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Antimalarial agents diminish while methotrexate, azathioprine and mycophenolic acid increase BAFF levels in systemic lupus erythematosus

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B cell activating factor belonging to the tumour necrosis factor family (BAFF), also known as B lymphocyte stimulator (BLyS), is believed to be important in the pathogenesis of systemic lupus erythematosus (SLE) [1]. Elevated levels of circulating BAFF have been associated with active SLE [1], and the anti-BAFF antibody belimumab has been approved as an add-on to standard of care SLE treatment [2], the latter mainly comprising glucocorticoids, antimalarial agents (AMA) and other immunosuppressive treatments [3].

AMA were initially developed for the treatment of malaria, but are now widely used in chronic inflammatory conditions, including SLE since the 1950's [4]. The mechanism of action of AMA is not fully delineated, but several pathways have been found to be affected. Being lipophilic weak bases, AMA can freely cross cell membranes and increase the lysosomal pH [4,5], resulting in alterations in the auto-antigenic peptide load being processed in the lysosomes of antigen-presenting cells and therefore impeding their binding to class II major histocompatibility complex (MHC) molecules [6]. Additionally, AMA are known to have photoprotective and antithrombotic effects [7,8]. Although the evidence hitherto is scarce, AMA have been shown to lower serum BAFF concentrations in SLE [9].

Methotrexate has not been approved by regulatory agencies for the treatment of SLE, but is commonly used [3], especially in articular SLE. In inflammatory arthropathies, methotrexate has been shown to lower the numbers of early B cells [10], but data on its impact on BAFF levels have been ambiguous, with positive correlations between methotrexate dose and BAFF levels in an observational setting [11], decreased BAFF levels following methotrexate therapy in another [12], and no evident effect of methotrexate on BAFF levels in a third study [10].

Azathioprine and mycophenolate mofetil or sodium are widely used in rheumatic diseases, including SLE. To our knowledge, the effect of these treatments on serum BAFF levels has not been investigated. The aim of this study was to determine whether and how the use of AMA, methotrexate, azathioprine and mycophenolic acid affect serum BAFF levels in the pooled SLE population ($n = 1684$) of the BLISS-52 ($n = 865$; NCT00424476) [13] and BLISS-76 ($n = 819$; NCT00410384) [14] phase III clinical trials of belimumab.

At baseline, the patients were on stable standard of care SLE treatment (Table 1). Serum samples were obtained prior to intervention and stored at −80 °C until BAFF level determination. A microtiter plate was coated with streptavidin and purified biotinylated Fab fragments of anti-BAFF monoclonal antibody 3D4 were used as capture reagent. The captured BAFF was detected by the addition of a HRP-conjugated goat anti-BAFF polyclonal antibody. Quantification of BAFF levels was not consummated in 19 patients.

Statistical analyses were performed using the IBM SPSS Statistics 25 software (New York, USA). The Mann-Whitney *U* test was used to compare BAFF level distributions between treatment groups. Linear regression was applied to determine independence and confounding potentiality. *P* values < .05 were considered statistically significant.

Serum BAFF levels were higher in patients receiving methotrexate (mean ± SD: 1835 ± 1617 pg/mL; $n = 212$; $P = .001$), azathioprine (mean ± SD: 1901 ± 1472 pg/mL; $n = 364$; $P < .001$) or mycophenolic acid (mean ± SD: 1994 ± 1544 pg/mL; $n = 175$; $P < .001$) compared with patients receiving no immunosuppressive therapy (mean ± SD: 1593 ± 1929 pg/mL; $n = 860$) at baseline; AMA were allowed in all groups (Fig. 1A, Table 1). In contrast, patients on AMA had lower BAFF levels (mean ± SD: 1654 ± 1318 pg/mL; $n = 1085$) than patients who did not use AMA (mean ± SD: 1942 ± 2408 pg/mL; $n = 580$; $P = .002$) (Fig. 1B, Table 1).

In linear regression analysis, AMA use was associated with lower BAFF levels (standardised coefficient, $\beta = -0.08$; $P = .002$); this association remained significant and independent of other factors in

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Table 1
Patient characteristics.

	All patients $N = 1684$	AMA $N = 1098$	No AMA $N = 586$	MTX $N = 231$	AZA $N = 389$	MPA $N = 189$
Female sex	1585 (94.1%)	1033 (94.1%)	552 (94.2%)	218 (94.4%)	368 (94.6%)	173 (91.5%)
Ethnic origin						
Indigenous American[a]	374 (22.2%)	254 (23.1%)	120 (20.5%)	63 (27.3%)	109 (28.0%)	25 (13.2%)
White/Caucasian	798 (47.4%)	491 (44.7%)	307 (52.4%)	122 (52.8%)	155 (39.8%)	100 (52.9%)
Black/African American	146 (8.7%)	98 (8.9%)	48 (8.2%)	23 (10.0%)	28 (7.2%)	28 (14.8%)
Asian	353 (21.0%)	243 (22.1%)	110 (18.8%)	21 (9.1%)	95 (24.4%)	35 (18.5%)
Multiracial	13 (0.8%)	12 (1.1%)	1 (0.2%)	2 (0.9%)	2 (0.5%)	1 (0.5%)
Age (years)	37.8 ± 11.5	36.8 ± 11.4	39.6 ± 11.5	40.9 ± 11.8	36.4 ± 11.0	36.0 ± 10.3
SLE disease duration (years)	6.4 ± 6.3	6.1 ± 6.2	7.0 ± 6.6	6.5 ± 6.4	6.1 ± 5.9	8.3 ± 6.7
Disease activity						
SLEDAI-2 K	10.0 ± 3.8	9.8 ± 3.8	10.3 ± 3.9	9.8 ± 3.7	10.2 ± 4.1	10.5 ± 3.8
SELENA-SLEDAI PGA	1.4 ± 0.5	1.4 ± 0.5	1.5 ± 0.5	1.5 ± 0.4	1.4 ± 0.5	1.5 ± 0.5
Prednisone eq. dose (mg/day)	10.8 ± 8.7	10.1 ± 8.5	12.1 ± 8.8	8.5 ± 8.0	11.8 ± 8.7	11.3 ± 7.9
Antimalarial agents	1098 (65.2%)	N/A	N/A	144 (62.3%)	221 (56.8%)	104 (55.0%)
Hydroxychloroquine	836 (49.6%)	N/A	N/A	N/A	N/A	N/A
Chloroquine	265 (15.7%)	N/A	N/A	N/A	N/A	N/A
Other antimalarial agents[b]	5 (0.3%)	N/A	N/A	N/A	N/A	N/A
Immunosuppressive agents	816 (48.5%)	476 (43.4%)	340 (58.0%)	N/A	N/A	N/A
Azathioprine	389 (23.1%)	221 (20.1%)	168 (28.7%)	N/A	N/A	N/A
Methotrexate	231 (13.7%)	144 (13.1%)	87 (14.8%)	N/A	N/A	N/A
Mycophenolate mofetil/sodium	189 (11.2%)	104 (9.5%)	85 (14.5%)	N/A	N/A	N/A
Other immunosuppressive agents[c]	65 (3.9%)	38 (58.5%)	27 (41.5%)	N/A	N/A	N/A

Data are presented as numbers (percentage) or mean values ± standard deviation (SD).
AMA: antimalarial agents; MTX: methotrexate; AZA: azathioprine; MPA: mycophenolic acid; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment version of the SLE Disease Activity Index; PGA: Physician's Global Assessment; N/A: not applicable or not available.
[a] Alaska Native or American Indian from North, South or Central America.
[b] Mepacrine, mepacrine hydrochloride, quinine sulfate.
[c] Cyclosporine, oral cyclophosphamide, leflunomide, mizoribine, thalidomide.

multivariable models adjusted for age, SLEDAI-2 K scores and other immunosuppressive agents. In contrast, use of each one of methotrexate ($\beta = 0.05$; $P = .029$), azathioprine ($\beta = 0.06$; $P = .010$) and mycophenolic acid ($\beta = 0.05$; $P = .037$) was associated with higher BAFF levels in adjusted models. Importantly, the daily prednisone dose was not found to impact serum BAFF levels ($\beta = -0.04$; $P = .124$) (Fig. 1C–F).

The observed discrepant associations may indicate differential effects of AMA and other immunosuppressive treatments on BAFF levels, reflecting the different mechanisms of action of the drugs. Defective apoptotic cell clearance is believed to play a central role in SLE, resulting in accumulation of nucleic fragments and debris. Such molecular patterns are recognised by toll-like receptors (TLRs), maintaining the inflammatory response. AMA have multiple functions; apart from impeding the binding of antiphospholipid antibody (aPL)-β_2-glycoprotein I complexes to phospholipid bilayers [8], they inhibit protein degradation by raising the lysosomal pH and hamper TLR-signalling, with a subsequent down-regulation of type I interferon and thereby BAFF mRNA production [15]. This provides a reasonable explanation for the lower BAFF levels observed in AMA-treated patients in the present study and in previous literature [9].

The effects of methotrexate, azathioprine and mycophenolic acid on BAFF levels are less understood. It is worth noting that treatment with methotrexate has previously been shown to diminish BAFF levels in a setting of patients with rheumatoid arthritis [12], and the contradicting results in the present study of SLE patients might imply disease-specific effects. These medications share a common final effect, *i.e.* inhibition of *de novo* purine synthesis and DNA replication in T cells and B cells. Due to suppression of B cell function and proliferation, BAFF receptor activity is diminished and serum BAFF levels are expected to increase, as shown following B cell depletion with the anti-CD20 antibody rituximab [16,17].

Patients with severe lupus nephritis and neuropsychiatric SLE were excluded from the BLISS trials; our findings might therefore not be applicable to these patient subsets. A major strength was the large study population. Our study provides strong implications of differential effects of AMA and other immunosuppressive agents on BAFF levels in SLE. It is worth mentioning that methotrexate and mycophenolic acid are not approved for the treatment of SLE. Considering the importance of BAFF in B cell homeostasis and SLE pathogenesis, exploration of the biological and clinical significance of our observations is merited.

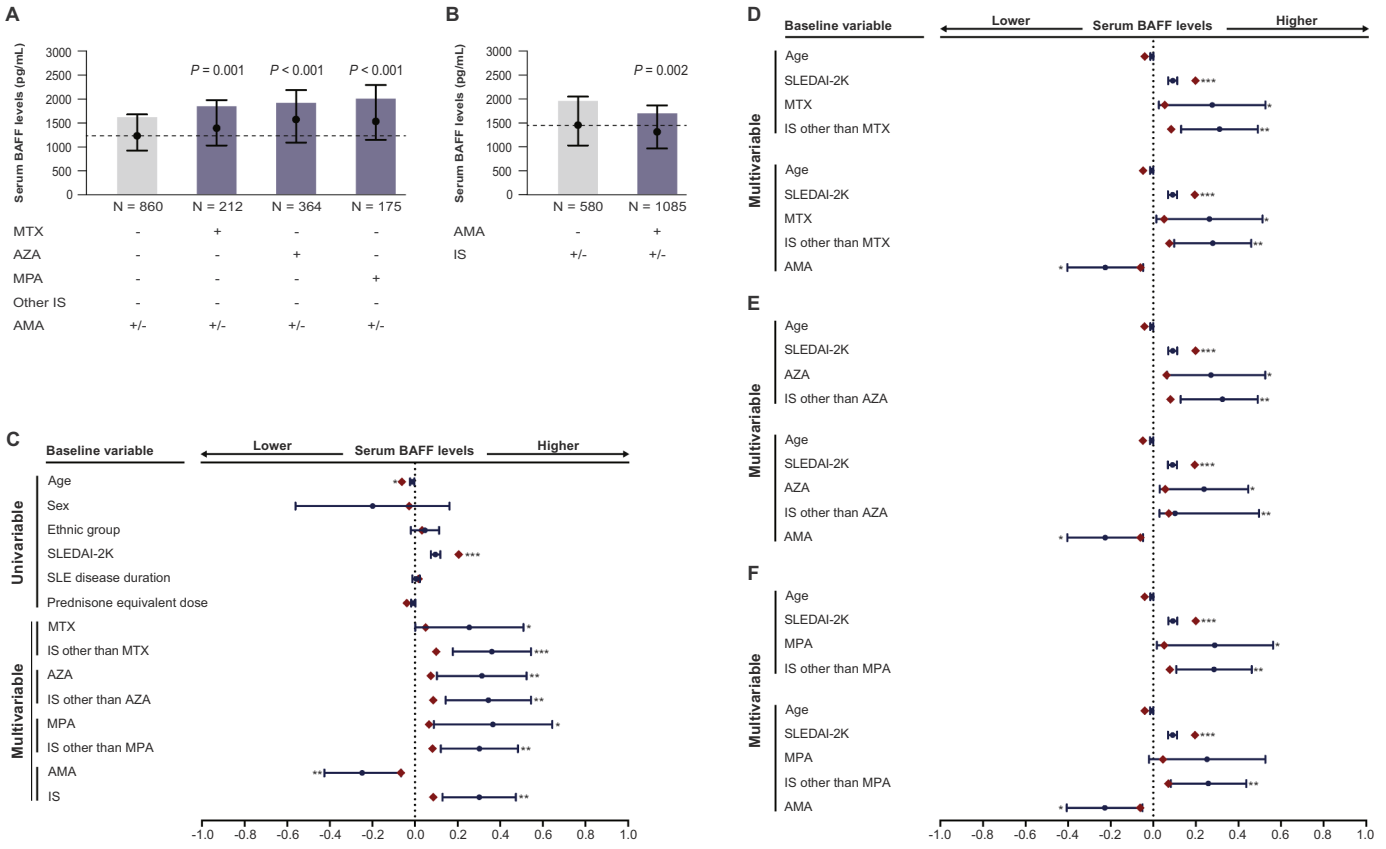

Fig. 1. Differential effects of antimalarial agents and other immunosuppressive treatments on serum BAFF levels. Panel A delineates comparisons between BAFF levels in patients receiving methotrexate (MTX), azathioprine (AZA) or mycophenolic acid (MPA) and BAFF levels in patients receiving no immunosuppressive agents (IS). Use of antimalarial agents (AMA) was allowed in all groups. Panel B depicts serum BAFF levels in SLE patients receiving *versus* not receiving AMA. Use of immunosuppressive agents other than AMA was allowed in both groups. The heights of the boxes represent mean BAFF levels, the dots indicate median BAFF levels, and the whiskers indicate interquartile ranges. The dashed lines represent the median level of the respective reference group. The forest plots in panels C–D illustrate results from linear regression analysis. Panel C illustrates the impact of different baseline factors on serum BAFF levels, including the use of MTX, AZA, MPA and AMA; the results were derived from linear regression models. The models of MTX, AZA and MPA were adjusted for the use of immunosuppressive agents other than the one investigated; AMA use was allowed. The model of AMA was adjusted for the use of other immunosuppressive agents. Panels D–F illustrate results from multivariable linear regression models, in which factors showing statistically significant associations with serum BAFF levels in the simple linear regression models of panel C (age, SLEDAI-2K scores) were included in models investigating the impact of MTX (panel D), AZA (panel E) and MPA (panel F) on BAFF levels, in order to assess independence, priority and confounding potentiality. Vertical lines group factors investigated in multiple models. All models were adjusted for immunosuppressive treatments other than the one investigated. The nether groups in panels D–F were also adjusted for the use of antimalarial agents. The dark blue diamonds represent the unstandardised coefficients, and the red diamonds represent the standardised coefficients. The dark blue whiskers represent the 95% confidence intervals. Asterisks indicate statistically significant associations.
Level of significance: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.
SLE: systemic lupus erythematosus; BAFF: B cell activating factor belonging to the tumour necrosis factor family.

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Authors' contributions

Study conception, design and coordination: BH, IP.
Acquisition of data: AG, SS, PJ, IP.
Statistics: BH, AG, SS, PJ, IP.
Interpretation of the results: BH, AG, IP.
Manuscript draft: BH, AG, IP.
All authors read and critically revised the manuscript for intellectual content, approved its final version prior to submission, and agree to be accountable for all aspects of the work.

Declaration of competing interest

None declared.

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Borja Hernández-Breijo[a], Alvaro Gomez[b,c], Sofia Soukka[b,c], Petter Johansson[b,c], Ioannis Parodis[b,c,*]
[a] *Immuno-Rheumatology Research Group, IdiPaz, La Paz University Hospital, Madrid, Spain*
[b] *Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*
[c] *Rheumatology, Karolinska University Hospital, Stockholm, Sweden*
E-mail address: ioannis.parodis@ki.se (I. Parodis).

[*] Corresponding author at: Rheumatology, Karolinska University Hospital, SE-17176 Stockholm, Sweden.