Cytokine/chemokine changes in plasma of patients with MPO-ANCA RPGN: Before and after IVIg therapy

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Abstract

Myeloperoxidase (MPO) is a subset of anti-neutrophil cytoplasmic antibodies (ANCA) and patients with this disease are highly susceptible to infection. Intravenous immunoglobulin (IVIg) therapy is considered beneficial for individuals with weak immune systems or for patients with other diseases who need to ward off infections. It has also demonstrated efficacy in suppressing disease activity in MPO-ANCA RPGN. The purpose of this study is to discuss how IVIg therapy helps to alleviate MPO-ANCA RPGN by shedding light on the mechanism of the treatment.

Cytokines/chemokines have been implicated in the pathogenesis of MPO-ANCA RPGN, therefore we determined patients' plasma cytokine/chemokine levels before and after IVIg therapy using 27 plex and 12 plex array to observe any important changes. Therapy was supplied in two dosages, full and mini dose. We observed that post-treatment levels of RANTES, IL-1α, IL-2Ra, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β decreased from their pre-treatment levels in both full dose and mini dose patients. These results suggest that these cytokines and chemokine become highly activated and are closely connected to acute MPO-ANCA nephritis, and that the efficacy of IVIg therapy is due to a reduction in these highly activated cytokine/chemokines.

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated rapidly progressive glomerulonephritis (RPGN) leads to renal failure through systemic vasculitis accompanying diffuse crescentic glomerulonephritis; the representative diseases of ANCA are granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). There are two known types of ANCA and proteinase-3 (PR3)-ANCA which is highly associated with GPA-ANCA and myeloperoxidase (MPO)-ANCA which is associated with MPA. This latter form is more prevalent among RPGN patients in Japan[1,2,3] and it tends to be associated with high susceptibility to infection, which frequently results in poor survival rates for these patients[4].

Ito-Ihara et al. demonstrated that IVIg therapy was effective in treating MPO-ANCA RPGN, and that in these patients, pre-treatment Plasma TNF-α levels decreased after IVIg therapy[1,5]. The objective of this study is to shed light on the characteristics and pathogenesis of MPO-ANCA and to discuss the mechanism of IVIg therapy through cytokine/chemokine fluctuations. To this end, we measured plasma cytokine/chemokine levels before and after IVIg therapy using bio-plex Group I and Group II multiplex array.

Methods

Subjects

Fifty-one patients with MPO-ANCA-RPGN were enrolled in this study. Patients were admitted to Kitano Hospital (Osaka, Japan) between January 2001 and February 2011. All patients were diagnosed with microscopic polyangitis (MPA) by the classification of Watt et al., which adheres to the definition of MPA described by the Chapel Hill Consensus Conference 2012[6]. Ethical approval was obtained from the Tazuke Kofukai Medical Research Institute Ethical Committee at Kitano Hospital.

Nineteen healthy subjects were selected from individuals who received routine health checks at Louis Pasteur Center for Medical Research (Kyoto, Japan) between January 2008 and February 2011. Healthy subjects were aged over 60, and had no history of cancer, chronic infectious diseases, autoimmune diseases, nephritis, nor asthma. Approval was obtained from the Louis Pasteur Center for Medical Research Ethical Committees (LPC.8). All participants gave written informed consent.

Treatment protocol for IVIg

Eight of the 51 MPO-ANCA -RPGN patients were treated with IVIg therapy, patient profiles are shown in Table 1. Four patients were given the full dose immunoglobulin administered intravenously once daily for 5 consecutive days (400 mg/kg/day) (Kenketus Venilon-I, Teijin Co., Ltd., Tokyo). The other four patients were treated with mini doses intravenously administered once daily for 3 consecutive days (500 mg /day). This 500 mg /day is the standard IVIg dose covered by the Japanese national health insurance for patients with severe diseases. Muso et al. separately reported clinical course and outcome which include these cases[7].

Cytokines/chemokines assay

Cytokines and chemokines were quantified using Bio-Plex 200, a multiplex cytokine array system (Bio-Rad Laboratories,

Table 1. Clinical data of patients before and after IVIg therapy

Patient No.	Age	F/M	Blood drawn interval (days)	Type of IVIg	WBC(x100/ml)		CRP(mg/dl)		Cre(mg/dl)	
					IVIg Before	IVIg After	IVIg Before	IVIg After	IVIg Before	IVIg After
1	68	M	15	400 mg/kg/day for 5 days	94	80	<0.1	0.13	2.07	2.53
2	81	F	6	400 mg/kg/day for 5 days	131	110	10.87	9.38	1.33	1.47
3	67	M	4	400 mg/kg/day for 5 days	187	137	12.84	3.47	2.77	2.13
4	88	F	5	400 mg/kg/day for 5 days	105	114	11.82	11.23	3.18	3.17
5	88	F	6	500mg/day for 3days	58	62	0.89	0.3	0.97	0.92
6	84	M	6	500mg/day for 3days	83	107	6.36	3.94	3.35	3.24
7	64	F	6	500mg/day for 3days	106	142	5.41	0.54	5.86	7.35
8	68	M	3	500mg/day for 3days	113	80	2.42	4.89	4.24	4.83

CA, USA) according to the manufacturer's instructions. Heparinized blood plasma from all 51 MPO-ANCA RPGN patients and healthy subjects were collected and centrifuged at 1,600 g for 10 min, and frozen at −80 °C until they were analyzed. We simultaneously quantified Bio-Plex Human Cytokine 27-Plex Panel and Group II -12plex array (IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-bb, RANTES, TNF-α, VEGF, IL-1α, IL-2Rα, IL-3, IL-12p40, IL-18, CTACK, HGF, M-CSF, MIF, MIG, SCF, TNF-β). Data acquisition and analysis were performed using Bio-Plex Manager software version 5.0.

The cytokine/chemokine values in MPO-ANCA RPGN patients and healthy controls were log-transformed and used for our analysis. All statistical analyses were carried out with JMP 9.0 software.

Results

Table 2 shows a comparison of cytokine/chemokine profiles in MPO-ANCA patients and healthy controls. Among 39 cytokine/chemokines measured, 23 were significantly higher in MPO-ANCA-patients than healthy controls.

Table 3 summarizes the clinical score and cytokine/chemokine characteristics of patients before and after full or mini dose IVIg therapy. Compared with patients treated with full dose IVIg, patients administered the mini-dose therapy showed lower cytokine/chemokine levels except for IL-12p40 and SGF (Table 3). Most cytokine/chemokine levels decreased after IVIg therapy, particularly RANTES, IL-1α, IL-2R α, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β significantly decreased in both full dose and mini dose IVIg therapy patients.

Patients treated with IVIg therapy had good prognosis and survived more than two years except one case who moved to another hospital (data not shown).

Discussion

MPO-ANCA RPGN patients are known to be highly susceptible to infection and often die due to complications arising from these infections. Ito-Ihara *et al*. have demonstrated that IVIg therapy resulted in good prognosis and low risk of renal failure[1]. In this paper, we compared MPO-ANCA patients' plasma cytokine/chemokine levels before and after IVIg therapy and compared these level with those of healthy subjects.

As shown in Table 2, pre-treatment cytokine/chemokine levels were higher in most MPO-ANCA RPGN patients than in healthy controls. Although it has been reported that several cytokine/chemokines are elevated in this disease, we believe

Table 2. Comparison of cytokine/chemokine values between MPO-ANCA patients and healthy subjects

	Healthy subjects	MPO-ANCA patients	p
log pg/ml	n=19	n=51	
IL-1b	−0.51±0.16	0.05±0.10	0.0031
IL-1ra	1.89±0.08	2.26±0.05	0.0001
IL-2	0.59±0.18	0.45±0.11	0.498
IL-4	−0.27±0.12	−0.03±0.08	0.106
IL-5	−0.37±0.14	0.41±0.09	<0.0001
IL-6	0.61±0.10	1.52±0.06	<0.0001
IL-7	−1.33±0.22	0.27±0.13	<0.0001
IL-8	1.16±0.07	1.32±0.04	0.069
IL-9	1.12±0.12	1.61±0.07	0.0008
IL-10	0.26±0.14	0.56±0.09	0.076
IL-12	−0.30±0.17	0.77±0.11	<0.0001
IL-13	−0.17±0.13	0.430±0.08	0.0003
IL-15	0.00±0.13	0.39±0.08	0.0096
IL-17	0.51±0.14	0.40±0.09	0.521
Eotaxin	1.39±0.15	1.24±0.10	0.415
FGF basic	0.95±0.19	1.06±0.11	0.605
G-CSF	−0.17±0.16	0.42±0.10	0.003
GM-CSF	1.19±0.15	1.50±0.09	0.082
IFN-g	0.50±0.22	1.22±0.14	0.0075
IP-10	2.37±0.06	2.90±0.04	<0.0001
MCP-1	1.75±0.06	1.71±0.04	0.637
MIP-1a	0.32±0.14	0.63±0.09	0.074
MIP-1b	2.00±0.53	2.03±0.03	0.659
PDGF-bb	2.19±0.13	3.00±0.08	<0.0001
RANTES	3.14±0.02	3.21±0.01	0.0024
TNF-a	0.82±0.20	0.88±0.12	0.778
VEGF	1.06±0.12	1.83±0.07	<0.0001
IL-1a	−0.50±0.13	−0.31±0.08	0.235
IL-2Ra	1.79±0.12	2.27±0.07	0.0007
IL-3	1.59±0.17	1.19±0.11	0.055
IL-12p40	0.93±0.23	1.22±0.14	0.296
IL-18	1.16±0.10	1.54±0.06	0.0012
CTACK	2.75±0.08	2.75±0.05	0.955
HGF	2.29±0.11	2.67±0.06	0.0029
M-CSF	1.32±0.09	1.79±0.06	<0.0001
MIF	1.58±0.11	2.20±0.07	<0.0001
MIG	2.68±0.09	3.51±0.06	<0.0001
SCF	2.13±0.08	2.39±0.05	0.0079
TNF-b	−0.15±0.14	0.36±0.09	0.0033

that to date this report demonstrates the most complete range of cytokine/chemokines that are elevated in MPO-ANCA RPGN.

Both full dose and mini dose therapies were effective and their outcomes were good even for patients who were over sixties. Our data demonstrated that RANTES, IL-1α, IL-2R α, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β, decreased significantly after IVIg therapy regardless of whether patients were administered a full or partial dose. Our previous study[3] demonstrated that TNF-α levels decreased significantly after IVIg therapy, and we observed the same tendency in this study. TNF-α levels decreased in three patients after IVIg therapy, two patients had levels that were unchanged and two patients showed increased TNF-α levels. A larger sample size would be ideal to fully assess the degree to which TNF-α levels actually decrease after IVIg therapy. Elevated IL-1α and IL-18 are well known to lead to cytokine storm and sepsis[8]. It is difficult to say which cytokine or chemokine is the most important to manipulate to halt the pathogenesis of MPO-ANCA

Table 3. Cytokine/chemokine values before/after IVIg therapy

	IVIg Before	IVIg After	Prob>|t|	Mini IVIg Before	Mini IVIg After	Prob>|t|	IVIgBefore vs Mini IVIg Before
log pg/ml	Mean	Mean	p	Mean	Mean	p	p
WBC(x100/μl)	129.6	110.3	NS	90.0	97.8	NS	NS
CRP(mg/l)	8.9	6.1	NS	3.8	2.4	NS	NS
Cre(mg/dl)	2.3	2.5	NS	3.6	4.1	NS	NS
IL-1b	0.68	1.01	NS	−0.19	−1.02	NS	NS
IL-1ra	2.47	2.30	NS	2.47	2.30	NS	NS
IL-2	1.04	0.95	NS	1.02	0.00	NS	NS
IL-4	0.37	0.68	NS	−0.27	−1.03	0.022	NS
IL-5	1.47	1.70	NS	0.34	−0.16	NS	0.045
IL-6	2.14	1.86	NS	1.54	0.93	0.031	0.009
IL-7	0.56	0.75	NS	0.08	−0.88	0.083	NS
IL-8	1.30	1.40	NS	1.44	1.13	0.021	NS
IL-9	1.70	1.30	NS	1.39	1.10	NS	NS
IL-10	1.30	1.26	NS	0.82	0.09	0.055	0.066
IL-12	1.17	1.30	NS	0.65	0.28	NS	NS
IL-13	0.96	1.05	NS	0.26	−0.45	NS	NS
IL-15	1.11	0.40	NS	0.94	0.00	NS	NS
IL-17	0.80	0.36	NS	0.54	0.00	NS	NS
Eotaxin	1.57	1.01	NS	0.64	0.31	NS	NS
FGF basic	1.75	1.21	NS	1.24	0.00	NS	NS
G-CSF	1.27	1.14	NS	0.39	−0.70	0.067	NS
GM-CSF	2.13	2.02	NS	1.22	0.93	NS	NS
IFN-g	1.89	2.15	NS	0.83	0.00	NS	NS
IP-10	2.70	2.79	0.012	2.97	2.88	NS	NS
MCP-1	2.00	1.91	NS	1.85	1.80	NS	NS
MIP-1a	1.37	0.93	NS	0.91	0.00	NS	NS
MIP-1b	2.10	2.06	NS	1.99	1.93	NS	NS
PDGF-bb	3.26	2.92	NS	2.63	2.95	NS	NS
RANTES	3.21	3.12	0.021	3.19	3.13	0.052	NS
TNF-a	1.78	2.04	NS	0.85	0.00	NS	NS
VEGF	2.25	1.99	NS	1.84	1.68	NS	NS
IL-1a	0.25	−0.50	0.004	−0.10	−0.69	0.035	NS
IL-2Ra	2.82	2.09	0.018	2.33	1.65	0.001	NS
IL-3	1.76	0.91	0.010	1.99	1.05	0.003	NS
IL-12p40	1.26	0.23	0.058	2.52	1.72	0.008	0.044
IL-18	1.97	1.28	0.004	1.74	1.12	0.015	NS
CTACK	2.83	2.25	0.018	3.14	2.52	0.004	0.090
HGF	3.12	2.53	0.025	3.06	2.28	0.001	NS
M-CSF	2..1593	1.31	0.002	2.06	1.08	0.002	NS
MIF	2.31	2.05	NS	2.01	1.67	NS	NS
MIG	3.59	3.05	0.001	3.70	3.14	0.002	NS
SCF	2.48	1.82	0.002	2.78	2.10	0.000	0.038
TNF-b	1.09	0.26	0.002	0.71	0.03	0.004	NS

RPGN. What we can say from the results of our study that a general decrease in elevated cytokine/chemokine tends to prevent the onset of cytokine storm.

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