Lymphocyte subset profile and clinical phenotype in lupus nephritis: abridged secondary publication

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KEY MESSAGES

1. Distinct B cell subsets and relevant cellular signatures are related to relapses in lupus nephritis.
2. Cyclophosphamide and mycophenolate induction confer different changes in lymphocyte subsets and cytokines in patients with active lupus nephritis.

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Introduction

Lupus nephritis is characterised by repeated nephritic flares intercalated with periods of quiescence. Repeated nephritic flares lead to attrition of nephron mass and hence compromise long-term renal outcomes.

Perturbation in lymphocyte subsets and the relevant serum cytokine profiles is worth investigating. Patients with systemic lupus erythematosus exhibit an expanded memory B cells and plasma cells in the peripheral blood as well as shrinkage in the number of naïve B cells.[1] Disturbed T-cell homeostasis plays an essential role in the development of systemic lupus erythematosus; T-helper cells and regulatory T-cells (T_{Reg}) appear to be the more crucial subsets involved.[1] Cytokines such as IL-2, IL-4, IL-6, IL-10, IL-18, and IFN-γ are more involved in the Th1/Th2 and T_{Reg} balance, whereas IL-6, IL-17, IL-21, and IL-23 are more involved in Th17 subsets.[1] B cell subsets are significantly influenced by the B lymphocyte activation factor (BAFF), IL-6, and IL-21.[1] Both cyclophosphamide (CTX) and mycophenolate mofetil (MMF) are established initial treatment for severe lupus nephritis; CTX induction is associated with fewer relapses than MMF, but the underlying mechanism remains to be elucidated.[2]

Methods

We compared the lymphocyte subsets and serum cytokine profiles between patients with multiple relapses (MR) [ie, ≥3 within 36 months, unrelated to treatment non-compliance] and those with no relapse (NR). Clotted blood samples during quiescent disease were obtained for analysis of lymphocyte subsets and serum cytokine levels. Healthy subjects were recruited as controls. B cell signatures (miRNA148a, BACH1, BACH2, and PAX5) were measured in the serum and B cells.

Patients with biopsy-proven active class III/IV±V lupus nephritis (both treatment-naïve and relapsed patients were eligible) from two renal units (Queen Mary Hospital and United Christian Hospital) were randomised to receive CTX or MMF, together with corticosteroids, as induction treatment. The commencing dose of prednisolone was 0.8 mg/kg/day and tapered to reach 7.5 mg/day at approximately 6 months. MMF was initiated at 1 g twice a day for the first 6 months and then tapered according to clinical status. CTX was administered orally at 2 mg/kg/day for 6 months and then substituted with oral azathioprine at 1.5 mg/kg/day.

Exclusion criteria were having class II or pure class V lupus nephritis, having received calcineurin or proliferation signal inhibitors or biological therapies in the preceding 12 months, having received CTX or MMF at a daily dose >500 mg in the preceding 12 months, having other medical conditions with associated immunological abnormalities or requiring treatment with corticosteroids or immunosuppressive medications, having leucopoenia (white cell count $<3.5 \times 10^9$/L) secondary to lupus activity, and being pregnant or lactating. Serial blood and urine samples were assessed for lymphocytes subsets and serum cytokine levels. Patients were followed at 12-week intervals for 24 months to monitor for relapse.

Results

A total of 48 patients with stable lupus nephritis (24 in the MR group and 24 in the NR group) were included for analysis (Table 1). Circulating naïve B cells were significantly lower in MR patients than NR patients (0.7% [0.1%-14.1%] vs 4.0% [0.4%-24.6%],

TABLE 1. Clinical characteristics of those with multiple relapses or with no relapse in lupus nephritis

	Multiple relapses group (n=24)*	No relapse group (n=24)*	P value
No. of female:male	20:4	21:3	0.683
Age, y	48.7±6.6	51.4±11.3	0.314
Duration of follow-up from last nephritic episodes, months	118.9±79.9	133.2±81.9	0.594
Class of lupus nephritis			
Class III/IV	11 (45.8)	17 (70.8)	0.079
Class III/IV± V	13 (54.2)	7 (29.2)	0.089
Maintenance treatment:			
Prednisolone alone	6 (25.0)	12 (50.0)	0.140
Prednisolone + mycophenolate mofetil	11 (45.8)	6 (25.0)	0.230
Prednisolone + azathioprine	7 (29.2)	6 (25.0)	0.750
White cell count, $\times 10^9$/mL	5.2±1.8	5.8±2.3	0.383
Serum C3, mg/dL	80.0±29.5	83.0±20.9	0.699
Anti-dsDNA, IU/mL	62.7±89.8	42.2±67.0	0.865
Estimated glomerular filtration rate, mL/min/1.73 m^2	59.6±29.2	75.6±21.6	0.070
Serum albumin, g/L	39.3±5.1	42.5±2.2	0.017
Urine protein excretion, g/D	0.6±1.0	0.2±0.3	0.01

* Data are presented as mean ± standard deviation or No. (%) of patients

TABLE 2. Serum cytokine levels in patients with multiple relapses or with no relapse

Serum cytokine levels, pg/mL	Multiple relapses group*	No relapse group*	P value
B cell activating factor	1338.0 (1059.9-1627.1)	1252.7 (809.8-1664.9)	0.555
IL-6	203.7 (121.6-405.2)	195.0 (129.4-221.4)	0.598
IL-21	9.7 (6.4-19.4)	6.7 (3.1-8.8)	0.121
IFN-α	26.9 (22.9-30.1)	27.7 (25.8-40.5)	0.555
IFN-γ	33.5 (24.1-279.1	64.3 (23.1-216.7)	1.000
IL-2	2150.7 (1577.7-8053.5)	2304.3 (1709.7-7041.9)	0.877
IL-4	41.0 (34.2-43.8)	41.8 (34.9-52.8)	0.555
IL-10	6.7 (6.0-7.6)	6.9 (6.3-7.2)	0.926
IL-17	350.8 (49.1-502.2)	141.5 (23.1-506.5)	0.475
IL-18	212.7 (191.1-313.4)	211.1 (153.3-486.1)	1.000
IL-23	12.3 (11.1-14.4)	11.9 (10.9-16.2)	0.828

* Data are presented as median (range)

P=0.017). The two groups were comparable in terms of percentage of circulating memory B cells (0.6% vs 1.0%) and plasma cells (0.2% vs 0.3%). The memory-to-naïve B cell ratio was significantly higher in MR patients than NR patients (0.8 [0.1-9.0] vs 0.2 [0.1-2.0], P=0.024). The percentage of circulating T cell subsets (Th1, Th2, Th17, and Treg) did not differ between the two groups. Lymphocyte subsets showed no association with serum anti-dsDNA antibody or C3 levels in both groups. Serum levels of BAFF, IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, IFN-α, and IFN-γ were similar in both groups (Table 2).

We investigated the relevant B cell signatures in serum and B cells to account for the changes in the circulating B cell subsets profile. MR patients showed significantly higher serum miRNA-148a expression than did NR patients or healthy controls (1.0±0.0 vs 0.7±0.2 vs 9.4 ± 6.9 fold difference, p<0.001). miRNA-148a expression was significantly higher in memory B cells in MR patients than NR patients or healthy controls (1.0±0.0 vs 0.8±0.3 vs 5.8±1.7 fold difference, p<0.001). BACH1, BACH2, and PAX5 expressions were also significantly lower in memory B cells from MR group.

18 patients with active class III/IV±V lupus nephritis were randomised to CTX group (n=8) or MMF group (n=10). The overall renal response was comparable at 6 months (62.5% vs 80.0%, P=0.41, Table 3). Only one patient in the CTX group had renal relapse. After 24 weeks, the CTX group showed numerically higher circulating cytotoxic T cells (51.2% [43.3%-53.7] vs 23.0% [22.5%-52.0%], P=0.700] and lower Th1 cells (1.0% [1.0%-2.0%] vs 6.4% [3.6%-8.7%], P=0.700). There was no significant difference in other B cell subsets and T-helper cells. After 24 weeks, in the CTX group, MMF induction was associated with significantly lower serum BAFF (855.0 [714.8-1094.7] pg/mL vs 2175.0 [1721.6-2786.2] pg/mL, P=0.016) but significantly higher IFN-γ (553.6 [353.2-756.6] pg/mL vs 26.5 [18.9-29.9] pg/mL, P=0.032). There was no significant difference in the serum levels of IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, and IFN-α.

Discussion

We demonstrated that during disease quiescence, MR patients had a higher memory-to-naïve B cell ratio and reduced circulating naïve B cells than NR patients had. Changes in B cell subset profile in MR patients may be related to repeated exposure to autoantigens, which promotes differentiation of naïve B cells to more mature B cell subpopulations. Memory B cells from patients with systemic lupus erythematosus also show reduced FcγRIIb expression, which leads to increased Ca^{2+} influx and diminished inhibitory signals for memory B cell activation.[3,4] We did not observe any significant difference in the frequency of circulating plasma cells in MR and NR patients. This may be because plasma cells in the bone marrow and circulating plasma cells are present in very low quantities. In addition, we did not observe any significant change in T lymphocyte subsets profile between MR and NR patients. Pro-inflammatory and anti-inflammatory cytokines (such as BAFF, IL-2, IL-4, IL-6, IL-10, IL-17, IL-18, IL-21, IL-23, IFN-α, and IFN-γ) are secreted by B and T cells to promote B cell maturation

and survival, immunoglobulin switching, defective apoptotic cell clearance, and Th1 differentiation. This may be explained by the collection of samples during quiescent disease.

We demonstrated that serum miRNA-148a level was significantly higher in memory B cells in MR patients than in NR patients. This was accompanied by a decrease in BACH1, BACH2, and PAX5 expression in B cells. Increased miRNA-148a expression can inhibit *Gadd45a*, *Bim*, and *PTEN* and prevent apoptosis of immature B cells, resulting in enhanced B lymphocyte autoreactivity. A recent GWAS meta-analysis identified BACH2 as a novel susceptibility locus in Chinese lupus patients. Other studies reported that BACH2 expression was reduced in B cells isolated from patients with systemic lupus erythematosus, and that transfection of BACH2 into B cells from these patients suppressed their proliferation and promoted apoptosis.

Our results suggested that patients receiving CTX induction showed numerically lower percentage of circulating Th1 cells but higher percentage of cytotoxic T cells. Our findings of lower circulating Th1 and IFN-γ in the CTX group supported the finding of a lower risk of relapse in patients receiving CTX induction.[2] CTX induction in some high-risk ethnic groups was reported to be associated with increased renal fibrosis and considerable incidence of chronic kidney disease.[5] Our finding of higher circulating cytotoxic T cells might provide some explanation for the more renal parenchymal damage. MMF induction was reported to be associated with earlier reduction of circulating plasma cells, but CTX induction was reported to confer preferential depletion of naïve B cells and pre-switched memory B cells. We reported significantly lower BAFF levels in patients receiving MMF induction, which might translate into less efficient B cell repopulation and proliferation during disease quiescence.

TABLE 3. Baseline clinical characteristics and circulating lymphocyte subsets profiles in patients with active lupus nephritis receiving cyclophosphamide or mycophenolate mofetil induction

	Cyclophosphamide group (n=8)*	Mycophenolate mofetil group (n=10)*	P value
No. of female:male	7:1	9:1	0.867
Age, y	41.3±12.7	44.6±16.2	0.639
Lupus nephritis class III/IV ± V	8	10	1.000
White cell count, $\times 10^9$/L	10.4±5.5	8.5±3.2	0.392
Serum creatinine, μmol/L	133.9±72.8	80.1±38.3	0.203
24-hr urine protein, g/D	2.2±0.8	2.7±1.4	0.414
Anti-dsDNA, IU/mL	144.8±132.4	221.8±118.7	0.277
Serum C3, mg/dL	67.1±20.5	49.8±13.0	0.093
Circulating lymphocyte subsets before induction treatment			
Naïve B cells, %	9.9 (2.1-23.1)	10.8 (1.0-24.4)	1.000
Memory B cells, %	5.3 (4.1-5.9)	8.8 (4.7-10.6)	0.343
Plasma cells, %	0.12 (0.05-1.58)	0.29 (0.04-0.52)	0.886
CD8+, %	47.2 (36.7-60.1)	25.0 (17.3-40.0)	0.629
Th1, %	3.3 (1.7-4.1)	8.9 (6.4-10.0)	0.229
Th2, %	1.1 (0.7-2.2)	0.3 (0.2-0.4)	0.057
Th17, %	0.9 (0.3-2.3)	0.8 (0.5-1.2)	1.000
Treg, %	1.0 (0.6-2.2)	3.5 (2.3-4.5)	0.114

* Data are presented as mean ± standard deviation or median (range)

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Disclosure

The results of this research have been previously published in:

1. Yap DY, Yung S, Lee P, Chan TM. Circulating lymphocyte subsets and disease relapse in lupus nephritis. J Am Soc Nephrol 2016;27(Suppl):15.
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