Two types of myeloperoxidase-antineutrophil cytoplasmic autoantibodies with a high affinity and a low affinity in small vessel vasculitis

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ABSTRACT

Objective. *Myeloperoxidase (MPO) -anti-neutrophil cytoplasmic autoantibodies (ANCAs) are detected at a high rate in microscopic polyangiitis and renal-limited vasculitis. MPO-ANCA titers are not always reflected in the disease activity. We studied the titer and affinity of MPO-ANCA in sera from patients in relation to vasculitis activity.*

Methods. *Blood samples were collected from 27 newly diagnosed or relapsed patients with MPO-ANCA-associated vasculitides. The MPO-ANCA titer was determined by a direct enzyme-linked immunosorbent assay (ELISA) using homogeneously purified human MPO of leukocytes. The MPO-ANCA affinity was expressed as IC50 that was determined by a competitive inhibition method using the ELISA.*

Results. *The MPO-ANCA affinity of 27 sera from 27 patients could be classified into a high-affinity type (14 sera) and a low-affinity type (13 sera). The mean values for IC50 in the two types were 0.15±0.06 μg/ml and 0.54±0.15 μg/ml, and the difference was statistically significant ($p<0.0000000684$). Between the two groups of patients divided by the affinity, there were differences in the Birmingham Vasculitis Activity Score (BVAS): and in C-reactive protein (CRP): ($p<0.00093$ and $p<0.00129$, respectively). However, the difference in titer was not statistically significant ($p<0.0265$). The affinity remained steady from the disease onset to remission or relapse.*

Conclusions. *The affinity of MPO-ANCA from patients with MPO-ANCA-associated vasculitides were largely distinguished into a high and a low affinity, irrespective of the level of MPO-ANCA titers, and may be helpful for assessment of vasculitis activity affecting mainly the kidney and the lung.*

Introduction

Proteinase 3 (PR 3) - anti-neutrophil cytoplasmic autoantibodies (ANCAs) are frequently detected in Wegener's granulomatosis, while myeloperoxidase (MPO)-ANCAs are detected at a high rate in microscopic polyangiitis and renal-limited form of vasculitis (RLV). These diseases are referred to as ANCA-associated vasculitides (1). The level of disease activity of these ANCA-associated vasculitides and the ANCA titer have been found to generally fluctuate in close correlation (1). There are patients with high MPO-ANCA titers but almost no disease activity, as well as patients with low or negative titers but a high level of vasculitis activity (2-4). However, the acute 3 or 4 fold increase in PR-3 and MPO-ANCA titer is used as an important index when monitoring disease activity (5, 6). MPO-ANCA-associated vasculitides are more common in Asian countries than in the USA or Europe where 80% of ANCA associated vasculitis is PR-3 ANCA (7). In Japan, MPO-ANCAs have been reported to account for 90% of patients with ANCA-associated vasculitides (8-10). The relationships between the MPO-ANCA titer and the affinity in patients with small vessel vasculitis are not comprehensively understood (11, 12). Here we report the two types of MPO-ANCA having a high affinity and a low affinity in small vessel vasculitis.

Materials and methods

Between January 2005 and August 2008, blood samples were collected from 27 patients with ANCA-associated vasculitides who could be periodically followed up at Tokyo Medical University Hachioji Medical Center. These patients were either newly diagnosed or relapsed cases of ANCA-associated vasculitides, and each had

given written informed consent to participation in the clinical study. The titer and affinity of the serum MPO-ANCA were determined prior to the start of therapy or at the time of relapse. The MPO-ANCA titer was determined by a standard procedure of a direct enzyme-linked immunosorbent assay (ELISA) (13) using a homogeneously purified human MPOIII of peripheral blood leukocytes (50 μl of 1 μg/ml) for coating solid phase surface (14). IC_{50} was taken as an MPO-ANCA affinity. IC_{50} was the concentration of MPO which produces 50% inhibition of MPO-ANCA binding at the dose response curve in the liquid phase of ELISA. Briefly, the MPO (1 μg/ml) (14) was immobilized on the surface of microtiter wells at 50 μl/well, and then 1% skimmed milk-phosphate buffered saline – 0.05% Tween 20 was added to each well as a blocking agent. Each of the serum samples was first suitably diluted to adjust its OD by the direct ELISA to about 1.0 (measured at 405 nm), and then each diluted serum sample (25 μl/well) and serially diluted MPO (0-100 μg/ml; 25 μl/well) were simultaneously added to the MPO-coated plates and incubated for 1h at 37°C. Then, the bound IgG was detected using the system consisting of anti-human IgG alkaline phosphatase conjugate (1 h 37°C) and colour development at 405 nm with substrate (0.5 h 37°C). The competitive inhibition rate (%) was calculated by defining the inhibition at the highest MPO concentration (50 μg/ml) as 100%. An approximation curve was drawn based on the assay results, and then the liquid-phase MPO concentration causing 50% competitive inhibition (IC_{50}) of MPO-ANCA binding to the solid-phase MPO was calculated from the curve.

Each assay was performed in duplicate and all serum samples were assayed at least 2-3 times.

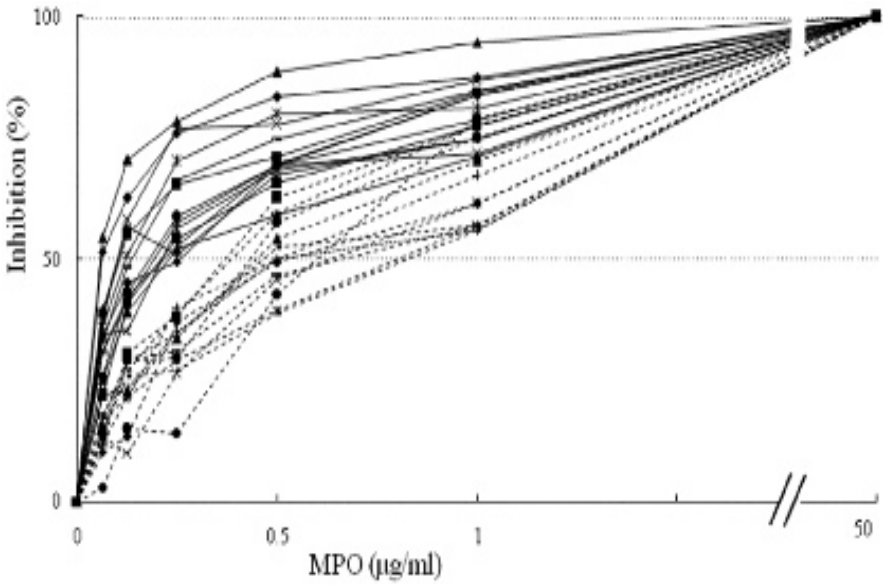
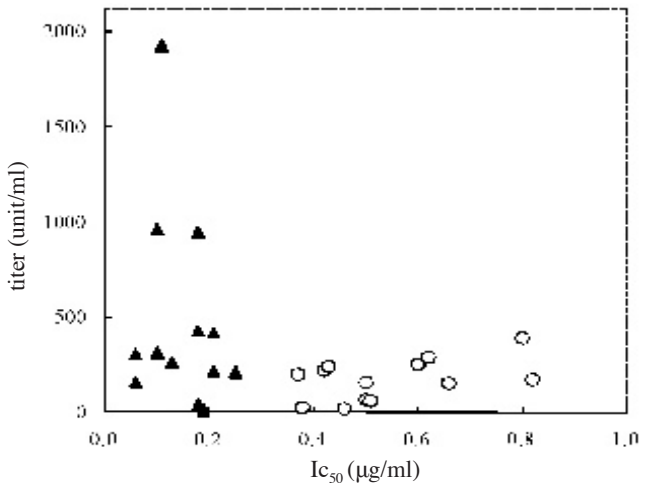


Fig. 1. MPO-inhibition of MPO-ANCA in ELISA.
The MPO-ANCA affinity (IC50) of the 27 patients with MPO-ANCA associated vasculitides could be classified into a high-affinity group (solid line 14 patients) and a low-affinity group (dotted line 13 patients). Each point was assayed in duplicate.

Fig. 2. Correlation of MPO-ANCA titer and affinity (IC_{50}).
solid triangle (▲), high affinity group (n=14)
open circle (○), low affinity group (n=13)



Results

Most of antigen specificity for MPO-ANCAs was confirmed on the basis of 100% inhibition by adding an excess antigen of 50 μg/ml to the liquid phase (Fig. 1). In few MPO-ANCAs, 5-10% MPO-uninhibitable binding activity was observed. This amount was excluded in 100% inhibition because the more excess antigen did not increase inhibition. Figure 1 showed that IC_{50} of sera of the 27 patients with MPO-ANCA-associated vasculitides could be classified into a high-affinity type (14 patients) ranging from 0.06 to 0.25 μg/ml and a low-affinity type (13 patients) ranging from 0.37 to 0.82 μg/ml. Figure 2 showed that correlation of MPO-ANCA titer and affinity (IC_{50}) of the 27 patients with MPO-ANCA associated vasculitides. As shown in Table I, the mean values ± standard deviation for IC_{50} in the high affinity type were 0.15±0.06 μg/ml and those in the low affinity type were 0.54±0.15 μg/ml, and the difference between the two types was statistically significant (p<0.0000000684). The Birmingham Vasculitis Activity Score (BVAS) (15) and C-reactive protein (CRP), which are clinical indicators of vasculitis activity (16, 17), were 18±6 and 10.8±7.7, respectively, in the high-affinity patient group (14 patients) and 11±8 and 2.8±4.1, respectively, in the low-affinity patient group (13 patients). There were statistically significant differences in BVAS and CRP scores between the two groups (BVAS: p<0.000933; CRP: p<0.00129). Mean MPO-ANCA titer in the high affinity group was 465±507 unit/ml (ranging from 13 to 1926 unit/

Table I. MPO-ANCA affinity and clinical data.

Patient	Age	Gender	BVAS	Renal symptoms RPGN/CRF	Respiratory symptoms ARDS/CIP	CRP (mg/dl)	MPO-ANCA titers (ELISA unit/ml)	MPO-ANCA affinity IC50 (μg/ml)	Treatment
Group 1. High affinity (n=14)									
1	79	F	15	+/-	-/-	4.60	316	0.10	CS
2	58	M	22	+/-	-/-	9.00	261	0.13	CS, IS
3	72	M	22	+/-	-/-	13.00	210	0.25	CS, IS, PE, HD
4	79	F	9	-/-	+/-	10.00	38	0.18	CS
5	56	F	9	-/-	+/-	7.00	307	0.06	CS
6	78	M	22	+/-	-/-	31.30	965	0.10	CS, IS, PE,
7	81	M	22	+/-	-/-	14.60	1926	0.11	CS, IS, PE,
8	88	F	15	+/-	-/-	3.50	947	0.18	CS, IS, PE,
9	65	M	15	-/-	+/-	20.00	13	0.19	CS, IS
10	89	F	22	+/-	+/-	8.10	429	0.18	CS, IS, PE, HD
11	74	M	9	+/-	-/-	1.60	418	0.21	CS, IS
12	81	F	21	-/+	+/-	15.12	158	0.06	CS
13	77	F	20	-/+	-/-	7.50	306	0.10	CS, IS
14	63	F	26	-/+	-/-	6.42	213	0.21	CS, IS
mean	74	M6:F8	● 18±6	8(57%)/3(21%)	5(36%)/0(0%)	■ 10.8±7.7	▲ 465 (±507)	◆ 0.15 (±0.06)	
Group 2. Low affinity (n=13)									
15	77	M	12	-/+	-/+	0.35	156	0.50	CS
16	68	M	3	-/+	-/+	0.10	19	0.46	not done
17	65	F	3	-/+	-/-	0.10	150	0.66	CS
18	56	M	8	-/+	-/-	0.10	254	0.60	CS
19	63	F	15	-/+	-/+	0.50	388	0.80	CS
20	61	M	12	+/-	-/-	0.10	222	0.42	CS
21	82	F	9	-/+	-/-	6.00	64	0.50	CS
22	76	F	14	+/-	-/-	8.00	171	0.82	CS, IS
23	63	F	4	-/+	-/-	1.30	22	0.38	not done
24	61	M	15	-/+	-/+	7.00	59	0.51	CS, IS
25	67	M	12	-/+	-/+	0.10	199	0.37	CS
26	63	M	19	-/+	-/+	12.00	241	0.43	CS, IS
27	77	F	12	+/-	-/+	0.10	288	0.62	CS
mean	68	M7:F6	● 11±8	3(23%)/10(77%)	0(0%)/7(54%)	■ 2.8±4.1	▲ 172 (±110)	◆ 0.54 (±0.15)	

CS: corticosteroid: prednisolone; PE: plasma exchange; IS: immunosuppresant (cyclophosphamide, azahathiopurine, mizoribine etc); HD: hemodialysis; RPGN: rapidly progressive renal failure; CRF: chronic renal failure; ARDS: acute respiratory distress syndrome (acute interstitial pneumonitis/lung bleeding); CIP: chronic intestitial pneumonitis.
Differences between Group 1 and Group 2; ● $p<0.000933$; ■ $p<0.00129$; ▲ $p<0.0265$; ◆ $p<0.0000000684$ (Welch T-test).
BVAS, CRP, MPO-ANCA at the time of first diagnosis or relapse.
All cases from 9 to 18 months long term observation of MPO-ANCA titer and affinity.

ml) and that in the low affinity group 172±110 unit/ml (ranging from 22 to 388 unit/ml). The criterion of the difference in titer between the two groups for statistically significance was the 0.03 level ($p<0.0265$). The high affinity group (n=14) included mainly cases with severe vasculitis disease activity (*i.e.* rapidly progressive glomerulonephritis (RPGN) 57% and/or acute respiratory distress syndrome (ARDS) 36%), while the low affinity group (n=13) included mainly cases with mild vasculitis activity (*i.e.* chronic renal failure (CRF) 77% and/or chronic interstitial pneumonitis (CIP 54%)). In addition, time-course determinations of the ANCA titer and IC_{50} were performed using serum samples from 10 of 27 patients. Figure 3 shows the representation time course in groups 1 and 2. In patient 6 of group 1, the MPO-ANCA titer decreased from 2800 (units/ml) to 250 (units/ml) during the 12-month period from the early stage of RPGN after onset until remission was achieved (serum creatinine from 14 mg/dl to 1.5 mg/dl and CRP from 31 mg/dl to 0.3 mg/dl), but IC_{50} was maintained at approximately 0.15 μg/ml during the same period. In patient 19 of group 2 of CRF (serum creatinine from 2.8 mg/dl to 2.1 mg/dl and CRP from 2.8 mg/dl to 2.0 mg/dl) and CIP, the MPO-ANCA titer decreased from 820 (units/ml) to 300 (units/ml) during an 10-month period with a relapse, but IC_{50} remained steady at 0.5 and 0.6 μg/ml, respectively, with no statistically significant change during the time-course (Fig. 3).

Discussion

We set out to determine whether MPO-ANCA activity could be a potential biomarker of the activity of ANCA-associated vasculitides. This study indicated the occurrence of the two types of MPO-ANCA in vasculitis: one with a high affinity (IC_{50}=0.15±0.06 μg MPO/ml) and one with low affinity (IC_{50}=0.54±0.15 μg MPO/ml). High

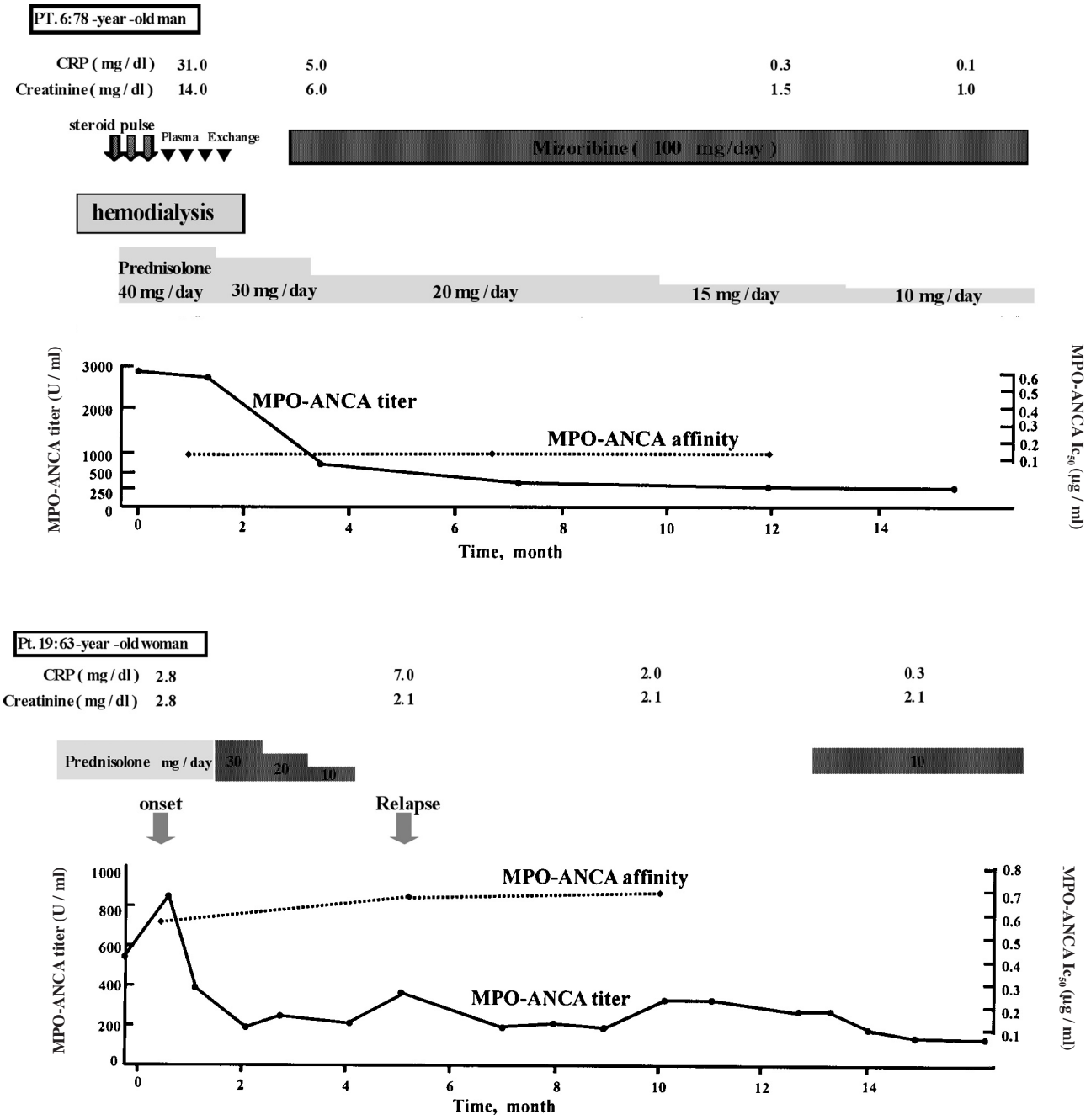


Fig. 3. Clinical course of titer and IC 50 of MPO-ANCA in patients with renal limited form of vasculitis.
Upper panel: patient 6 (Group 1) with rapidly progressive glomerulunephritis;
Lower panel: patient 19 (Group 2) with chronic renal failure.

affinity MPO-ANCAs were found in patients with mean BVAS of 18±6 with severe vasculitis disease activity (16, 17) (*i.e.* RPGN and/or ARDS) and low affinity MPO-ANCAs in patients with mean BVAS of 11±5 with mild vasculitis disease activity (16, 17) (*i.e.* CRF and CIP). The distinctive difference in affinity between the two types suggests that the number of antibody clones produced during disease was limited. In addition, this notion is also supported by the observation in which no change in affinity of MPO-ANCA was longitudinally found from disease onset to remission or to the time of relapse. Native MPO is an N-glycosylated heme protein, which is a form of homodimer each consisting of a light chain and a heavy chain (18). Epitope mapping for MPO-ANCAs has indicated that epitopes are restricted to mostly either an N-terminal 131 amino acid residues region or a C-terminal 228 amino acid residues region in the heavy chain, depending on the use of a panel of recombinant polypeptides or a series of recombinant human-mouse chimeric forms in a whole molecule (19, 20), respectively. These findings are also consistent with the notion that the antibody producing clones in each individual patient are limited. However,

whether these epitopes are associated with the affinities given for native MPO in this work remains to be clarified. Importantly, the finding that MPO ANCAs with high or low affinity are found in patients with vasculitides will aid in mapping pathogenic epitope(s). As shown in this work, no correlation between MPO-ANCA titer and affinity was found as similarly as reported by others (11). In the longitudinal study the affinity remained unchanged during the follow-up. On the contrary, the two other groups reported that high apparent affinity constants aK of MPO-ANCAs in systemic vasculitis and propylthiouracil-induced vasulitis decrease to very low values within the short period of follow-up (11, 12). Discrepancies in affinity among these studies remain unsolved. The impurity of used MPO preparations and the presence of non-specific IgG binding to MPO-ELISA wells in some serum samples may affect affinity determination.

The present report is the first regarding the contribution of MPO-ANCA affinity in ANCA-associated vasculitides. Our results suggest that the affinity may be a useful index for assessment of the activity of vasculitis in the early period after disease onset and at the time of relapse. They also suggest that the chronological determinations of both titer and affinity of MPO-ANCA from the disease onset to the time of remission or relapse may be helpful for the assessment of the disease activity.

Conclusions

The affinity of MPO-ANCAs from patients with MPO-ANCA associated vasculitides were largely distinguished into a high and low affinity, irrespective of the level of MPO-ANCA titers.

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