Cytokine profiles in intraocular fluids of patients with acute retinal necrosis

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Background: Acute retinal necrosis (ARN) is one of the most serious ocular diseases that cause uveitis. The clinical picture of ARN was first described by Urayama et al. in 1971 in the Japanese literature, and the disease later became known in Japan as Kirisawa type uveitis. Immune mediators play a critical role in the pathogenesis of ARN. A predominant Th1 cytokine profile characterized by high blood concentration of interferon-γ (IFN-γ) and low concentration of interleukin 4 (IL)-4 has been reported. However, limited data on cytokine concentrations in ocular fluids of patients with ARN are available, and they are based on studies with small sample sizes. In this study, we measured a panel of cytokines in the ocular fluids of patients with ARN as well as in controls.

Patients & Methods: We performed a retrospective medical chart review of all patients diagnosed with ARN and treated at the Ophthalmology Clinic of Tokyo Medical University Hospital (TMU) between 2003 and 2017, who were followed for a minimum of one year (total 36 patients) . Polymerase chain reaction (PCR) analysis was performed to determine the causative virus of ARN. Aqueous humor (AH) samples were obtained from 23 eyes with ARN (HSV: 5, VZV: 18) and 31 control eyes with cataract, epiretinal membrane or macular hole. Vitreous humor samples were obtained by vitrectomy from 28 eyes with VZV-ARN and 21 control eyes with macular hole or epiretinal membrane. AH samples from 16 patients with ARN were collected at the first visit to TMU before intravenous acyclovir therapy combined with systemic corticosteroids. AH samples from 14 patients with ARN were collected at the start of vitrectomy with lensectomy after intravenous acyclovir therapy combined with systemic corticosteroids. The vitreous samples from 28 patients with ARN were collected after intravenous acyclovir therapy combined with systemic corticosteroids, during vitrectomy with or without encircling prophylactic scleral buckles, in an attempt to prevent posterior pole detachment. Vitrectomy was performed using the three-port system to remove as much of the vitreous cortex as possible. Finally, the vitreous was substituted with silicone oil. Each sample was centrifuged (3000 rpm for 5 min), separated into the cellular component and supernatant, and frozen at -80ºC until use.

Twenty-four cytokines (IFN-γ, IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL10, IL-12p70, TNF-α, TNF-β, MCP-1, MIP-1α, MIP-1β, IP-10, RANTES, Mig, eotaxin, Fas ligand, VEGF, angiogenin, basic FGF, and GM-CSF) were measured using the Cytometric Bead Array Flex kit. All patients were immunocompetent.

Results:

- Intraocular fluid concentrations of IFN-γ, IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, IP-10, Mig, RANTES, bFGF, angiogenin, Fas L and VEGF were significantly higher in patients with ARN compared to controls.
- In patients with ARN, post-acyclovir treatment aqueous humor concentrations of IFN-γ, IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β and angiogenin were significantly lower compared to pre-treatment concentrations. However, these post-acyclovir treatment cytokine concentrations were still significantly higher compared to controls.

24 Cytokines & chemokines in this study

Th1 cytokines
- IFN-γ
- IL-1β
- IL-2
- IL-12p70
- TNF-α
- LT-α(TNF-β)

Th2 cytokines
- IL-5
- IL-10

Chemokines
- IL-8
- MCP-1
- MIP-1α
- MIP-1β
- IP-10
- RANTES
- Mig
- Eotaxin

Apoptosis
- Fas ligand

Growth Factors
- basic FGF
- GM-CSF
- IL-6
- IL-7
- IL-9

Neovascularization
- VEGF
- Angiogenin

Table 1. Patients characteristics

	ARN	Aqueous samples Controls	Vitreous samples controls
N	n=36	n=31	n=21
M:F ratio	23:13	11:20	7:14
Age (years)	47.9±16.7	67.1±9.0	69.3±8.8
disease	VZV:31 HSV: 5	Cataract: 20 MH: 5 ERM: 6	MH: 7 ERM: 14
Duration of acyclovir treatment(day)	8.8±16.9	0	0

Table 2 . Aqueous humor cytokine concentrations in two study groups

	ARN (pre-acyclovir)	Controls	p-value
IFN-γ (pg/ml)	471.0±547.4	0.9±1.8	<0.0001
IL-8 (pg/ml)	4745.0±6942.0	3.6±3.7	<0.0001
IL-10 (pg/ml)	157.0±149.3	0	<0.0001
MCP-1 (pg/ml)	15058.0±24570.4	400.4±187.8	<0.0001
MIP-1α (pg/ml)	49.0±44.6	0	<0.0001
MIP-1β (pg/ml)	338.0±351.2	8.2±8.2	<0.0001
IP-10 (pg/ml)	51287.0±67719.8	106.2±91.9	<0.0001
RANTES (pg/ml)	114.0±156.9	0.2±0.8	0.0007
bFGF (pg/ml)	18.0±33.3	0	0.108
IL-6 (pg/ml)	379763.0±1383943.5	2.7±4.0	<0.0001
Fas L (pg/ml)	1331.0±4748.2	5.3±6.9	<0.0001
VEGF (pg/ml)	234.0±314.1	25.7±20.1	0.383
Angiogenin (pg/ml)	20061.0±13679.2	5500.6±1669.4	<0.0001
Mig (pg/ml)	>100000±2.8	42.2±39.0	<0.0001
IL-1β, IL-2, IL-5, IL-7, IL-9, IL-12, TNF-α, GM-CSF, Eotaxin,	0	0	

Table 3. Aqueous humor cytokine concentrations in post-acyclovir treatment group and controls.

	ARN (post-acyclovir)	Controls	p-value
IFN-γ (pg/ml)	165.0±260.0	0.9±1.8	0.0012
IL-8 (pg/ml)	466.0±785.0	3.6±3.7	<0.0001
IL-10 (pg/ml)	66.0±92.0	0	<0.0001
MCP-1 (pg/ml)	3268.0±4315.0	400.4±187.8	<0.0001
MIP-1α (pg/ml)	13.0±18.0	0	0.0167
MIP-1β (pg/ml)	78.0.0±103.0	8.2±8.2	0.0781
IP-10 (pg/ml)	37808.0±65037.0	106.2±91.9	<0.0001
RANTES (pg/ml)	9.0±14.0	0.2±0.8	0.0951
bFGF (pg/ml)	3.0±9.0	0	0.425
IL-6 (pg/ml)	1805.0±2461.0	2.7±4.0	<0.0001
Fas L (pg/ml)	529.0±1434.0	5.3±6.9	0.0002
VEGF (pg/ml)	18.0±23.0	25.7±20.1	0.274
Angiogenin (pg/ml)	9727.0±4465.0	5500.6±1669.4	0.014
Mig (pg/ml)	307008.0±512878.0	42.2±39.0	<0.0001
IL-1β, IL-2, IL-5, IL-7, IL-9, IL-12, TNF-α, GM-CSF, Eotaxin,	0	0	

Table 4. Aqueous humor cytokine concentrations in pre- and post-acyclovir treatment groups.

	Pre-acyclovir	Post-acyclovir	p-value
IFN-γ (pg/ml)	471.0±547.4	165.0±260.0	0.0199
IL-8 (pg/ml)	4745.0±6942.0	466.0±785.0	0.001
IL-10 (pg/ml)	157.0±149.3	66.0±92.0	0.0126
MCP-1 (pg/ml)	15058.0±24570.4	3268.0±4315.0	0.0362
MIP-1α (pg/ml)	49.0±44.6	13.0±18.0	0.0167
MIP-1β (pg/ml)	338.0±351.2	78.0.0±103.0	0.0016
IP-10 (pg/ml)	51287.0±67719.8	37808.0±65037.0	0..228
RANTES (pg/ml)	114.0±156.9	9.0±14.0	0.0053
bFGF (pg/ml)	18.0±33.3	3.0±9.0	0.3604
IL-6 (pg/ml)	379763.0±1383943.5	1805.0±2461.0	0.0041
Fas L (pg/ml)	1331.0±4748.2	529.0±1434.0	0.2891
VEGF (pg/ml)	234.0±314.1	18.0±23.0	0.674
Angiogenin (pg/ml)	20061.0±13679.2	9727.0±4465.0	0.0159
Mig (pg/ml)	>100000±2.8	307008.0±512878.0	0.2148
IL-1β, IL-2, IL-5, IL-7, IL-9, IL-12, TNF-α, GM-CSF, Eotaxin,	0	0	

Table 5. Vitreous humor cytokine concentrations in two study groups.

	ARN	Controls	p-value
IFN-γ (pg/ml)	235.0±355.2	1.0±2.4	<0.0001
IL-8 (pg/ml)	1121.0±1836.3	8.6±13.8	<0.0001
IL-10 (pg/ml)	123.0±110.8	0	<0.0001
MCP-1 (pg/ml)	8884.0±7821.3	264.2±122.7	<0.0001
MIP-1α (pg/ml)	43.0±45.2	7.4±8.2	<0.0001
MIP-1β (pg/ml)	337.0±279.7	12.9±14.4	<0.0001
IP-10 (pg/ml)	30652.0±98157.2	552.8±2160.8	<0.0001
RANTES (pg/ml)	104.0±201,5	1.0±2.7	<0.0001
bFGF (pg/ml)	30.0±41.5	16.3±21.4	0.004*
IL-6 (pg/ml)	6921.0±20561.2	11.6.0±21.4	<0.0001
Fas L (pg/ml)	341.0±1153.9	3.2±7.2	<0.0001
VEGF (pg/ml)	40.0±138,3	19.5±66.1	0.002*
Angiogenin (pg/ml)	13894.0±13243.6	2182.8±1344.1	<0.0001
Mig (pg/ml)	>100000±8.3	96.6±323.6	<0.0001
IL-1β, IL-2, IL-5, IL-7, IL-9, IL-12, TNF-α, GM-CSF, Eotaxin,	0	0	*t-test

Conclusions: This study suggests that increases of several cytokines in the aqueous and vitreous humor of eyes with ARN may indicate a state of activation during inflammation. Since the concentrations of several cytokines were still high after acyclovir treatment, vitrectomy may be necessary for these patients. Further studies including correlation of cytokine profile with viral load and visual outcome will be planned.