Multiplex immunoassay of the cytokine profile in systemic lupus erythematosus: Relationship with disease activity and level of antinuclear antibodies

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Systemic lupus erythematosus is an autoimmune disease characterized by pathological activation of B- and T-cells, the formation of antinuclear antibodies, and dysregulation of cytokine production. The aim of the work was to study the cytokine profiles in patients with this disease in comparison with the disease activity and levels of antinuclear antibodies when using multiplex immunoassay technology, which has a high analytical sensitivity and provide the possibility of simultaneous determination of a large number of biomarkers. Hyperproduction of chemokines IP-10 and MCP-1, regulated by IFN, was associated with high activity of the disease and increased production of antinuclear antibodies. Various immunological subtypes of systemic lupus erythematosus were identified according to the cytokine profile, which reflects the heterogeneity of this multifactorial disease.

Keywords: systemic lupus erythematosus; cytokine profile; antinuclear antibodies; SLE-DAI-2K; multiplex immunoassay.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by pathological activation of B- and T-cells, the formation of antinuclear antibodies (ANA), and dysregulation of cytokine production. ANA directly or by the formation of immune complexes with nuclear antigens (generated during netosis, apoptosis and necrosis of the cells) stimulates membrane receptors (TLR 7, TLR 9; FcγRIIa) on plasmacytoid dendritic cells, monocytes/macrophages, neutrophils, T- and B- lymphocytes. Also they enhance production of IFN-α, BLyS and other pro-inflammatory cytokines (IL-6, -8, -1β, -12, -21, -22, -23, -17, -18, TNF-α), activate the complement system, complement-dependent and antibody-dependent cellular cytotoxicity, which leads to inflammation and tissue damage [1]. The most significant cytokines in pathogenesis of SLE are: BLyS, IFN-α, IL-6, -17, -12, -23, TNF-α, IP-10, and GM-CSF. These cytokines enhance the production of autoantibodies, support the inflammatory process and serve as potential targets for biological therapy [2]. Increased serum concentrations of chemokines: IP-10, MCP-1 and MIP-3B, regulated by IFN, are correlated with lupus nephritis activity and disease relapse. Other markers of SLE activity are: BLyS, TNF-α, IL-1ra, IFN-α, IL-6, -10, -18, and CD40L [1]. In patients

with active SLE, a high level of expression of genes induced by type I IFN correlates with an elevated concentration of IgG antibodies towards: dsDNA, nucleosomes, U1 RNP, SS-A/Ro, and SS-B/La [3]. The use of multiplex technology (with its high analytical sensitivity and the possibility of simultaneous determination of a large number of biomarkers) allows the identification of cytokine and ANA profiles associated with various SLE subtypes.

The aim of the work was to study the cytokine profiles in patients with SLE in comparison with the disease activity and levels of ANA when using multiplex immunoassay (MIA) of these biomarkers.

Materials and methods

We examined serum samples from 80 patients with SLE (2012 SLICC classification criteria) (8M/72 F), median and interquartile range (25^{th}–75^{th} percentile) of age 31.5 (16.0-65.0) years, disease duration 48.0 (2.0-432.0) months, SLEDAI 2K score 9.7 [0-40.0]; SLICC damage index score 1.6 (0-18.0), as well as samples of 28 healthy donors. The levels of cytokines were determined using multiplex bead-based immunoassay system Bio-Plex® 200 (Human Grp I Cytokine 27-plex panel; Bio-Rad Laboratories Inc., USA). ANA (anti-dsDNA, anti-Sm, anti-chromatin, anti-SS-A/Ro, anti- SS-B/La, anti-RNP-70, anti-ribosomal P — anti-RibP) were analyzed by BioPlex® 2200 technology (ANA Screen; Bio-Rad Laboratories Inc., USA).

Results

SLE patients had decreased levels of IL-1β, -1ra, -2, -9, -10, eotaxin, G- CSF, IFN-γ, MIP-1α, TNF-α, FGF, PDGF-BB, and VEGF; but increased concentrations of IL-4, -6, -8, -12, GM-CSF, MCP-1, MIP-1β, and RANTES compared with healthy donors ($p < 0.05$) (Table 1). The levels of IL-5, -7, -13, -15 and IP-10 did not differ from normal values ($p > 0.05$). Thus, the cytokine profile in SLE was characterized by low or normal values of the median concentrations of most pro-inflammatory, anti-inflammatory, Th-1, Th-2 cytokines, colony-stimulating and angiogenic factors (except for high levels of IL-6, -12 and GM-CSF) and overexpression of chemokines IL-8, MCP-1, MIP-1β, RANTES, as well as the main Th-2 cytokine — IL-4. The hyperproduction of IFN-inducible chemokines — IP-10 and MCP-1 was associated with increased SLEDAI-2K scores and high anti-dsDNA ($r = 0.3$), anti-chromatin ($r = 0.5$), anti-Sm ($r = 0.5$), anti-SS-B/La ($r = 0.3$), anti-RibP ($r = 0.4$) ($p < 0.05$) and anti-Sm ($r = 0.3$), anti-SS-B/La ($r = 0.3$), and anti-RibP ($r = 0.3$) ($p < 0.05$) antibodies levels. The levels of IL-1β, -15 and IL-8 negatively correlated with the concentration of antibodies to dsDNA ($r = -0.3$), RibP ($r = -0.3$) and SS-A/Ro ($r = -0.3$) ($p < 0.05$). A positive correlation was observed between the levels of anti-inflammatory cytokines IL-10, -1ra and antibodies to Sm ($r = 0.3$), SS-B/La ($r = 0.3$), SS-A/Ro ($r = 0.3$) ($p < 0.05$). Elevated concentration of GM-CSF was negatively correlated with serum level of anti-Sm antibodies ($r = -0.3$) ($p < 0.05$).

Discussion

Similar cytokine levels and ANA profiles data in SLE patients were obtained using multiplex technologies by other authors [4; 5]. Y. Pacheco et al. [4] describe elevated serum levels of IFN-α, IL-6, -8, -10, -12 / 23p40, -17A, TNFα, and G-CSF; but normal con-

Table 1. **The concentration of cytokines in the sera of patients with SLE and healthy donors**

Cytokine (pg/ml)	SLE (n = 80)	Healthy donors (n = 28)	p
	Median (25^{th}–75^{th} percentile)		
Proinflammatory			
IL-1β	1.7 (1.3–2.9)	4.3 (2.6–5.1)	0.000075
TNF-α	15.5 (11.9–21.4)	38.9 (21.8–66.0)	0.000764
IL-2	14.5 (9.3–17.7)	10.8 (5.0–14.4)	0.023539
IL-6	13.7 (9.0–21.9)	6.8 (4.3–13.1)	0.000097
IL-15	11.9 (0.0–28.4)	7.8 (4.0–19.1)	0.935177
Th1-related			
IL-12	16.8 (10.8–30.9)	5.6 (2.2–9.6)	0.000001
IFN-γ	92.5 (63.4–140.3)	175.9 (112.3–966.0)	0.000007
Th2-related			
IL-4	11.0 (8.8–13.2)	2.5 (0.2–5.8)	0.000001
IL-5	2.0 (1.7–2.7)	1.5 (0.2–5.2)	0.478648
IL-9	10.5 (7.7–19.8)	34.2 (27.2–41.7)	0.000001
IL-13	11.1 (4.8–29.7)	16.7 (9.1–22.7)	0.633549
Eotaxin	22.5 (11.2–39.7)	88.6 (18.1–590.0)	0.000084
Anti-inflammatory			
IL-1ra	57.3 (44.6–113.3)	145.2 (109.1–234.4)	0.00001
IL-10	8.5 (5.9–11.4)	13.2 (5.7–44.5)	0.052989
Colony stimulating factors			
IL-7	3.9 (2.6–6.4)	6.3 (0.4–20.0)	0.73118
GM-CSF	129.1 (53.7–226.8)	39.9 (15.4–56.5)	0.000066
G-CSF	6.1 (1.2–13.0)	12.0 (2.4–21.4)	0.042468
Stromal and angiogenic factors			
FGF	10.3 (8.1–15.1)	27.3 (19.8–42.3)	0.000001
PDGF-BB	2335.3 (1698.2–3176.2)	16338.8 (5320.5–56472.8)	0.000001
VEGF	61.9 (34.1–116.3)	205.6 (91.1–313.8)	0.000054
Chemokines			
IL-8	29.6 (13.4–149.7)	12.5 (4.7–15.9)	0.000003
IP-10	772.0 439.0 1450.4	349.3 (188.1–3452.1)	0.47024
MCP-1	130.1 (57.8–246.2)	51.5 (22.0–123.6)	0.000203
MIP-1α	3.9 (2.2–11.1)	10.8 (8.8–16.6)	0.000084

centrations of IL-1β, -2, -4, -5, -9, -13, and IFN-γ in 67 patients. Four cytokine clusters reflecting the activity of SLE by the SLAQ index (p = 0.022) were defined: (1 — *neutral*, with low cytokine levels; 2 — *chemotactic*, with dominance of IL-8; 3 — *G-CSF dominant*; 4 — *IFNα/pro-inflammatory*, with dominance of IFN-α, IL-12/23p40, TNF-α, IL-17A, G-CSF, and IL-10). Three integrative clusters of cytokines and autoantibodies were described in patients with active SLE. The first cluster was characterized by low levels of cytokines and a predominance of ANA, the second — by the high levels of chemokine IL-8 and antiphospholipid antibodies and the third — by the high levels of IFN-α and anti-dsDNA antibodies. We revealed elevated serum levels of IL-6, -8, and -12; but low or normal concentrations of IL-1β, -2, -5, -9, -13, and IFN-γ in SLE patients, while the hypoproduction

of IL-1β, -8, and -15 was associated with an increase ANA titers (anti-dsDNA, anti-SS-A/Ro and anti-RibP), however, cytokine levels (except IP-10 and MCP-1) did not correlate with SLEDAI-2K scores. J. A. Reynolds et al. [5] divided SLE patients (n = 96) on three groups. Two of them — were characterized by high disease activity (according to SLEDAI-2K and BILAG-2004) and high levels of IFN-α, BLyS (group I) or CXCL10, and CXCL13 (group II). Group III had low disease activity and low serum cytokines' levels. In group I, the high level of IFN-α and BLyS was combined with an increase in IL-10, -17, and -21 concentration; in group II — there was hyperproduction of chemokines — with a high anti-dsDNA concentration. This information was confirmed by our data about positive correlation between IP-10 and MCP-1 concentrations and SLEDAI-2K as well as levels of antibodies to dsDNA, nucleosomes, Sm, and RibP.

Conclusions

Hyperproduction of chemokines IP-10 and MCP-1, regulated by IFN, is associated with high activity of SLE and increased production of ANA. MIA of cytokine profiles identifies various immunological subtypes of SLE, reflecting the heterogeneity of this multifactorial disease.

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