IL-15 in the culture supernatants of PMN and PBMC and the serum of patients with Lyme disease

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Abtract

Purpose: The aim of this study was to estimate the production of interleukin-15 (IL-15) by neutrophils (PMNs) and peripheral blood mononuclear cells (PBMC) confronted with the serum levels of IL-15 in patients with Lyme disease.

Material and methods: PMN and PBMC were isolated from heparinized whole blood of patients. The cells were incubated for 18hs at 37°C in a humidified incubator with 5% CO_2. After 18hs incubation, supernatant was removed and assessed for IL-15 using ELISA kits.

Results: The results obtained showed significant increase in the ability of patient's PMNs and peripheral blood mononuclear cells (PBMC) to release IL-15. Although PBMC produced higher concentrations of IL-15 than PMN, the quantitative dominance of PMN in the peripheral blood suggest a significant role for these cells in the defense reactions controlled by this cytokine. Similar changes in the secretion of IL-15 by PMN and PBMC in patient group may be caused by the same regulatory mechanisms which influence the functional abnormalities of the cells examined.

Conclusions: A change in the ability of PMN and PBMC to release IL-15 may have various implications for the cellular and humoral response to the Borrelia burgdorferi (B.b.) infection in patients with Lyme disease.

Key words: interleukin-15, neutrophil, peripheral blood mononuclear cells.

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Introduction

Interleukin-15 (IL-15) is a pleiotropic cytokine which shares biological activities with IL-2. IL-15 uses both β- and γ-chains of the IL-2 receptor for binding and signalling. The IL-15 receptor (IL-15R) complex also includes a specific α subunit (IL-15Rα), distinct from the IL-2Rα chain. IL-15R is expressed on various cells of the immune response, including T and B cells, NK cells and more recently, peripheral blood neutrophils [1,2].

IL-15 plays an important role in both innate and adaptive immunity. It induces T cell proliferation and cytokine production, stimulates the locomotion and chemotaxis of T cells and delays its apoptosis. IL-15 has been shown to stimulate the growth and cytotoxicity of NK cells and to induce antibody-dependent cell-mediated cytotoxicity. It was also demonstrated that this cytokine induces macrophages function and B cell proliferation [1-3]. Recent investigation have shown that IL-15 potentiated several antimicrobial functions of normal neutrophils (PMNs) involved in the innate immune response against invading pathogens [4]. IL-15 was observed to enhance phagocytosis, NF-κB activation, IL-8 production and to delay apoptosis of these cells. In addition, IL-15 has been shown to prime the metabolic burst of PMN in response to N-formyl-methionyl-leucyl-phenylalanine (fMLP) [4-8].

Numerous in vitro and in vivo studies have suggested that neutrophils play a critical role in controlling spirochete Borrelia burgdorferi (B.b.) persistence that is responsible for Lyme disease. It has also been demonstrated that infectivity of the B.b. correlates with resistance to elimination by phagocytic cells [9,10].

The main source of IL-15 are macrophages/monocytes and lymphocytes as well as neutrophils [1,4,11,12]. There is no available data refering to PMN and peripheral blood mononuclear cells (PBMC) ability to release IL-15 in patients with Lyme disease. The changes in the secretion of IL-15 by these cells might modulate the PMN and PBMC activity in response to B.b. infection through autocrine or/and paracrine mechanisms.

Table 1. The mean concentrations of IL-15 in the culture supernatants of PMN and PBMC and serum of control and patients with Lyme disease.

	PMN unstimulated $\bar{X} \pm SD$	PMN LPS-stimulated $\bar{X} \pm SD$	PBMC unstimulated $\bar{X} \pm SD$	PBMC LPS-stimulated $\bar{X} \pm SD$	Serum (pg/ml) $\bar{X} \pm SD$
Control n=15	0.82±0.56	1.68[a]±0.69	1.94±0.79	3.21[a]±0.95	1.97±0.9
Patient group n=30	2.32*±1.62	2.76*±1.66	3.67*±1.85	3.86±1.61	5.46*±0.15

* – statistical differences with control ($p<0.05$)
[a] – statistical differences between unstimulated and LPS-stimulated cells ($p<0.05$)

The aim of this study was to estimate the production of IL-15 by neutrophils and peripheral blood mononuclear cells in comparison to the serum levels of IL-15 in patients with Lyme disease. Studies, involving estimation of IL-15 production by immunocompetent cells, such as PMN and PBMC, may be useful and would widen knowledge about IL-15 role in the course of B.b. infection.

Material and methods

Patients

We examined 45 patients (21 males and 14 females) aged between 44 and 72 years (mean ±58) with Lyme disease recognized according to EUCALB. The patients were treated in the Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok. The diagnosis of Lyme borreliosis was based on the epidemiological and case history, and interpretation of serologic tests using ELISA and Western-blot methods. Of 30 patients in the study 13 were classified as early localized Lyme borreliosis in the form of erythema migrans, 13 as Lyme arthritis form. Blood samples were taken from each patient at presentation.

Control subjects (n=15) were healthy people aged from 32 to 53 years (mean 42.5 year).

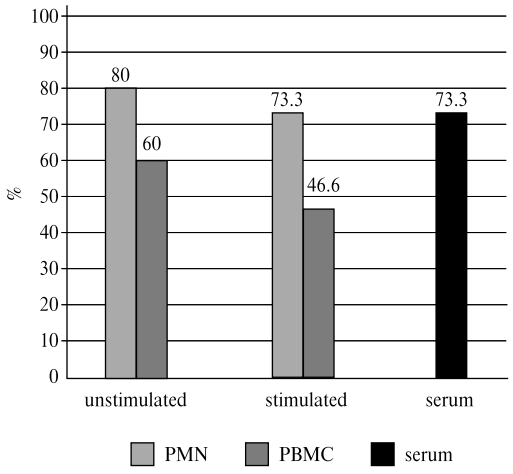
PMN and PBMC cultures

Cells were isolated from heparinized (10 U/ml) whole blood by Gradisol G gradient (1.115 g/ml). This method enables simultaneous separation of two highly purified leukocyte fractions: mononuclear cells (PBMC), containing 95% lymphocytes and polymorphonuclear cells (PMNs) containing 94% PMNs. The purity of isolated PMNs was determined by May-Grunewald-Giemsa-staining. The PMN were washed three times with RPMI-1640 medium (RPMI-1640 Medium, Gibco) and were suspended in the culture medium to provide $5x10^6$ cells/ml. Following culture, the viability of PMN was >93%, PBMC>94% as determined by trypan blue exclusion.

Culture medium consisted of RPMI-1640 medium supplemented with 10% autologous serum, 100 U/mL penicillin and 50 ng/mL streptomycin.

The cells were cultured in flat-bottomed 96-weel plates (Falcon) with LPS (100 ng/ml, Sigma-Chemical Co). The cells were incubated for 18 hs at 37^0C in a humidified incubator with 5% CO_2. After 18hs incubation, supernatant was removed and assessed for IL-15 using ELISA kits (Biosource).

Figure 1. The precentage of patients with the concentrations of parameters examined above the mean concentrations of control group.



Results

Unstimulated PMN derived from patients with Lyme disease secreted more IL-15 than those from normal subjects (*Tab. 1*). In 80% of patients, the concentration of IL-15 in the culture supernatants of PMN was significantly higher than in healthy persons (*Fig. 1*). In contrast to control group, LPS stimulation of patients PMN did not lead to a significant enhance in the production of IL-15 (*Tab. 1*). In 73.3% of patients, the concentration of IL-15 in the culture supernatants of LPS--stimulated PMN was higher than in control group (*Fig. 1*). There was no difference between patient groups with Lyme arthritis and erythema migrans.

To investigate the PMN contribution to the secretion of IL-15 among the leukocytes of peripheral blood, we also measured the IL-15 release by autologous PBMC under the

same culture conditions. The concentrations of IL-15 in the culture supernatants of PBMC from patients were higher than of those in control group (*Tab. 1*). In 60% of patients, unstimulated PBMC secreted more IL-15 than those from healthy subjects (*Fig. 1*). Similar to PMN, LPS stimulation did not result in higher IL-15 production by PBMC from patients (*Tab. 1*). In 46.6% of patients, the concentration of IL-15 in the culture supernatants of LPS-stimulated PBMC was higher than in control group (*Fig. 1*). The differences between IL-15 values in the culture of PBMC obtained from patients with different forms of Lyme disease were not significant.

There was no significant differences between the amounts of IL-15 secreted by unstimulated and LPS-stimulated PMN and PBMC (*Tab. 1*).

The mean concentrations of IL-15 in the serum of patients with Lyme disease were significantly higher than in control group (*Tab. 1*). The increased level of IL-15 has been found in 73.3% of patients (*Fig. 1*).

There was a correlation between the concentration of IL-15 in the culture supernatants of PBMC and the serum levels ($r=0.62$, $p<0.05$).

Discussion

PMNs are the primary effector cells in inflammation that extent beyond their capacities to function as phagocytes and cells releasing cytotoxic compounds. It was established that PMN have ability to synthesize and release the proinflammatory cytokines such as: IL-1, IL-6, OSM, IL-8, IL-12, IFN-α, TNF-α, GM-CSF, G-CSF, Gro-α, MIP-1α and MPI-1β and anti-inflammatory mediators such as TGF-β, IL-1Rα, sTNFRs [4,6,13,14]. The synthesis and secretion of these mediators by PMN can be changed at different pathological conditions [15,16].

The present study showed increase in the ability of PMN derived from patients with Lyme disease to release IL-15. Additionally, we have also found that autologous PBMC exhibited the same changes in the IL-15 secretion. Although PBMC produced higher concentrations of IL-15 than PMN, the quantitative dominance of PMN in the peripheral blood suggest a significant role for PMN in defense reactions controlled by IL-15 in patients with borreliosis. Similar changes in the release of IL-15 by PMN and PBMC may be caused by the same regulatory mechanisms which influence the functional abnormalities of the cells examined.

The high levels of IL-15 secreted by unstimulated PMN and PBMC from patient group, and simultaneous lack of response to additional LPS-stimulation in vitro, could be caused by the earlier activation of these cells in vivo due to direct or indirect effects of B.b. antigens. There are different data indicating that the outer surface lipoprotein of B.b., OspA and OspB are potent inducers of several inflammatory cytokines or chemokines [10,17-19]. It has been demonstrated that OspA can stimulate the production of IL-1, IL-6, IL-12, IFN-α, and TNF-α by mononuclear cells [17-19]. Furthermore, Oksi et al. showed that production of IL-4 by these cells was decreased but production of IFN-α was increased in patients with Lyme disease [20]. On the other hand, Diterich et al. demonstrated reduced release of TNF-α and IFN-α and enhanced secretion of IL-10 and GM-CSF by white blood cells [21]. Recent examination of Infante-Duarte et al. showed that B.b. and lipopeptides derived from B.b. outer surface lipoproteins induce IL-17 expression in Th cells that were different from "classical" Th1 and Th2 and were characterized by coexpression of the proinflammatory cytokines IL-17, TNF-α and GM-CSF [22].

A change in the ability of PMN and PBMC to release IL-15 may have various implications for cellular and humoral reactions in patients with Lyme disease. IL-15 secreted by PMN and PBMC may lead to the stimulation of each other's function in response to B.b. infection. The increase in IL-15 production by PMN as well as PBMC can also influence other cytokines secretion. IL-15 has been shown to stimulate mononuclear cells to release of IL-1, MCP-1, GM-CSF, IFN-α and TNF-α [6,23]. Moreover, Musso et al. showed that IL-15 induces secretion of the neutrophil chemotactic factor IL-8 that is effective in neutrophil activation suggesting an indirect role of IL-15 in the early steps of the inflammatory response to B.b. through increased amount of PMN infiltrating the tissues [8]. Additionally, our previous studies demonstrated that IL-15 induces in neutrophils not only IL-1β but also its regulatory protein – IL-1 receptor antagonist (IL-1Rα) [15]. Miller et al. reported that the balance between IL-1β and IL-1Rα influenced the pathogenesis of Lyme arthritis [24].

The changes in secretion of IL-15 by PMN and PBMC can lead to changes in their concentration in body fluid. The results of our study demonstrate markedly elevated serum levels of IL-15 in patients with Lyme disease. A direct correlation between the release of IL-15 by PBMC and the sera levels indicated significant influence of these cells on the level of IL-15 in blood. Athough there was no correlation between IL-15 release by PMN and the serum levels in patients, a coincidence between them was found.

In conclusion, the observations made in this study suggest an additional role of PMN and PBMC in the immune response of patients with Lyme disease. However, further examination, involving estimation the IL-15 secretion together with its regulatory protein, such as soluble IL-15Rα by these cells, in patients before and after treatment, might be helpful to confirm the clinical significance of the above observations.

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