TEMPORAL DYNAMICS OF CYTOKINES IN THE BLOOD OF RATS WITH EXPERIMENTALLY INDUCED AUTOIMMUNE ENCEPHALOMYELITIS

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In this work we explore the temporal dynamics of cytokines in Dark Agouti rats with experimentally induced autoimmune encephalomyelitis (EAE). The main group consisted of 11 animals who were injected with 100 µl (per leg) of spinal cord homogenate obtained from random-bred rats and combined with incomplete Freund's adjuvant to the hind footpads. The control group included 7 animals who received 100 µl of normal saline mixed with incomplete Freund's adjuvant. Blood samples (500 µl) were collected daily, starting from day 1 through day 7. We ran a Bio-Plex-based multiplex cytokine assay on the samples using the Bio-Plex Pro Rat Cytokine 24-plex Assay kit. EAE in rats was shown to simulate progression of multiple sclerosis in humans in terms of temporal dynamics of lymphoproliferative and hematopoietic factors IL-1b, IL-2, IL-4, IL-5, IL-6, and IL-7. The studied model satisfactory imitates the dynamics of factors stimulating migration of lymphocytes, monocytes and other immune cells, including IL-17, RANTES (CCL-5) and MCP-1 (CCL-2) but excluding GRO/KC (CXCL1), which shows a different dynamics. The model also resembles patterns of human multiple sclerosis in terms of factors affecting cytotoxic and apoptotic reactions, including IFNγ, IL-6 and IL-17, but excluding TNFα.

Keywords: multiple sclerosis, experimental autoimmune encephalomyelitis, myelin, immunization, multiplex cytokine assay

Acknowledgements: the authors thank Boris Shevelev for help in immunization of the animals.

Funding: this work was supported by the Ministry of Education and Science of the Russian Federation (Grant agreement 14.607.21.0133 dated October 27, 2015, ID RFMEFI60715X0133).

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Received: 15.12.2017 **Accepted:** 20.12.2017

ВРЕМЕННАЯ ДИНАМИКА ЦИТОКИНОВ В КРОВИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АУТОИММУННОМ ЭНЦЕФАЛОМИЕЛИТЕ У КРЫС

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Изучена динамика содержания цитокинов у крыс линии Dark Agouti с индуцированным экспериментальным аутоиммунным энцефаломиелитом (ЭАЭ). В экспериментальную группу включили 11 животных, которым в подушечки задних лап инъецировали гомогенат спинного мозга беспородных крыс, смешанный с неполным адъювантом Фрейнда. В контрольную группу включили 7 животных, которым в подушечки задних лап вводили по 100 мкл физиологического раствора, смешанного с неполным адъювантом Фрейнда. У животных ежедневно с 1 по 7 сутки отбирали по 500 мкл крови. Был выполнен мультиплексный цитокиновый тест с помощью набора реагентов Bio-Plex Pro Rat Cytokine 24-plex Assay на платформе Bio-Plex. Показано, что в контексте цитокинового профиля модель ЭАЭ у крыс отражает течение рассеянного склероза у человека в части динамики содержания системных лимфопролиферативных и гемопоэтических факторов: IL-1b, IL-2, IL-4, IL-5, IL-6 и IL-7. В части динамики факторов таксиса лимфоцитов, моноцитов и других клеток иммунной системы изученная модель удовлетворительно имитирует динамику содержания IL-17, RANTES (CCL-5) и MCP-1 (CCL-2), но отличается по динамике GRO/KC (CXCL1). В отношении факторов, влияющих на цитотоксические и апоптотические реакции, сходство модели с заболеванием человека было выявлено по таким ключевым факторам, как IFNγ, IL-6 и IL-17, но не по TNFα.

Ключевые слова: рассеянный склероз, экспериментальный аутоиммунный энцефаломиелит, миелин, иммунизация, мультиплексный цитокиновый тест

Благодарности: авторы благодарят Бориса Шевелева за участие в иммунизации животных.

Финансирование: исследование поддержано Министерством образования и науки Российской Федерации (Соглашение о предоставлении субсидии № 14.607.21.0133 от 27.10.2015, уникальный идентификатор RFMEFI60715X0133).

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Статья получена: 15.12.2017 **Статья принята к печати:** 20.12.2017

Multiple sclerosis (MP) is a sever neurodegenerative autoimmune disorder. Due to its high prevalence and the severity of symptoms causing partial or complete loss of mobility, multiple sclerosis remains a pressing problem, prompting a search for new therapies. Most patients with MS completely loose the mobility 25 years after the onset of the disease. More than a half of MS patients become dependent on crutches 15 years after appearance of the first symptoms. To date, there is no effective causal treatment for MS.

Usually the disease strikes at young age: 70 % to 80 % of patients suffer the first symptoms of MS between 20 and 40 years of age [1]. MS is diagnosed by neurological examinations, magnetic resonance imaging of the central nervous system, and by biopsy or autopsy [2]. MS has numerous clinical manifestations indicating damage to the spinal cord, the brain, cranial nerves, the cerebellum, and cognitive function. Current diagnostics are insufficient for accurate estimation of MS severity. MRI, electroencephalography and lumbar puncture can still be inconclusive, in spite of providing valuable information about patient's condition. In patients with MS, many symptoms can be caused by infection, vascular pathology, or autoimmune comorbidities [3].

There are four types of MS: relapsing-remitting (RRMS, alternating periods of relapses and remissions) occurring in 80 % to 85 % of patients; primary progressive (PPMS) occurring in 10 % to 15 % of patients; progressive-relapsing (PRMS) — in 5 % of patients; and secondary-progressive (SPMS) [4, 5]. About half of patients with RRMS develop symptoms of SPMS 10 years after the onset of the disease. Over 90 % of patients with RPMS eventually demonstrate SPMS symptoms [6].

The hallmark of MS is destruction of the myelin sheaths of neurons in the central nervous system caused by clustering T- and B-cells. Another typical feature of this disease is accumulation of oligoclonal antibodies in the cerebrospinal fluid. It is not clear, though, how and where the clonal expansion of lymphocytes specific for myelin basic protein is initially triggered. We do not know yet whether it happens in the CNS, where the myelin sheath is directly involved, or outside of it, with autoreactive species migrating to the CNS from other places [7].

Development of effective MS treatments is impossible without animal models accurately replicating the course of the disease in humans, such as experimental autoimmune encephalomyelitis (EAE) of rats and mice. EAE is induced by injecting myelin or basic myelin protein (MBP) suspensions in incomplete Freund's adjuvant into the hind footpads of rodents [8]. One month after immunization the mice develop hind limb paralysis which lasts for 4–6 months [9]. In Dark Agouti (DA) rats, EAE progresses more rapidly (paralysis sets in on days 10–11 and lasts until day 14). The key difference of EAE in animals from MS in humans is full recovery of rodents, which is absolutely unattainable for humans at this point.

An interesting study [10] reports cytokine profiles of 19 patients with MS, including 16 patients with RRMS, 1 individual with PPMS, and 2 — with SPMS. The patients were distributed into groups based on disease duration from the moment of diagnosis: 4.2 ± 0.8 months in group 1 and 76.6 ± 14.3 months in group 2. The study showed that in earlier stages of MS (in comparison with later stages and the absence of the disease), interferon gamma (IFNγ) and the anti-inflammatory lymphokine IL-10 dominate in the cytokine profiles. In the late stage, the levels of IL-1RA, IL-8, IL-12(p70), CCL-3, CCL-7, CCL-11, CXCL-10, FGF, and IFNγ go down. Later stages are also characterized by elevated levels of IL-1a, IL-1b, IL-2RA, IL-3, IL-4, IL-7, IL-12(p40), IL-18, CCL-5 (RANTES), CCL-27, HGF, MIF, M-CSF and TRAIL. Interestingly, MS patients were shown to have elevated blood levels of IL-17, known to play a key role in triggering development of psoriatic skin lesions [11]. In addition, patients with RRMS exhibited elevated IL-22 levels. Dynamics of cytokine profiles in the cerebrospinal fluid drove the researchers [10] to the conclusion about the crucial role of the accumulating IFNγ and MIF (a key factor of joint capsule degeneration in osteoarthritis) and a few other factors stimulating migration of lymphocytes: CCL-5 (RANTES), CCL-2 and CCL-27, induced by IFNγ and MIF. The study also revealed accumulation of proapoptotic TNF-α and TRAIL-ligand in the cerebrospinal fluid (but not blood) of MS-stricken patients.

These data suggest a few patterns typical for MS, including increased long-term systemic activity of hematopoietic growth factors, in particular those targeting granulocytes, sustained Th1-response, and overrepresentation of lymphocyte/monocyte migration factors in the absence of pronounced proinflammatory response (factors stimulating production and taxis of neutrophils). The study [10] could provide an insight into how cytokine levels observed in the cerebrospinal fluid and blood change in patients with MS, but due to the limitations of the applied statistical methods, significance of the identified patterns is questionable.

Considering the above said, our study aimed to

1) investigate the short-term dynamics of cytokines in rats with rapidly progressing induced EAE;

2) compare the data on cytokine levels in patients with MS and in rats with induced EAE in order to assess the feasibility of the EAE rat model for testing anti-MS candidate drugs.

METHODS

Induction of EAE in rats

Experiments involving laboratory animals were carried out in compliance with the "Regulations for the use of Experimental Animals" (Addendum to Order 755 of the Ministry of Health of the USSR dated August 12, 1977) and the principles of the Declaration of Helsinki (2013).

Homogenates of the spinal cord of random-bred rats were prepared as described in [12]. Further *in vivo* experiments were carried out in Dark Agouti rats weighing 220–250 g. The main group included 11 animals. On day 0 the animals were injected with the spinal cord homogenate mixed with incomplete Freund's adjuvant in the ratio of 1 : 1 into the hind footpads. The total volume of the injected mixture was 100 µl per paw. The controls (n = 7) received 100 µl of normal saline mixed with incomplete Freund's adjuvant in the ratio of 1 : 1. From day 1 through day 7, except for day 6, blood samples were collected from the tail vein (500 µl of blood daily) and immediately used for serum preparation. Briefly, blood was placed into Vacuette Z serum sepclot activator vacuum test tubes and centrifuged for 15–20 min at 2,500 rpm and +4 °C. The obtained serum (about 100 µl) was transferred to microcentrifuge tubes and frozen at –20 °C. The animals were weighted daily, and the severity of the disease was assessed using the following scale: 0 points — no symptoms, 1 point — decreased tail tone, 2 points — impaired righting reflex, 3 points — partial paralysis, 4 points — complete paralysis, 5 points— moribund or dead. In borderline cases, a lower index value was opted. Clear signs of EAE appeared in the controls starting from day 8 to day 14 of the experiment. On days 11–14 the disease reached its peak, which lasted for 2–3 days.

Multiplex cytokine assay

Serum samples were analyzed on the Bio-Plex platform (Bio-Rad, USA) using the Bio-Plex Pro Rat Cytokine 24-plex Assay (Bio-Rad). This assay employs magnetic beads coated with monoclonal antibodies to rat cytokines. It was performed according to the manufacturer's recommendations and the protocol published in [13]. Serum was divided into 50 µl aliquots for the analysis. Mean fluorescence intensity of each sample was measured on Luminex 200 analyzer (Luminex Corporation, USA). Data were processed using MasterPlex CT and MasterPlex QT analysis software (Hitachi Solutions America, USA). For each analyte a calibration curve was constructed using 7 concentrations expressed as pg per 1 ml serum.

Statistical analysis

Two quartiles and median values of cytokine levels in each group were calculated daily for each cytokine. Then, significance of differences between the groups was tested using the nonparametric Mann-Whitney test and Statistica 8.0 for Windows. At p-value > 0.05 the differences were considered insignificant; we also used 3 significance thresholds: $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

RESULTS

The data on the short-term dynamics of cytokine levels in human and animal blood are still scarce. Multiplex assays are expensive, and daily blood tests in MS patients and lab animals can be technically challenging or raise ethical concerns. Data obtained from the controls in the course of our experiment demonstrate that although incomplete Freund's adjuvant injected into the footpads does not induce EAE, it still causes considerable fluctuations of cytokine levels in animals' blood, rendering less reliable the assessment of the impact of the spinal cord homogenate on the course of the disease. Therefore, special statistical methods are needed to analyze the dynamics of cytokine profiles.

All animals included in the main group developed paralysis of the hind legs. The rising phase of the disease was observed on days 11–13, while the decline — on days 12–17. By day 18 all animals had recovered from the paralysis. Blood was collected on days 1 through 7 in the absence of visible signs of EAE.

Tables 1 and 2 show that on day 1 of the experiment the levels of 13 of total 24 analytes (IL-1a, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12(p70), IL-17, IL-18, G-CSF, IFN-γ, RANTES (CCL-5), and MCP-1 (CCL-2)) were significantly higher (by up to 220 % for IL-4) in the main group than in the controls in terms of the second and third significance thresholds (Fig. 1). On day 2 no significant differences were observed for all studied cytokines. On day 3 differences were observed for IL-1b and VEGF (≤ 0.05), but on day 4 again no differences were found. On day 5 the main group demonstrated a considerable decrease in the levels of IL-1a, IL-1b, IL-13, and erythropoietin (Fig. 2). On day 7 the differences between the groups were observed for 14 of 24 studied cytokines. Those were practically the same cytokines that showed differences on day 1, although statistical significance was confirmed for IL-10 and erythropoietin GM-CSF only and was not confirmed for IL-12(p70) and G-CSF (Fig. 3) Of note, the levels of 13 of 14 cytokines in the main group were higher than in the controls. The only exception was GM-CSF that dropped from 8.17 pg/ml to 2.00 pg/ml.

DISCUSSION

A cytokine burst on day 1 of the experiment followed by a drop on day 2 should be interpreted as a manifestation of acute clonal nonspecific response to excess myelin outside the CNS. The response to the myelin manifested as simultaneous release of several lymphoproliferative factors is likely to be stimulated by hyperproduction of IL-1b originating from macrophages, dendritic cells and skin fibroblasts.

Increased cytokine synthesis on days 5 and 7 is, most probably, the result of the step-by-step accumulation of various clonal-specific lymphocytes, including those with autologous reactivity to myelin. Such longitude of the reaction is typical for the systemic clonal expansion of T-cells and eventually leads to visible physiological symptoms.

The most significant differences between the main and the control groups on day 7 were observed for the levels of IL-18 (2,475.85/4,182.05 pg/ml), RANTES (756.78/1,310.78 pg/ml), MCP 1 (CCL 2) (1,909.68/3,300.50 pg/ml) and IL-2 (743.52/1,091.57 pg/ml). Considering that IL-2 has been proved to induce production of other growth and hematopoietic factors [14], an assumption can be made that IL-2 triggers synthesis of such nonspecific immune factors as VEGF and erythropoietin, as well as IL-13, whose synthesis lagged in phase with respect to IL-2. Considering persistently high levels of IL-2 typical for patients with MS [10], this lymphokine seems to play a key role in the mass proliferation of lymphocytes

Table 1. Significance of differences between the main and the control groups of animals calculated by using the Mann–Whitney test with Yates's correction for continuity. Hypothesis tested: the absence of significant differences between the samples. The result is presented as Fisher's p with three significance thresholds: $p > 0.05$ — the difference is insignificant; $0.01 < p \leq 0.05$ — the first significance threshold; $0.001 < p \leq 0.01$ — the second significance threshold; $p \leq 0.001$ — the third significance threshold

Cytokine	Days of the experiment					
	1	2	3	4	5	7
IL-1a	3	–	–	–	2	2
IL-1b	–	–	1	–	2	-
IL-2	2	–	–	–	–	1
IL-4	3	–	–	–	–	2
IL-5	2	–	–	–	–	2
IL-6	3	–	–	–	–	1
IL-7	2	–	–	–	–	2
IL-10	–	–	–	–	–	2
IL-12	2	–	–	–	–	–
IL-13	–	–	–	–	2	–
IL-17	3	–	–	–	–	2
IL-18	3	–	–	–	–	2
Erythropoietin EPO	–	–	–	–	1	2
G-CSF	3	–	–	–	–	–
GM-CSF	–	–	–	–	–	3
GRO/KC	–	–	–	–	–	–
IFN-γ	2	–	–	–	–	1
M-CSF	–	–	–	–	–	–
MIP-3a	–	–	–	–	–	–
RANTES	2	–	–	–	–	2
TNFα	–	–	–	–	–	-
VEGF	–	–	1	–	–	–
Leptin	–	–	–	–	–	–
MCP-1	2	–	–	–	–	2

Table 2. Statistical analysis of changing cytokine levels in the main group of rats with induced autoimmune encephalomyelitis and the controls

Cytokine	Parameter	Day of the experiment											
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 7	
		Controls	Main group	Controls	Main group	Controls	Main group	Controls	Main group	Controls	Main group	Controls	Main group
IL-1a	Mean	195	387	182	206	268	181	193	144	319	138	203	359
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	87	92	65	61	148	122	113	76	279	75	84	134
	Q25	132	304	133	170	160	68	98	83	182	90	139	218
	Median	188	347	175	204	218	200	195	155	184	121	184	411
	Q75	288	487	226	264	396	237	274	210	396	187	269	462
IL-1b	Mean	433	401	412	274	1033	493	469	265	819	250	319	571
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	386	382	244	184	427	362	432	157	861	164	180	288
	Q25	251	236	211	148	701	134	119	101	279	151	180	300
	Median	315	270	399	222	967	441	412	310	607	214	204	536
	Q75	411	367	550	370	1 477	782	553	409	730	307	504	821
IL-2	Mean	356	607	366	436	367	500	472	414	579	373	744	1092
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	103	138	127	207	209	303	292	180	286	215	245	348
	Q25	278	579	285	319	186	311	213	260	396	205	543	776
	Median	374	597	345	331	332	432	308	362	557	284	657	1145
	Q75	443	626	463	629	523	608	762	647	668	509	914	1368
IL-4	Mean	11	36	9	19	9	24	14	18	19	16	41	90
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	6	16	4	19	8	23	12	16	13	15	27	40
	Q25	6	27	5	5	4	4	4	5	9	4	17	57
	Median	11	30	8	8	6	17	8	12	15	14	33	76
	Q75	17	38	12	37	10	32	29	34	33	23	64	128
IL-5	Mean	77	128	59	69	56	88	69	74	91	68	136	193
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	25	27	33	51	47	59	62	45	43	49	46	34
	Q25	55	110	26	22	23	26	22	20	43	22	106	174
	Median	78	123	63	54	30	83	23	67	88	83	130	193
	Q75	97	147	78	121	113	133	144	106	118	106	176	212
IL-6	Mean	232	503	351	273	468	1595	515	540	600	316	668	1145
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	79	204	226	255	404	3960	572	743	284	269	292	496
	Q25	172	379	169	63	170	96	76	85	414	46	456	640
	Median	224	444	340	182	349	428	190	240	584	261	521	1240
	Q75	287	463	540	470	758	635	1 132	559	713	556	877	1624
IL-7	Mean	103	254	68	123	70	178	111	114	125	106	228	612
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	50	96	62	128	83	169	129	115	77	98	146	236
	Q25	58	181	14	16	12	13	11	16	50	10	119	428
	Median	102	226	63	72	23	144	20	64	109	108	161	724
	Q75	145	350	90	246	159	281	239	224	209	165	391	787
IL-10	Mean	149	240	105	180	121	215	163	157	208	132	403	634
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	70	80	53	131	93	200	129	99	70	106	160	226
	Q25	91	208	61	57	56	63	56	58	144	44	254	420
	Median	142	220	101	183	67	149	88	116	214	116	413	557
	Q75	217	280	129	284	237	277	294	272	287	208	550	821

Продолжение табл. 2

IL-12	Mean	46	125	35	56	43	81	58	54	68	47	155	288
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	29	62	35	61	56	102	68	61	50	59	98	143
	Q25	16	88	5	6	5	6	6	6	15	4	81	173
	Median	49	99	32	23	10	53	12	29	59	26	125	277
	Q75	74	133	46	123	98	118	135	70	104	81	212	425
IL-13	Mean	31	32	15	16	20	22	22	13	19	13	33	61
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	28	31	8	15	8	20	26	11	6	8	21	37
	Q25	18	16	9	6	13	9	7	6	15	6	19	29
	Median	21	20	14	9	20	13	13	9	18	11	23	44
	Q75	28	37	22	20	23	28	26	15	22	17	58	95
IL-17	Mean	24	55	17	25	19	36	26	27	36	25	67	119
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	12	20	15	28	24	36	32	23	22	24	38	43
	Q25	12	43	3	3	3	3	3	4	16	3	38	84
	Median	24	49	16	15	4	34	5	21	35	28	62	108
	Q75	37	56	24	44	50	52	52	42	58	45	102	168
IL-18	Mean	1110	2360	909	1480	976	2004	1351	1829	1628	1562	2476	4182
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	340	822	345	828	600	1513	895	1390	455	1557	774	1495
	Q25	846	1671	623	707	591	582	615	779	1494	426	1663	3219
	Median	1009	2167	877	1444	609	1677	1064	1157	1616	1152	2334	3808
	Q75	1462	2766	1094	2243	1701	3435	2055	2367	1831	2632	3217	4100
Erythropoietin EPO	Mean	202	263	186	242	197	310	238	246	281	175	342	745
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	68	60	77	141	62	240	71	95	86	91	117	345
	Q25	154	235	127	110	158	127	173	153	209	107	249	504
	Median	175	258	179	219	202	272	209	223	247	166	360	585
	Q75	235	287	225	346	241	352	300	346	373	197	415	1060
G-CSF	Mean	3	6	3	4	3	6	4	4	5	4	9	15
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	1	3	1	3	2	6	3	2	1	2	6	9
	Q25	3	4	2	2	2	2	2	2	3	2	4	8
	Median	3	6	3	3	2	4	2	3	4	3	6	12
	Q75	4	7	3	6	5	8	6	5	6	5	14	24
GM-CSF	Mean	5	7	5	5	6	9	8	5	6	4	8	2
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	3	3	3	3	4	10	6	2	4	2	2	1
	Q25	2	6	3	2	3	4	4	3	2	2	7	1
	Median	5	7	6	5	6	9	9	5	5	5	8	2
	Q75	6	10	8	8	9	10	10	7	11	5	10	3
GRO/KC	Mean	161	237	156	165	217	153	201	90	153	116	163	178
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	93	104	79	65	134	97	115	31	128	43	62	57
	Q25	72	124	101	135	75	64	84	62	82	74	103	153
	Median	142	267	140	157	239	133	198	96	90	119	155	170
	Q75	270	331	191	189	341	272	299	116	324	145	184	222
IFNγ	Mean	36	79	30	42	32	63	61	45	53	40	107	215
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	14	46	13	40	23	77	62	41	19	35	45	122
	Q25	27	52	20	14	17	14	17	15	36	12	65	87
	Median	30	64	27	20	20	43	24	28	52	27	98	206
	Q75	46	74	41	60	56	64	95	66	76	69	148	290

Продолжение табл. 2

M-CSF	Mean	132	174	92	89	91	121	100	79	93	104	113	164
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	42	44	46	38	70	83	116	49	54	60	55	74
	Q25	99	143	64	55	32	53	32	33	39	41	52	110
	Median	124	159	80	88	71	106	40	69	69	105	141	149
	Q75	175	205	122	111	174	183	164	121	147	164	165	234
MIP-3a	Mean	70	88	55	49	45	88	69	64	76	45	88	128
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	19	38	44	40	44	95	80	59	45	40	36	44
	Q25	52	47	19	13	12	21	14	13	25	11	65	107
	Median	76	94	41	31	21	53	19	51	91	34	83	139
	Q75	86	122	85	83	99	134	161	83	122	90	102	171
RANTES	Mean	685	1070	342	470	295	696	364	505	574	456	757	1311
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	230	247	277	422	306	541	403	381	208	389	147	388
	Q25	545	966	80	73	108	60	37	51	437	39	726	814
	Median	665	1052	334	387	139	679	57	545	634	660	758	1495
	Q75	805	1129	558	855	481	1212	821	777	687	735	846	1580
TNFα	Mean	58	39	25	30	29	33	30	28	33	27	54	82
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	26	10	15	18	18	19	20	14	11	19	26	42
	Q25	42	29	17	16	16	16	17	16	21	14	32	51
	Median	57	39	19	21	19	27	23	25	36	24	57	72
	Q75	72	49	26	47	46	44	49	34	39	29	82	106
VEGF	Mean	130	87	88	55	128	73	107	46	133	65	92	110
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	110	90	57	79	73	80	78	24	149	49	44	50
	Q25	41	52	42	20	53	37	54	32	33	29	57	57
	Median	119	60	88	35	129	44	78	36	97	46	84	100
	Q75	132	74	128	44	178	84	185	58	136	89	146	139
Leptin	Mean	158	217	59	131	121	264	129	90	231	213	239	460
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	161	240	77	184	163	255	202	167	219	199	237	358
	Q25	10	1	20	11	11	16	3	4	12	17	0	20
	Median	150	137	24	27	21	312	21	20	246	286	324	620
	Q75	301	437	83	273	304	482	375	30	447	419	404	745
MCP-1	Mean	1979	2662	2277	2317	2428	3097	2071	2468	2117	2254	1910	3300
	N	6	10	8	12	6	11	7	11	7	11	7	11
	SD	413	365	949	754	576	1120	830	1141	830	623	557	879
	Q25	1592	2353	1606	1729	2130	2126	1501	1575	1651	1889	1340	2986
	Median	2102	2606	2155	2063	2428	3108	1849	2192	2012	2448	1946	3411
	Q75	2317	2864	2909	3047	2904	3762	2369	3021	2189	2750	2405	3846

outside the CNS. Increasing levels of lymphoproliferative and hematopoietic IL-4, IL-5, IL-6, Il-7, and IL-13 in the backdrop of decreased GM-CSF can be described as a cascade induced with IL-2 participation.

Unlike MS of humans, EAE in rats is not accompanied by production of proapoptotic TNF-α, regardless of the increased synthesis of its classic inducers IL-12, IL-18 and IFNγ [14]. Therefore, elevated levels of TNF-α in patients with MS are rather a result and not the cause of myelin destruction. At the same time, TNF-α can contribute significantly to the damage of astrocytes and neurons in the late stages of MS.

According to the pattern described in [10], simultaneous increase and decrease of IFNγ and RANTES (CCL-5), respectively, in rats with EAE simulate similar processes occurring in humans with MS. The early stages of EAE in rats are not accompanied by an increase in GRO/KC (CXCL1) responsible for lymphocyte infiltration in the CNS, which renders the rat model different from MS in humans [10].

Both rats with EAE and humans with MS have hyperproduction of IL-17 which can contribute to the accumulation of specific lymphocytes in the CNS and activate their toxic function.

In spite of IL-1b hyperproduction, MS in humans shows no signs of neutrophil involvement in the pathology, which is also true for the factors regulating neutrophil taxis and activation. This pattern turned to be no different in the studied rat model.

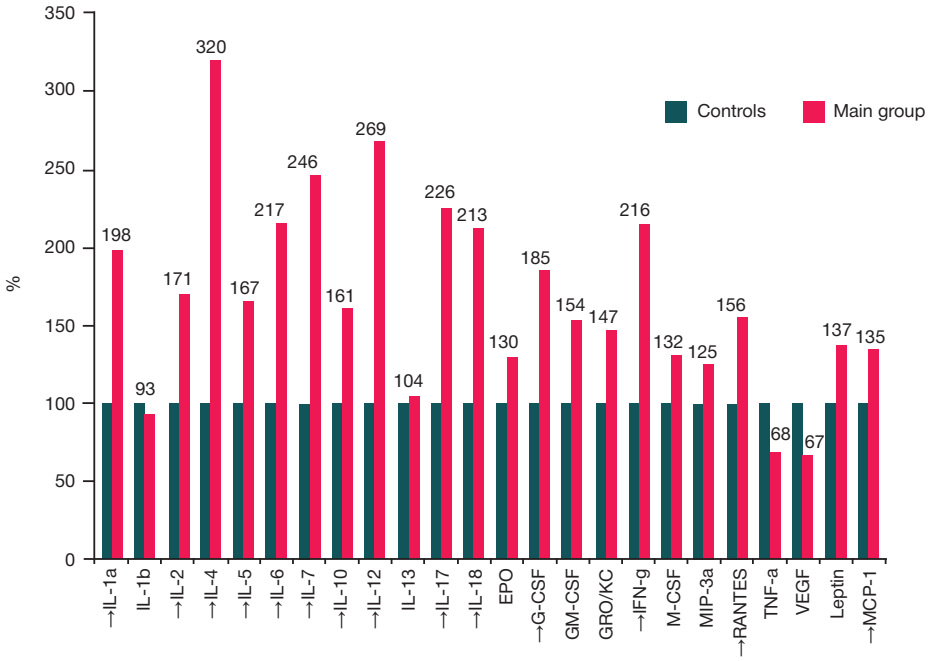

Fig. 1. Changes in cytokine levels in the blood serum of rats with induced EAE in comparison with the controls 1 day after the injection. Cytokine levels in the controls were taken as 100 %. Significant differences are marked with arrows

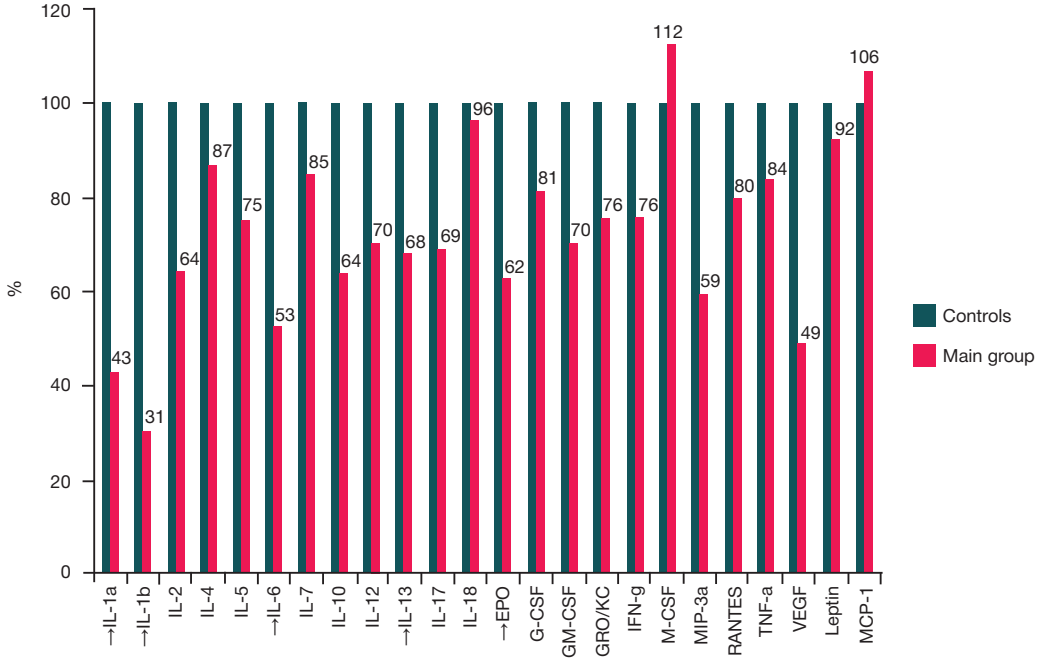

Fig. 2. Changes in cytokine levels in the blood serum of rats with induced EAE in comparison with the controls 5 days after the injection. Cytokine levels in the controls were taken as 100 %. Significant differences are marked with arrows

The levels of M-CSF stimulating proliferation of neutrophil precursors did not change throughout the experiment. The same pattern was observed for MIP-3a (CCL20) that protects mucosa from bacterial infection and for leptin that raises body temperature in infected individuals.

Hyperproduction of IL-4 and IL-10 in rats with EAE in the background of elevated IL-5, IL-13, and GM-CSF should be considered a factor stimulating proliferation of B-cells. In theory, this set of cytokines can trigger synthesis of oligoclonal antibodies, but this effect has not yet been described in the literature.

Our experiment proves that proliferation of myelin-specific lymphocytes can be triggered outside the CNS. However, the course of EAE in rats and the course of MP in humans differ considerably. We cannot rule out that the first event occurring at the onset of the disease is infiltration of the CNS by lymphocytes that do not undergo clonal expansion but do undergo further selection in the presence of excess myelin. Abnormal behavior of lymphocytes observed in the rat model can be a result of their primary clonal-nonspecific hyperproliferation triggered by systemic or local excess of lymphoproliferative factors or/and lymphotaxis factors originating in CNS. Another possibility is induction of abnormally rapid degradation of myelin in CNS leading to a massive release of degradation products into the systemic circulation. In this case the rat model seems to be quite adequate to the early stages of MS in humans.

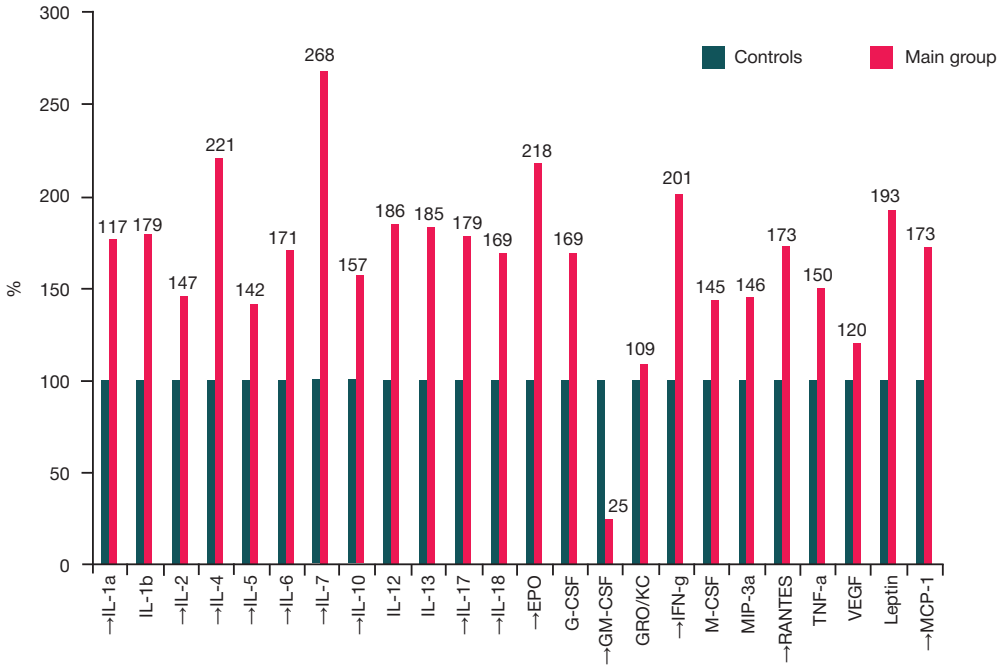

Fig. 3. Changes in cytokine levels in the blood serum of rats with induced EAE in comparison with the controls 7 days after the injection. Cytokine levels in the controls were taken as 100 %. Significant differences are marked with arrows

CONSLUSIONS

Data on the dynamics of cytokine production in rats with EAE obtained with the multiplex cytokine assay suggest that the rat model adequately imitates the course of MS in humans with respect to the levels of systemic lymphoproliferative and hematopoietic factors IL-1b, IL-2, IL-4, IL-5, IL-6 and IL-7. With respect to factors regulating taxis of lymphocytes, monocytes and other immune cells, the model fairly well imitates behavior of IL-17, RANTES (CCL-5) and MCP-1 (CCL-2), but exhibits a different dynamics for GRO/KC (CXCL1) levels. The model resembles the course of MS in humans in terms of IFNγ, IL-6 and IL-17 involved in cytotoxic and apoptotic reactions, but exhibits a different dynamics for TNF-α.

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