Cytokins in the Pathogenesis of Astrakhan Spotted Fever and North Asian Scrub Typhus: Problems of Immunocorrection

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Comparative study of interleukin-1 and tumor necrosis factor production under conditions of experimental rickettsial infection caused by agents of Astrakhan spotted fever and North Asian scrub typhus showed that therapy with galavit reduced manifestations of the disease, decreased mortality of experimental animals, and decreased the concentrations of interleukin-1 and tumor necrosis factor to normal values.

Key Words: *rickettsial infection; interleukin-1; tumor necrosis factor*

The main immunoregulatory mechanisms in the development of systemic inflammation are cytokins: interleukin-1 (IL-1), tumor necrosis factor (TNF), interleukin-8, and some other chemokins. Proinflammatory cytokins promote the development of oxidative stress, systemic autophagocytic pathology, disseminated intravascular coagulation, and, eventually, polyorgan failure. The role of cytokines in clinical and experimental infections is extensively studied; they are also involved in subsequent elimination of the agent from the body [1,4-6,9].

We investigated the role of IL-1 and TNF during immunomodulating therapy with an aminophthalhydrazide (APH) derivative galavit in the dynamics of acute infectious process in random-bred albino mice experimentally infected with agents of Astrakhan spotted fever (ASF) and North Asian scrub typhus (ST).

MATERIALS AND METHODS

R. astrakhani strain AR-1 was isolated from the blood of a patient with ASF in 1990 [3]. Infective material was prepared by routine culturing of the isolated rickettsia in developing 6-day chick embryos with consideration for the characteristic features of the group of rickettsia causing scrub spotted fever [2]. *R. astrakhani* strain AR-1 in a dose corresponding to 10^5 minimum infective doses was injected intraperitoneally (1 ml) to random-bred albino mice (n=10, 10-12 g). Control group consisted of 10 intact mice. Blood for measuring IL-1 and TNF was collected from the orbital sinus of control and experimental animals on days 4, 6, and 8 after infection.

Experiments with ST were carried out on similar animals. Mice (n=10) were intraperitoneally infected (1 ml) with 10% suspension of chick embryo yolk sacks infected with *R. sibirica* (Netsvetaev strain). Controls (n=10) were injected intraperitoneally with 1 ml normal saline at the same terms. Blood for measurements of TNF and IL-1 was collected from the orbital sinuses on days 4, 6, and 8 postinfection. The activity of IL-1 was measured in biological test using murine (C57Bl/6) thymocytes as the indicator cells [10]. TNF content was estimated by the lysis of TNF-sensitive L-929 cells [8].

Immunomodulator galavit (offered for trials by Abidofarma company) was injected intramuscularly (8 mg/kg) starting from day 4 of the disease.

RESULTS

Clinical symptoms of the disease were observed in animals infected with ASF starting from day 4 post-

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TABLE 1. Serum Levels of Proinflammatory Cytokines IL-1 and TNF in Mice with ASF ($M\pm m$, n=10)

Parameter	Control	Day after infection		
		4	6	8
IL-1 activity, arb. units	1.25±0.3	1.53±0.31	1.80±0.35	1.75±0.30
TNF activity, %	12.6±2.1	17.3±3.3	21.1±2.6*	15.0±2.5

Note. Here and in Table 2: *p<0.05 compared to the control.

TABLE 2. Serum Levels of Proinflammatory Cytokines IL-1 and TNF in Mice with ST ($M\pm m$, n=10)

Parameter	Control	Day after infection		
		4	6	8
IL-1 activity, arb. units	1. 25±0.30	1.9±0.3	2.1±0.2*	1.7±0.2
TNF activity, %	12.6±2.1	21.1±2.8*	23.1±4.0*	19.6±2.0*

inoculation: dim ruffled fur, dyspnea, loss of appetite. Animals died mainly on days 4-6 postinfection (47% mortality). The course of infection was more severe in animals infected with *R. sibirica*, (100% mortality, mainly on days 1-6 postinfection).

The concentrations of IL-1 and TNF increased by day 4 of ASF, the peak concentrations were observed on day 6. By the end of the experiment these parameters trended to decrease, but statistically significant differences were observed only for TNF on day 6 of the study (Table 1).

In animals infected with ST agent the concentrations of IL-1 and TNF increased starting from day 4 postinfection. Statistically significant changes in the content of IL-1 were observed starting from day 6, while on day 8 this parameter tended to decrease. TNF concentration increased significantly throughout the experiment (Table 2).

In is noteworthy that the concentrations of IL-1 and TNF were significantly higher in animals infected with *R. sibirica*, and the clinical course of experimental infection was more severe in this group.

Hence, experimental ASF and ST are associated with enhanced production of IL-1 and TNF by macrophages. The highest levels of proinflammatory cytokins (in both ASF and ST) were observed on day 6 of the experiment, when manifestations of the toxic syndrome were most pronounced.

By the start of galavit treatment the main signs of rickettsial infection persisted in animals infected with *R. astrakhani*, but dynamic study showed that the drug rapidly (by 2 days compared to the control) reduced clinical manifestations of the disease. The mortality decreased to 25%. Clinical signs were less pronounced and were absent in 20% animals treated with galavit.

Dynamic observations of animals infected with *R. sibirica* showed that galavit rapidly reduced clinical manifestations of the infection, animal mortality decreased by 50%.

Serum concentrations of IL-1 and TNF in mice infected with ASF and ST and treated with galavit tended to decrease on days 4-8 of the study and virtually did not differ from the control.

IL-1 is a proinflammatory cytokine. Together with TNF it plays the leading role in the immunoregulation and induces a complex of phenomena and reactions intrinsic for infectious process in inflammation foci [5]. It is noteworthy that the favorable effects of IL-1 and TNF are observed only if their concentrations increased slightly and for a short time [1,4,6], while in high concentrations they produce negative effects, and sometimes fatal consequences for the organism [1,7]. IL-1 and TNF are produced by monocytes/macrophages. These cells are present in zones of phagocytosis and microorganism destruction. In acute experimental rickettsial infection caused by ASF and ST strains the monocytes/macrophages are involved in the pathological process and produce a complex of bioactive substances aggravating cell and vascular disorders, which represents a universal component of inflammation. Modulation of this key component provides the basis for pathogenetic treatment of the disease caused by these agents. High efficiency of APH derivative galavit can be explained by reversible inhibition (during the acute period of disease) of monocytes/macrophages hyperactivity and excessive production of IL-1 and TNF.

REFERENCES

1. A. F. Vozianov, A. L. Butenko, and L. P. Zak, *Cytokins* [in Russian], Kiev (1998).
2. P. F. Zdrodovskii and E. M. Golinevich, *The Science of Rickettsia and Rickettsiosis* [in Russian], Moscow (1972).

3. V. A. Makarova, N. F. Fetisova, and I. V. Tarasevich, *Vopr. Rikketsiol.* [in Russian], Moscow (1994), pp. 70-73.
4. I. A. Shchepetkin, I. V. Cherdyntseva, and N. V. Vasil'ev, *Immunologiya*, No. 1, 4-6 (1994).
5. C. A. Dinarello and J. M. Krueger, *Fed. Proc.*, **45**, 2545-2548 (1986).
6. C. A. Dinarello, *Blood*, **87 (86)**, 2095-2147 (1996).
7. A. Cerami and B. Bentler, *Immunol. Today*, **13**, 29-31 (1988).
8. H. Fish and G. E. Gifford, *Int. J. Cancer*, **32**, 105-112 (1983).
9. L. I. Old, *Nature*, **330**, 602-603 (1987).
10. T. Uede, Y. Kocda, Y. Ibayashi, *et al.*, *J. Immunol.*, **135**, 3243-3251 (1986).