Lymph and Blood Cytokines in Fever of Different Severity

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Experiments on rats showed that pyrogenal-induced fever is associated with elevation of IL-1β, IL-6, and IL-10 content in the thoracic duct lymph and blood plasma, whereas low-grade fever induced by administration of Freund's complete adjuvant is associated with elevation of only IL-6 concentration. The increase in IL-1β concentration during fever and IL-6 concentration in both processes was more pronounced in the central lymph than in blood plasma. Unchanged concentrations of IL-1β and IL-10 in low-grade fever apparently reflect differences in the mechanisms of these pathological processes.

Key Words: *cytokines; lymph; blood; fever; low-grade fever*

Cytokine network is a self-regulating system. Imbalance in this system results in excessive or insufficient synthesis of certain cytokines, which in turn, can affect the development of pathological processes. Some cytokines appear as pyrogenic compounds and others are the elements of the antipyretic system [1,3,4,7,15]. Considering multiplicity, synergism, and pleiotropy of cytokines involved in various pathological processes, simultaneous assessment of several mediators is more correct. On the other hand, since cytokines are just local mediators, it is reasonable to measure their levels not only in the blood, but also in fluid that outflows from the tissue, *i.e.* in the lymph.

We established levels of IL-1β, IL-6 and IL-10 in thoracic duct (TD) lymph and in blood plasma in low-grade fever and in fever in rats.

MATERIALS AND METHODS

Experiments were carried out on outbreed rats. Low-grade fever was modeled by single administration of Freund's complete adjuvant (FCA; Difco laboratories) containing 0.1% of killed and dried *Mycobacterium butyricum,* into the pad of the rear paws in a dose of 0.15 ml per animal [6]. Fever was reproduced by daily intramuscular pyrogenal injection in a dose of 100 μg/kg body weight for 5 days. Control rats (two groups) were administered with apyrogenic solution in the same volume, with the same frequency and in the same way as in experimental animals. On day 5 of low-grade fever and on day 6 of pyrogenal fever the animals were narcotized with sodium ethaminal (50 mg/kg body weight). The lymph was sampled by puncture of TD orifice neat the venous angle, the blood was taken from the vena cava inferior. The animals were sacrificed by administration of a lethal dose of anesthetic drug. Cytokine levels in biological fluids were measured by ELISA. The data was statistically treated using parametric Student's *t* test.

RESULTS

In low-grade fever, IL-6 level increased more than twofold in the lymph and only by 25.8% in blood plasma. IL-1β and IL-10 concentrations exhibited no dynamics in both fluids. In contrast to low-grade fever, pyrogenal fever was characterized by increased levels of all studied cytokines both in the lymph and blood. However, augmentation of IL-1β and IL-6 in the lymph was more pronounced. Lymph IL-1β and IL-6 levels increased by 93 and 69%, respectively, whereas their plasma levels increased by 27 and 24%. After LPS administration, IL-10 levels increased almost equally in both biological fluids: by 65.7% in the lymph and by 68.2% in blood.

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It should be noted that IL-1β and IL-6 are early inducible cytokines rapidly accumulating in circulation at pathological states. They play the key role in the mononuclear phase of inflammation providing proinflammatory effect by activation of adhesion molecule expression in the endothelium and by induction of leukocyte chemotaxis, increase in vascular endothelium permeability, intensification of fibroblast functional activity, activation of acute phase response induction of C-reactive protein synthesis, serum amyloid and fibrinogen synthesis in the liver, and by pyrogenic effect [3,4,7,13,14]. Taking into account more pronounced increase in IL-1β and IL-6 levels in the central lymph in comparison with the blood, we assume that the lymph deliver them from the site of generation to the circulation during pyrogenal fever. On the other hand, IL-1β and IL-6 possess procoagulant activity, which is determined by their ability to increase tissue factor expression, reduce thrombomodulin expression, and stimulate tissue-type plasminogen activator synthesis [1,13,14]. Hence, one may assume that increased lymph IL-6 level promotes activation of procoagulant reactions in it, which eventually appears as a factor of slower lymph circulation in TD during low-grade fever [9].

Speculating on possible mechanism for the absence of IL-1β changes in the lymph and blood during low-grade fever, we suppose that it is associated with the production of IL-1 receptor antagonist that blocks cytokine binding to lymphocyte and fibroblast receptors. Induction of receptor antagonist synthesis was established to occur in response to the same stimuli as IL-1 synthesis itself; however, the former is a unique cytokine among IL-1 cytokine family because is normally in present in blood plasma in relatively high concentrations. It is associated with constitutive expression of IL-1 receptor gene and with its permanent synthesis by tissue macrophages and hepatocytes. It can be hypothesized that IL-1 receptor in the circulation plays a role of a particular buffer blocking the effects of endogenous IL-1 and protecting the body from rapid increase in its level [1,3,11]. Moreover, high blood IL-6 level is known to inhibit IL-1β and TNF-α production, which in their turn appear as active inducers of IL-6 synthesis [13].

As the main anti-inflammatory cytokine, IL-10 inhibits secretion of proinflammatory cytokines, acts as a macrophage inhibiting factor due to inhibition of antigen presentation and phagocytosis, decrease in killing of the microorganisms ingested by macrophages. IL-10 blocks the release of various chemokines by neutrophil granulocytes, cyclooxygenase-2 activation, and E_2 prostaglandin synthesis [3,12,15]. On the other hand, acute increase in IL-10 synthesis results in altered anti-infectious protection, chronic infection development and unfavorable inflammation course. In addition, the inhibition of antigen presenting and cytotoxic macrophage functions occurs. Therefore, IL-10 overproduction is believed to be one of the substantial defects of cytokine network, that shifts emphasis of immune reactions toward antibody production [3,7,15]. Moreover, IL-10 overproduction during inflammation results in dramatic increase in oxygen radical and NO concentrations, which worsens the in-

TABLE 1. Cytokine Content (pg/ml) in TD Lymph and Blood Plasma in Rats during FCA-Induced Low-Grade Fever and Pyrogenal Fever (*M*±*m*)

Cytokine		Control 1	FCA	Control 2	Pyrogenal
Thoracic duct lymph	IL-1β	12.61±1.03 (*n*=7)	14.12±1.89 (*n*=9)	13.14±0.98 (*n*=6)	25.34±1.10** (*n*=7)
	IL-6	51.89±7.74 (*n*=7)	109.19±3.31** (*n*=9)	52.76±6.12 (*n*=6)	89.16±4.23* (*n*=7)
	IL-10	10.44±1.13 (*n*=7)	9.59±1.39 (*n*=9)	11.06±1.00 (*n*=6)	18.33±1.25** (*n*=7)
Blood plasma	IL-1β	20.83±1.91 (*n*=7)	19.04±1.45 (*n*=7)	20.11±2.55 (*n*=6)	45.61±2.89* (*n*=7)
	IL-6	144.33±4.15 (*n*=6)	181.53±18.35* (*n*=6)	136.57±5.66 (*n*=6)	169.40±9.05* (*n*=7)
	IL-10	18.72±2.17 (*n*=7)	20.12±2.08 (*n*=7)	20.10±2.72 (*n*=6)	33.81±3.13** (*n*=7)

Note. *$p<0.05$, **$p<0.001$ in comparison with the corresponding control.

toxication [5]. At the same time, being the most potent IL-1β synthesis inhibitor, IL-10 is produced only in the absence of the latter [3]. Therefore, unchanged levels of IL-10 in the lymph and blood during low-grade fever is apparently determined by unchanged IL-1β levels in both fluids, on one hand, and is the factor protecting from excessive activation of peroxidation processes, on the other.

Being immunosupressors, steroid hormones inhibit cytokine gene expression, block their synthesis, and prevent cytokine levels from surpassing the limit values [2,3]. This apparently is an efficient negative feedback mechanism to control excessive cytokine overproduction under our experimental conditions, because glucocorticosteroid production increased in fever [6].

Thus, pyrogenal fever is associated with increased levels of cytokines IL-1β, IL-6, and IL-10 in biological fluids, whereas low-grade fever was accompanied only by increased IL-6 levels. Augmentation of IL-1β concentration in fever and IL-6 concentrations in both processes was more pronounced in the lymph than in the plasma. Unchanged concentration of IL-1β and IL-10 in low-grade fever, apparently, is indicative for the differences in the mechanisms of the development of these pathological processes.

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