beads. Sensitization status was defined by the IgG complement-dependent lymphocytotoxicity panel-reactive antibodies (PRA) at the time of transplantation. Patients were deemed sensitized with an IgG PRA value 15% or more and highly sensitized with an IgG PRA value 85% or more.

Informed consent and approval for this study was obtained from the local ethics committees at Cambridge and Warwick.

BAFF Enzyme-Linked Immunosorbent Assay

Capture and detection antibodies and BAFF standards were obtained from Biosupply (Bradford, UK) and used according to the manufacturer's instructions. Streptavidin-horseradish peroxidase was obtained from BD Biosciences (San Jose, CA).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism. *P* values were calculated using Mann–Whitney test and log-rank (Mantel–Cox) test used to compare survival curves (as indicated in the figure legends).

Multivariate logistic regression analysis adjusting for previous transplant number, pretransplantation donor-specific antigen, and HLA mismatch was performed using R software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Elevated pretransplantation serum BAFF in Ai transplant recipients. A, baseline serum BAFF levels (predesensitization and pretransplantation) in Ai transplant recipients ranged from 0 to 2200 ng/mL, with a trend toward a higher median value in those who experienced AMR. B, patients within the highest serum BAFF tertile had an increased frequency of AMR and reduced rejection-free survival. C, $P=0.01$ based on a chi-square test for trend indicating an association between increasing BAFF levels and rejection. D and E, there was no association between pretransplantation BAFF levels and DSA titre (mfi, median fluorescence intensity). F, total lymphocyte count and serum BAFF. Horizontal bars represent median values. Shaded area in F indicates laboratory normal range. P values

calculated using Mann–Whitney (A), log-rank (Mantel–Cox; C) and Kruskal–Wallis (D and E) tests, and linear regression analysis (F).

FIGURE 2.

Pretransplantation serum BAFF in Ac transplant recipients. A, baseline serum BAFF ranged from 0 to 539 ng/mL (horizontal lines represent median values). B, a higher proportion of patients with a pretransplantation BAFF level more than 100 ng/mL experienced an episode of AMR compared with those with a pretransplantation BAFF level less than 100 ng/mL, but this did not reach statistical significance. There was no association between pretransplantation BAFF levels and subsequent T-cell-mediated acute rejection (C), pretransplantation total lymphocyte count (D), or pretransplantation sensitization status (E).

P values calculated using Mann–Whitney (A), chi-square test for trend (B, C, and E), and linear regression analysis (D).

FIGURE 3.

Pretransplantation serum BAFF and long-term allograft outcomes. Elevated pretransplantation serum BAFF was not associated with an increase in serum creatinine at 1 and 2 years after transplantation in either cohort 1 (Ai; A) or cohort 2 (Ac; B). GL, grafts lost (Cr recorded as 500 μ L/mL); N, number included; n/a, follow-up time not reached; PD, patient deaths; UK, number with creatinine unknown. Pretransplantation BAFF did not significantly influence allograft survival in either cohort 1 (C) or cohort 2 (D). *P* values calculated using Kruskal–Wallis (A and B) and log-rank (Mantel–Cox; C and D) tests.

TABLE 1
Patient demographics for patient cohorts investigated

	Cohort 1 (antibody incompatible)	Cohort 2 (antibody compatible)	<i>P</i>
Number of patients	32	319	
Age, mean (range)	40.3 (18–64)	46.0 (19.2–73.1)	0.01
Transplant number, n (%)			
1	10 (31.3)	272 (85.3)	0.0001
2	20 (62.5)	38 (11.9)	
3	1 (3.1)	7 (2.2)	
4	1 (3.1)	2 (0.6)	
Donor type, n (%)			
Living	31 (96.9)	67 (21.0)	0.0001
DBD ^a	1 (3.1)	102 (32.0)	
DCD ^b	0 (0)	150 (47.0)	
HLA mismatch			
(A, B, DR)	(0.84, 1.06, 0.90)	(1.04, 0.94, 0.66)	NS
Total	2.81	2.64	

^a DBD, deceased brainstem death.

^b DCD, deceased circulatory death.