examined (Fig. 3). fMLP-induced MPO release from neutrophils was enhanced after CAWS injection (Fig. 3b), while spontaneous MPO release was not changed (Fig. 3a). In addition, both fMLP and PMA-induced superoxide generation was enhanced following in vivo CAWS injection (Figs. 3c, d).

CAWS effects on proinflammatory cytokine production

Since activation of neutrophils was observed, after in vivo injection of CAWS, proinflammatory cytokines levels in plasma were measured (Fig. 4). IL-12 p70 production was increased IL-1β (b), IL-10 (c), 1L-6 (d) significantly increased CAWS injection. Levels of MIP-2 and G-CSF in plasma increased after CAWS injection and 16 h after CAWS injection (Figs. 4e, f). On the other hand, IL-18, TNF-α, INF-γ, GM-CSF were not detected up to 16 h after CAWS injection (data not shown). Since IL-1β, IL-10, IL-6 was significantly increased, production of these cytokines by casein-induced neutrophils was also measured (Fig. 5). IL-6 production was enhanced by exposure of neutrophils to CAWS (Fig. 5c), while IL-11L-10 was nearly the same in presence or absence of CAWS (Figs. 5a, b).

CAWS effect of ICAM-1 expression and soluble ICAM release

Sine ICAM-1 is a marker of activation of endothelial cells, we ICAM-1 gradually increased in plasma after CAWS injection (Fig. 6a). In addition, ICAM-1 the thoracic aortic wall was also significantly increased 16 h after CAWS injection (Fig. 6b).

CAWS C3 activation

Activation of C3 was examined (Fig. 7). C3 decreased time dependently after CAWS injection and gradually (Fig. 7a). This was confirmed by Western blotting analysis (Fig. 7b). These results suggest that neutrophil activation triggered through complement activation by a single injection.

Discussion

In study, we focused on neutrophil activation related to development of coronary arteritis. Single injection of CAWS at a dose of 4 mg/mouse induced coronary arteritis 4 weeks featuring neutrophil accumulation in the coronary arterial wall. This observation was similar to the

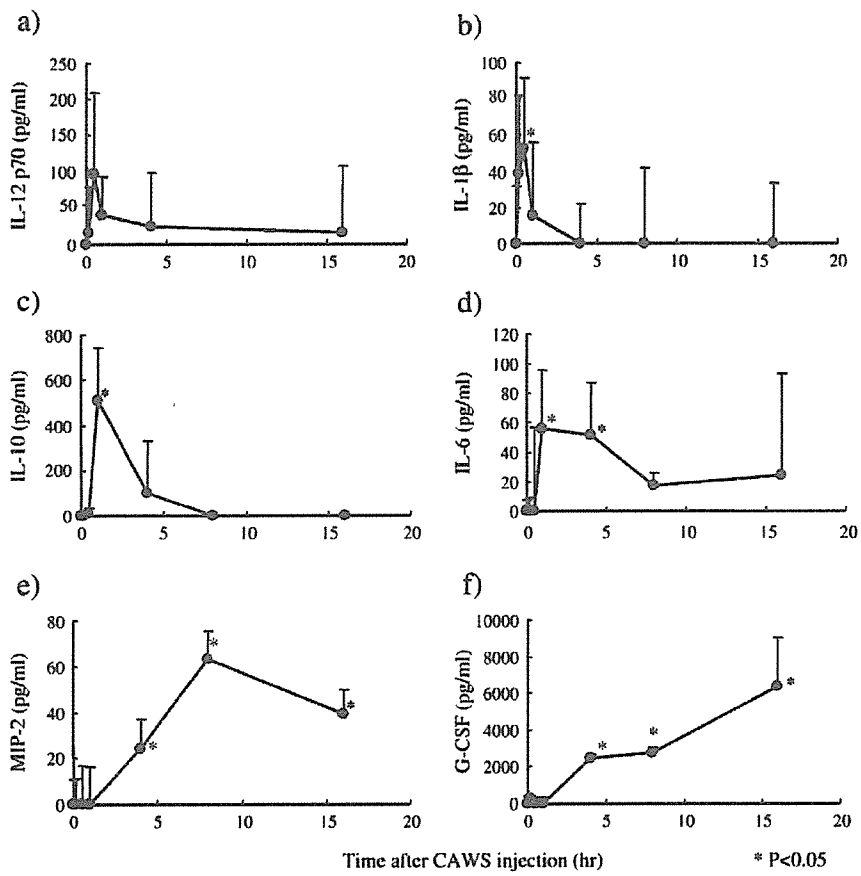

Fig. 4. Cytokine after CAWS injection. Heparinized blood was obtained from heart after CAWS injection. Plasma was separated and proinflammatory cytokines level was measured by ELISA kit. IL-12 p70, (b) IL-1β, (c) IL-10, (d) IL-6, (e) MIP-1, (f) G-CSF, respectively. $N=6$ in each group.

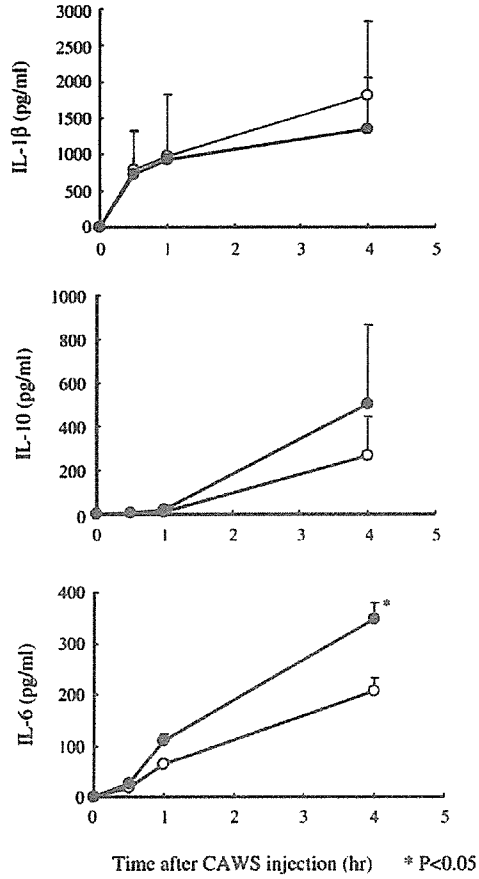

Fig. 5. Cytokine production by neutrophil. Casein-induced neutrophil (5×10^6 cells/ml) suspended in RPMI-1640 containing 0.3 mM PMSF and 1.4 μg/ml aprotinin and co-cultured with 1 mg/ml CAWS for 0.5 to 4 h. culture supernatant was prepared by centrifugation and level of cytokine. (a) IL-1β, (b) IL-10 and (c) IL-6, respectively. ○ not treated by CAWS ● 1 mg/ml of CAWS. N=3–6 in each group.

development of coronary arteritis daily injection of CAWS (4 mg/mouse/day) 5 days (Nagi-Miura et al., 2004; Ohno, 2003).

Blood neutrophils and subsequently monocyte increased, while lymphocyte counts decreased. In addition, the ratio of Gr-1 cells in bone marrow decreased, suggesting migration of neutrophils from bone marrow into peripheral blood after CAWS injection. G-CSF after CAWS injection, fMLP-induced MPO release from neutrophils was enhanced as was PMA and fMLP-induced superoxide generation in vivo injection. These results suggest that number of blood neutrophils their activation early after CAWS injection.

Levels of IL-1β, IL-6, and IL-10 significantly increased in plasma after CAWS injection. These cytokines are also blood leukocytes live *C. albicans* (Netea et al., 2002; Gasparoto et al., 2004). The of cytokines seems to be different depend on *Candida* strain, component and virulence (Villar et al., 2005). With regard to casein-induced neutrophils, IL-6 was significantly enhanced by co-culture with CAWS, but IL-1β and 1L-10 production virtually unchanged in presence of CAWS. These results suggest IL-6 production especially enhanced by CAWS. MIP-2 and G-CSF in plasma were maintained for 16 h, neutrophil levels in blood MIP-2 and G-CSF in blood.

Because of increase and activation of peripheral neutrophils, complement activation products may well be candidate because C5a is a chemoattractant for neutrophils (Guo and Ward, 2005). Ohno et al. have already demonstrated activation of the lectin complement pathway by CAWS (unpublished). Mullick A. et al. have confirmed dysregulated cytokine response during *C. albicans* infection (Mullick et al., 2004).

Soluble ICAM-1, which is a marker of activated endothelial cells (Iiyama et al., 1999), in blood increased after CAWS injection. In addition, ICAM-1 message was significantly increased in the thoracic aortic wall 16 h after CAWS injection. Both systemic and local increases in ICAM-1 could be involved to subsequent endothelial cell lesion development (Di Lorenzo et al., 2004).

In summary, increased numbers and activation of peripheral blood neutrophils are the initial events after CAWS injection, perhaps followed by macrophage activation and adaptive immune responses. Neutrophil a primary role in biodefense

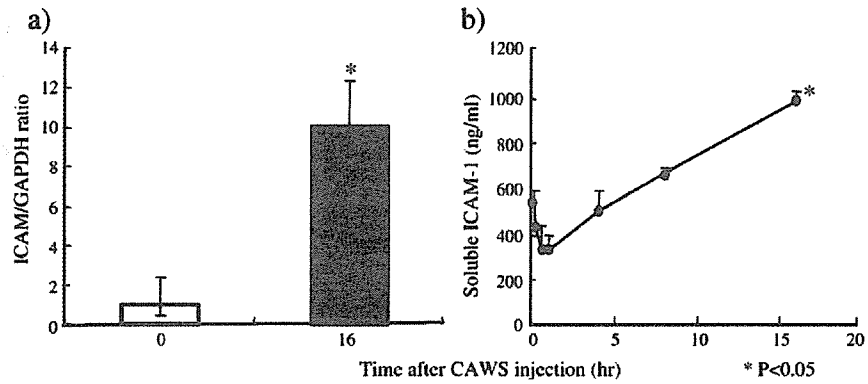

Fig. 6. Soluble ICAM-1 in plasma and ICAM-1 mRNA in aorta after CAWS injection. (a) Heparinized blood was obtained from heart after CAWS injection. Plasma was separated and ICAM-1level was measured by ELISA kit. (b) Total RNA was extracted and mRNA isolated. cDNA was prepared from 1 μg of mRNA. Real-time PCR was performed and analyzed. GAPDH was used as an internal control. White bar shows not treated by CAWS, black bar shows treated by 4 mg/mouse of CAWS, respectively. N=6 in each group.

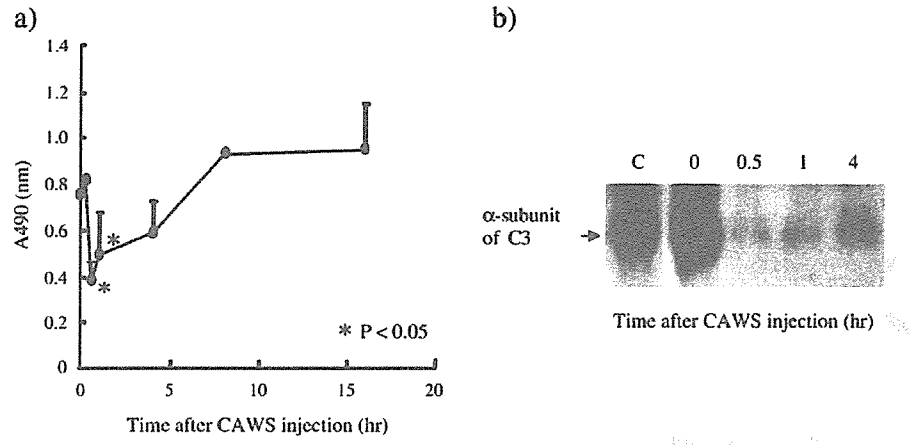

Fig. 7. Mouse complement 3 (C3) was assessed in plasma by sandwich ELISA and Western blotting. 10 mM EDTA treated blood was prepared from heart after CAWS injection. (a) C3 protein was detected by sandwich ELISA using monoclonal and peroxidase-labeled polyclonal antibody, (b) α chain of C3 was detected by monoclonal antibody to mouse C3.

against *C. albicans* or its products. Arteritis induced by CAWS might be.

Uncited reference

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Acknowledgments

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Incidence of ANCA-Associated Primary Renal Vasculitis in the Miyazaki Prefecture: The First Population-Based, Retrospective, Epidemiologic Survey in Japan

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Clinicoepidemiological manifestations of the vasculitides differ geographically. According to a nationwide, hospital-based survey in Japan, the prevalence of microscopic polyangiitis (MPA) and/or renal-limited vasculitis (RLV) is much higher than that of Wegener's granulomatosis (WG). However, little is known about the incidence of antineutrophil cytoplasmic autoantibodies (ANCA)-associated primary renal vasculitis (PRV) in Japan. The incidence of PRV was retrospectively determined by a population-based method in Miyazaki Prefecture in Japan between 2000 and 2004. PRV was defined according to the following criteria from the European Systemic Vasculitis Study Group: (1) new patients with WG, MPA, Churg-Strauss syndrome (CSS), or RLV, (2) renal involvement attributable to active vasculitis, and (3) ANCA considered positive if the disease was not histologically confirmed. The numbers of patients with PRV in the years 2000, 2001, 2002, 2003, and 2004 were 9, 9, 9, 16, and 13, respectively. The male to female ratio was 24:32 and the average age was 70.4 ± 10.9 (mean ± SD) yr. The estimated annual incidence of PRV was 14.8 (95% confidence interval [CI] 10.8 to 18.9) and 44.8 (95% CI 33.2 to 56.3) per million adults (>15 yr old) and seniors (>65 yr old), respectively. Ninety-one percent of the patients were myeloperoxidase (MPO)-ANCA positive, but none were positive for proteinase 3 (PR3)-ANCA. There were no WG or CSS patients. The incidence of PRV did not differ between Japan and Europe, but WG was not widespread in Japan. Furthermore, the ratio of serum MPO to PR3-ANCA among Japanese with PRV was much higher than that found among European and US patients.

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Differences among several clinicoepidemiologic manifestations among vasculitides, such as Takayasu arteritis, giant cell arteritis, and Kawasaki arteritis, have been identified between Japan and European and/or American countries (1–3). The incidence of Wegener's granulomatosis (WG) among the anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated systemic vasculitides is higher than that of microscopic polyangiitis (MPA) in northern Europe (4–7). Conversely, two nationwide Japanese surveys demonstrated that the prevalence of patients with WG is very low compared with that of patients with MPA (8) and/or renal limited vasculitis (RLV) (9). The annual prevalence (*i.e.*, the estimated number of patients treated in 1997) of WG is only 2.3 per million, whereas that of MPA and/or RLV is approximately 13.8 per million (8,9). Therefore, the number of patients with MPA and/or RLV is six-fold higher than that with WG in Japan. Furthermore, myeloperoxidase-ANCA (MPO-ANCA) was identified in 79 to 93% of patients with MPA and/or RLV in Japan, whereas reports from Europe described the ratio as being 44 to 69% (6,10–15). Therefore, the ANCA-associated systemic vasculitides epidemiologically and serologically differ between Japan and European countries.

The annual incidence of ANCA-associated vasculitides in Japan remains obscure because the two previous nationwide surveys were conducted using hospital-based, retrospective methods that determined the prevalence but not the incidence of diseases (8,9). Therefore, the precise annual incidence of ANCA-associated vasculitides in Japan should be determined and compared with that of European countries and the United States. We examined whether the incidence of ANCA-associated vasculitides in Japan is lower than that in European counties, whether MPA and/or RLV is more prevalent than WG in Japan, and whether the ratio of MPO-ANCA/proteinase 3-ANCA (PR3-ANCA) among Japanese patients with ANCA-associated vasculitides differs from that among Europeans.

We conducted a population-based survey of primary renal vasculitis (PRV) in Miyazaki Prefecture on the basis of recent

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epidemiologic methods (5,6,16) and the subclassification of ANCA-associated vasculitides by the European Systemic Vasculitis Study Group (EUVAS) (17–19). We also considered the advice and findings from a direct visit and an inspection of the population and of medical facilities in Miyazaki Prefecture in February 2005 by UK collaborators. This is the preliminary study for a prospective, population-based, clinicoepidemiologic investigation that should define the epidemiologic differences between Japanese and European patients with ANCA-associated vasculitides.

Materials and Methods

Before the study, several candidate areas were considered for investigation. We conferred with our UK collaborators as to whether the area population was suitably defined and whether the area has only a few medical facilities to which patients could be referred. After inspecting the medical facilities with UK collaborators Drs. D. Scott, R. Watts, and D. Jayne during February 2005, central, west, and south Miyazaki Prefecture, Kyushu, Japan, was considered suitable (Figure 1). The case records of all patients who had received a diagnosis of PRV at four hospitals in central Miyazaki Prefecture (Miyazaki Medical College, Miyazaki Prefectural Hospital, Koga General Hospital, and Miyazaki Social Insurance Hospital) from January 2000 through December 2004 were reviewed. These institutions are the only referral centers in Miyazaki Prefecture for patients with renal diseases that require investigation, renal biopsy, and introduction into dialysis therapy. We identified patients with PRV from the renal biopsy registers and discharge summaries of the Departments of Internal Medicine and Otorhinolaryngology, as well as the pathologic specimen records at each hospital. The dialysis center belongs to the Department of Internal Medicine in all four hospitals. The population in these areas seldom undergoes medical examinations in the other parts of Miyazaki or other prefectures. In fact, only one "cross-boundary" referral from the north of Miyazaki Prefecture or adjoining prefectures was identified during 5 yr, and that patient was excluded from the study.

Patients with PRV were defined according to the following criteria (Table 1) in accordance with EUVAS (17–19): New patients with WG, MPA, Churg-Strauss syndrome (CSS), or RLV and renal involvement (elevated serum creatinine, hematuria, proteinuria, or red cell casts) attributable to active vasculitis with or without other organ involvement. The Chapel Hill Consensus Conference (CHCC) nomenclature

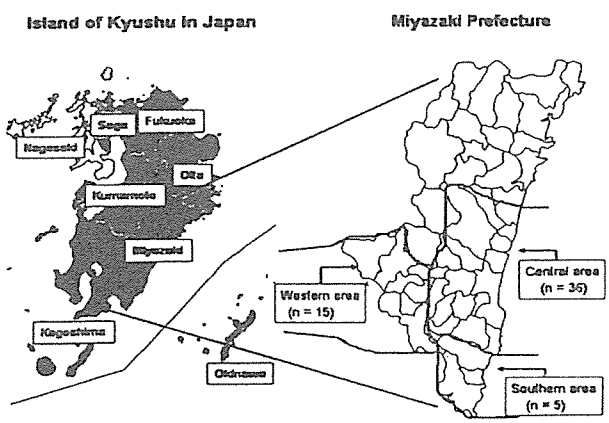

Figure 1. Map of study region and number of patients with primary renal vasculitis (PRV) between 2000 and 2004. Central, west, and south Miyazaki Prefecture.

Table 1. Inclusion criteria[a]

1. New patients with WG, MPA, CSS, or RLV, with or without histologic confirmation[b]
2. Renal involvement[c] with or without other organ involvements, attributable to active WG, MPA, CSS, or RLV
3. Positive serology for ANCA[d]

[a]1, 2, and 3 are required. CSS, Churg-Strauss syndrome; MPA, microscopic polyangiitis; RLV, renal limited vasculitis; WG, Wegener's granulomatosis.

[b]Histologic confirmation: Findings of necrotizing vasculitis and pauci-immune necrotizing, crescentic glomerulonephritis.

[c]Renal involvement: Elevated serum creatinine (>1.3 mg/dl), or hematuria (>30 red blood cells per high-power field), or proteinuria (>1 g/24 h), or red cell casts.

[d]ANCA negativity is allowed if the disease is confirmed histologically.

(20) was used to define MPA, and American College of Rheumatology (ACR) criteria were (21) to define WG and CSS. RLV was defined as necrotizing vascular injury confined to the kidneys (11,18,22,23). All patients underwent serology tests for PR3-ANCA, MPO-ANCA, and anti-glomerular basement membrane (anti-GBM) antibody using an ELISA. When the ELISA results were negative, both cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA) were tested further using indirect immunofluorescence. We excluded patients with anti-GBM antibodies and documented episodes of PRV before 2000. In addition, patients with Henoch-Schönlein purpura, systemic lupus erythematosus, or other connective tissue diseases were excluded. The month of symptom onset was taken as the date of PRV onset.

The numbers of adults (older than 15 yr) and seniors (older than 65 yr) who lived in Miyazaki Prefecture were 987,186 and 264,802, respectively, during 2004. In central, west, and south parts of the prefecture, the two populations comprised 767,988 (male *versus* female, 356,247 *versus* 411,741) and 200,962 people, respectively. The study population was relatively static during the study period, and we estimated the total immigration rate out of the study area during 2000 to 2004 to be <5%. However, for those who were older than 50 and older than 65 yr, the estimates fell to <1.5 and <0.8%, respectively. The 5-yr population decreased by only 7057 people. Urban areas were defined as cities (resident population >30,000), and rural areas were those that surrounded the urban areas. Agriculture is the main occupation of 19.1% of the rural adults and seniors and of 9.2% of these groups in the urban areas. These demographic data were obtained from the home page of the Miyazaki Prefectural office (http://www.pref.miyazaki.lg.jp/contents/org/honbu/toukei/jinko-setai/index.htm).

Continuous data are presented as means ± SD. Annual incidences are presented as simple proportions with 95% confidence intervals (CI). Seasonal differences were considered with the use of a Poisson regression model with covariates including season (February through April, May through July, August through October, and November through January), place of residence (rural/urban), and year (2000 through 2004). Incidence rate ratios across seasons and their 95% CI were estimated. The Poisson model was calculated by S-Plus (Insightful Corp., Seattle, WA).

Results

Fifty-six new patients with PRV were identified during the period from January 1, 2000, to December 31, 2004. All of them

Table 2. Laboratory data at admission (n = 56)[a]

CRP (mg/dl)			Creatinine (mg/dl)			Proteinuria (g/d)		
<0.5	0.5 to 2.0	>2.0	<1.0	1.0 to 3.0	>3.0	<1.0	1.0 to 2.0	>2.0
6	12	38	5	20	31	15	24	17

[a]C-reactive protein (CRP) normal range <0.5 mg/dl; creatinine normal range <1.0 mg/dl; proteinuria normal range <0.3 g/d.

Table 3. Glomerular lesions on renal biopsy specimens (n = 33)

Global Sclerosis				Crescents			
<10%	10 to 20%	20 to 30%	>30%	<25%	25 to 50%	50 to 75%	>75%
14	6	6	7	1	7	13	12

had systemic manifestations of vasculitis, such as fever of unknown origin, weight loss, myalgia, arthralgia, and malaise with or without multisystem involvement. C-reactive protein was elevated (5.8 ± 6.2 mg/dl), and surrogate markers of renal vasculitis, such as elevated serum creatinine (3.9 ± 2.8 mg/dl), proteinuria (1.8 ± 2.3 g/d), hematuria, and/or red cell casts, are outlined in Table 2. However, the kidneys, measured using abdominal echo and/or computed tomography, were not decreased in size. At the first admission, dialysis therapy was introduced into 17 patients, three of whom recovered and did not require further dialysis. Twenty-three patients had pulmonary symptoms with abnormal findings on chest x-ray films, three had mononeuritis multiplex, two had gastrointestinal bleeding, and one had otitis media. None had nasal lesions. Ten patients died at the first admission. Histologic evidence of pauci-immune, necrotizing, and crescentic glomerulonephritis and/or vasculitis was obtained from 35 patients, 33 of whom had undergone renal biopsy (Table 3). Five of the 33 patients who underwent biopsy were proved to be C-ANCA and P-ANCA/MPO-ANCA negative, whereas the other 51 patients were positive for MPO-ANCA. None of the patients was positive for either C-ANCA or PR3-ANCA (Table 4). The ACR criteria and/or CHCC definition did not uncover any patients with WG or CSS.

The numbers of recently registered patients with PRV were 9, 9, 9, 16, and 13 in 2000, 2001, 2002, 2003, and 2004, respectively. The male-to-female ratio was 24:32, and the average age was 70.4 ± 10.9 yr. Fifty-five and 45 patients were aged >50 and >65 yr, respectively, and the peak age group was 70 to 74 yr (Figure 2). All patients were from central, west, and south Miyazaki Prefecture (Figure 1). The annual incidence of PRV during the 5-yr period was 14.8 (95% CI 10.8 to 18.9) and 44.8 (95% CI 33.2 to 56.3) per million adults and seniors, respectively. These values increased during the last 2 yr to 18.9 and 57.2 per million people, respectively. The annual incidences per million male and female adults (13.5 [95% CI 11.4 to 15.5] *versus* 15.5 [95% CI 8.9 to 22.2]) did not differ. The annual incidence in urban (population 516,149) and rural (population 251,839) areas did not differ significantly (13.9 per million [95% CI 10.1 to 17.8] *versus* 15.9 per million [95% CI 10.4 to 21.4]).

Twenty-two of the 56 patients were aware of the onset of symptoms during the summer months, and two could not define the time of onset (others noticed symptoms during the following periods: February through April, 18.5%; May through July, 29.6%; August through October, 40.7%; November through January, 11.1%). The seasonal variation in annual incidence of PRV was observed in the result of the Poisson regression analysis. Compared with the incidence rate in November through January, higher rate ratios of 3.67 (95% CI 1.49 to 9.03) in August through October and 2.67 (95% CI 1.04 to 6.81) in May through July were observed, respectively (Figure 3). The annual incidence of PRV, accounting for the seasonal

Table 4. Maximum MPO-ANCA units during admission and results of indirect immunofluorescence assay for C-ANCA (n = 56)[a]

MPO-ANCA Titers (EU)				C-ANCA	
<20	20 to 200	200 to 640	>640	−	+
5	21	20	10	56	0

[a]Myeloperoxidase (MPO)-ANCA normal range <20 EU; cytoplasmic ANCA (C-ANCA) normal is negative.

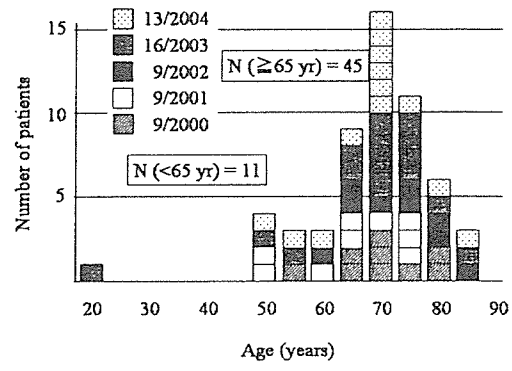

Figure 2. Number and age distribution of new patients with PRV from 2000 to 2004.

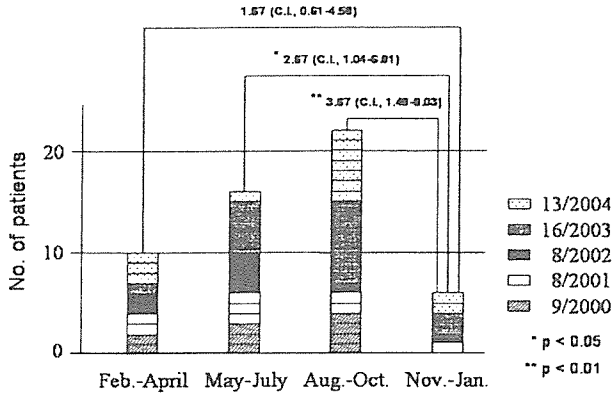

Figure 3. Number of patients with PRV, grouped by the onset time (the month of symptom onset) between 2000 and 2004, and estimated incidence rate ratios across seasons with their 95% confidence intervals by the Poisson regression model.

variation, was not statistically different between urban and rural areas.

Discussion

Previous nationwide surveys of ANCA-associated vasculitides in Japan used a retrospective, hospital-based method (8,9). Because the medical expenses of patients with MPA and WG were supported by the Ministry of Health, Labor and Welfare of Japan, previous nationwide government-supported surveys were directed to investigate prevalence (*i.e.*, the estimated number of patients treated in a year) but not the annual incidence of new ANCA-associated vasculitides. However, problems are associated with hospital-based surveys in that patients from tertiary and/or university referral centers usually are based on ill-defined denominator populations, and patients who are seen in tertiary hospitals might not be representative of those who are seen either at district hospitals or in the community, especially in terms of disease severity or age spectrum (5,10). Therefore, we used a population-based survey to investigate the incidence of ANCA-associated vasculitides.

The results showed that the estimated annual incidence of PRV in Miyazaki Prefecture was 14.8 per million, which was similar to that of ANCA-associated systemic vasculitides identified in several European studies (Table 5). The incidence rate in our study does not seem to be lower than expected in comparison with other epidemiologic studies, because it is considered that few patients with PRV died without reaching the hospital in the Japanese medical care system. However, we cannot completely deny a small possibility that patients who had rapidly progressive glomerulonephritis without positive ANCA and did not undergo biopsy would be excluded in this survey. Data from our 1998 nationwide survey showed that the annual prevalence of patients with ANCA-associated vasculitides is 17.1 per million (WG 2.3, CSS 1.0, and MPA/RLV 13.8 per million) (8,9). We therefore predicted a much lower incidence. The reasons could be that disease diagnosis has improved, hospital- and population-based methods differ, and the incidence of PRV has increased over time (5,24).

We also questioned whether MPA and/or RLV is more common than WG among the ANCA-associated vasculitides in Japan. However, the incidence of ANCA-associated vasculitides in Miyazaki Prefecture was very similar to that of the total incidence among European countries, but none of our patients was classified as having WG and CSS. A geographic difference in the incidence of systemic vasculitides has been suggested (1–3), and this theory seems to explain our results. The latitude of Miyazaki Prefecture is almost 30°, which is located south of Lugo, Spain (latitude 43°), where the incidence of WG, MPA, and CSS is 3.0, 7.9, and 1.3 per million, respectively (10). The incidence of WG and MPA might be latitudinal (7,10,25) (Table 5). To our knowledge, there are no reports regarding the incidence of WG in a more southern area than latitude 40° N. In the Middle East country of Bahrain, Saudi Arabia (latitude 26° N; population approximately 500,000), the very first case of WG was reported only in 1998 (26). Race differences also might

Table 5. Incidence of ANCA-associated vasculitides with renal involvement[a]

Location, Country (Reference)	Latitude	Annual Incidence (per million)				Population (millions)	Study Period
		MPA	WG	CSS	Total		
1. Orebro, Sweden (12)	59°N	ND	ND	ND	16.0	0.20 to 0.21	1975 to 1995
2. Lund, Sweden (16)	55°N	2.5[b]	2.1[b]	ND	4.6[b]	1.2	1971 to 1993
3. Norfolk, UK (7)	52°N	7.5	7.9	1.3	18.0	0.41	1992 to 1997
4. Schleswig-Holstein, Germany (8)	51°N	2.7[c]	7.9[c]	1.1[c]	11.7[c]	2.78	1988 to 2002
5. Devon, UK (25)	50°N	ND	ND	ND	12.4	0.85	2 yr
6. Lugo, Spain (11)	43°N	7.9[c]	3.0[c]	1.3[c]	12.2[c]	0.21	1988 to 2001
7. Miyazaki, Japan (this study)	30°N	14.8	0	0	14.8	0.77	2000 to 2004
8. Al-Jahra, Kuwait (14)	26°N	20.9	ND	ND	ND	0.12	1993 to 1996

[a]1, 5, 7, and 8 include RLV; 2, 3, 4, and 6 may not include RLV. 1, 2, 6, and 8 are hospital-based studies; 3, 4, 5, and 7 are population-based studies. ND, not done.
[b]All cases with biopsy-proven renal involvement.
[c]ANCA-associated vasculitides without renal involvement are included.

contribute to the proportion of type of vasculitis. In a US cohort study, white individuals composed >90% of all patients with WG, whereas black, Hispanic, and Asian individuals together represented only 1 to 4% of patients (27,28). The population in the European studies was white (10,25). We reported that the prevalence of Japanese patients with ANCA-associated vasculitides is 2.3, 13.8, and 1.0 per million with respect to WG, MPA/RLV, and CSS, respectively (9). Therefore, WG is not widespread in Japan. The incidence of WG and MPA and/or RLV among Europe, the United States, and Japan should be differentiated by the prospective study.

We examined whether the ratio of MPO-ANCA/PR3-ANCA among Japanese patients with ANCA-associated vasculitides differs from that in European counties. Only five patients were ANCA negative, whereas sera from 51 (91%) of 56 patients were MPO-ANCA positive, and none was serologically positive for C-ANCA or PR3-ANCA. Among Japanese patients with MPA and/or RLV, 79 to 93% are positive for MPO-ANCA (8,9), compared with 44 to 69% of European patients (6,10–15). Our preliminary examination of the type of ANCA in sera from patients with ANCA-associated vasculitides revealed that the results of commercially available ELISA kits that were used in EUVAS and Japan did not differ (Drs. T. Ihara [Department of Medicine, Kyoto University, Graduate School of Medicine, Kyoto, Japan] and E. Muso [Division of Nephrology & Dialysis, Department of Medicine, Kitano Hospital, Osaka, Japan], personal communication, August 2005). Even among European countries with the higher incidence of MPA and/or RLV, the maximal proportion of MPO-/PR3-ANCA was 5.5:1 (5,10,11,13), whereas that in Japan was 9:1. Therefore, MPO-ANCA–positive patients seem to be more widespread than those with PR3-ANCA among Japanese with ANCA-associated vasculitides. The genetic background of the patients should be closely related to the differences in rates of MPO-ANCA *versus* PR3-ANCA between Europe and Japan. Our previous study demonstrated that HLA-DR0901 is much more prevalent among patients with MPA than in healthy control subjects (29,30).

Approximately 80% of our patients were aged at least 65 yr, and the incidence peaked in the 70- to 74-yr age group (Figure 2). The mean age of 70 yr was higher than that in a previous study, which found that the average age of Japanese patients with ANCA-associated vasculitides was 10 yr younger (9). Recent studies revealed an increasing incidence of ANCA-associated vasculitides in the older population (5,24). Compared with European demographic data regarding MPA with renal involvement and/or RLV, our study was consistent with recent findings (12) showing that the mean age was 72 yr at presentation, and there was no significant gender difference (female 57%). Whether this reflects a real increase in incidence among the elderly, more accurate diagnosis, increased recognition, or an increase in the mean population age remains unclear.

Several reports have shown that more patients with C-ANCA and/or WG develop symptoms during the dark winter months (11,31–33). In contrast, this study showed that symptoms developed predominantly during the summer months. A significant association between farming and P-ANCA and/or MPA (odds ratios 4.3 [95% CI 1.5 to 12.7] and 6.6 [1.9 to 21.6], respectively) was identified (33). One explanation may be high silica exposure in the form of agricultural dust, because silica plays a role in the onset of ANCA-associated primary systemic vasculitides (34–38). Crops are harvested during the summer months in the areas of Miyazaki. The mean age of agricultural workers in Miyazaki prefecture is 58.5 yr, and more elderly residents frequently are involved in farming. Furthermore, Miyazaki prefecture is predominantly agricultural with no heavy industry. Further investigation obviously is required to clarify the association between symptom onset and seasonal variations or occupational exposure to silica.

Conclusion

This is the first epidemiologic study to elucidate the annual incidence of PRV in Japan, especially using the population-based survey applied by European investigators. The incidence of PRV did not differ between Japan and European countries. We found that WG was not widespread in Japan and that the ratio of serum MPO-/PR3-ANCA among patients with PRV is much higher than that reported for European and American countries. Our preliminary results will form the basis for a prospective study of Miyazaki prefecture, and our survey will be distributed in northern Japan. We also plan to determine the epidemiology of the ANCA-associated vasculitides in Asian countries.

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Contribution of the myeloperoxidase-dependent oxidative system to host defence against *Cryptococcus neoformans*

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The *in vivo* contribution of reactive oxygen species produced by neutrophils against *Cryptococcus* infection is not widely recognized. Myeloperoxidase (MPO) is a neutrophil-specific enzyme that catalyses the production of hypohalous acids such as HOCl from H_2O_2. This study investigated the role of MPO in immunological defence against *Cryptococcus neoformans* in an MPO-deficient ($MPO^{-/-}$) mouse model. The survival of $MPO^{-/-}$ mice infected either intranasally or intravenously with *C. neoformans* was lower than that of identically challenged wild-type mice. The $MPO^{-/-}$ mice that received intranasal injection of *C. neoformans* had significantly larger lung fungal burdens than wild-type mice. On day 7, $MPO^{-/-}$ mice had a significantly higher lung concentration of interleukin (IL)-4 and lower concentrations of IL-2, IL-12p70 and interferon (IFN)-γ than wild-type mice, suggesting a weak Th1 response in the $MPO^{-/-}$ mice to *C. neoformans*. Pathologically, the $MPO^{-/-}$ mice with intranasal infection showed more severe pneumonia than wild-type mice, which was associated with an increase in the levels of IL-1α/β in the lungs. In addition, in $MPO^{-/-}$ mice, the pulmonary infection disseminated to the brain with occasional meningitis. The keratinocyte-derived cytokine (KC) level in the brain of infected $MPO^{-/-}$ mice was higher than that of control mice. Both intranasal and intravenous infections resulted in a higher number of fungi in the spleen of $MPO^{-/-}$ mice compared to wild-type, suggesting decreased resistance to *C. neoformans* not only in the lungs but also in the spleen in the absence of MPO. Taken together, these data suggest a major role of MPO in the response to cryptococcal infection.

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INTRODUCTION

Cryptococcus neoformans is an encapsulated fungus that causes life-threatening infections, particularly in patients with impaired cell-mediated immunity (Chuck & Sande, 1989). The lung is the initial site of *C. neoformans* infection and elicits an influx of macrophages, lymphocytes and neutrophils. Although the infection is typically restricted to the lung, *C. neoformans* disseminates to the central nervous system in the immunosuppressed patient and occasionally in the normal host (Murphy, 1996), and leads to a potentially life-threatening meningoencephalitis (Kozel, 1993). Successful eradication of *C. neoformans* is largely mediated by T cells (Buchanan & Doyle, 2000), and is dependent on protective Th1 cell-derived cytokines (Kawakami *et al.*, 1996; Yuan *et al.*, 1997).

In addition to cell-mediated immunity, neutrophils have been implicated in protection against *C. neoformans* (Graybill *et al.*, 1997; Miller & Mitchell, 1991; Retini *et al.*, 1996; Vecchiarelli *et al.*, 1998). Whereas normal human neutrophils kill *C. neoformans*, those isolated from patients with chronic granulomatous disease lack fungicidal activity (Diamond *et al.*, 1980). Since chronic granulomatous disease is associated with a defect in the phagocyte NADPH oxidase, this suggests that normal neutrophil anti-cryptococcal activity depends on an intact NADPH oxidase. However, the contribution of neutrophil-derived reactive oxygen species (ROS) to host defence against *C. neoformans* has not been fully characterized.

Abbreviations: H&E, haematoxylin and eosin; IFN, interferon; IL, interleukin; KC, keratinocyte-derived cytokine; MPO, myeloperoxidase.

Myeloperoxidase (MPO) (Hansson *et al.*, 2006; Klebanoff, 2005) is found mainly in neutrophils and to a lesser degree in monocytes. In the presence of hydrogen peroxide (H_2O_2), MPO oxidizes chloride to produce the potent microbicidal agent hypochlorous acid. MPO also catalyses the oxidation of bromide, iodide and the pseudohalide ion thiocyanate. MPO deficiency (Lehrer & Cline, 1969; Nauseef, 1998) is the most common inherited neutrophil defect, with an estimated incidence of 1 in 2000–4000 in Europe and the USA (Parry *et al.*, 1981), and of 1 in 55 000 in Japan (Nunoi *et al.*, 2003). Phagocytes deficient in MPO express a mild to moderate defect in bacterial killing but a marked defect in fungicidal activity *in vitro* (Diamond *et al.*, 1980; Parry *et al.*, 1981). Using MPO-deficient ($MPO^{-/-}$) mice, we have previously demonstrated that the MPO-dependent oxidative system is important for *in vivo* host defence against a variety of micro-organisms (Aratani *et al.*, 1999, 2000, 2002a, b). The aim of the present study was to define the contribution of the MPO-dependent antimicrobial system to the *in vivo* host defence against *C. neoformans*.

METHODS

Animals. Animal experimentation was carried out in accordance with the guidelines of Kihara Institute for Biological Research, Yokohama City University. All mice used were 10- to 12-week-old females. C57BL/6 mice were purchased from Japan SLC (Hamamatsu). Homozygous mutant mice for MPO (Aratani *et al.*, 1999) were backcrossed more than ten times with C57BL/6 to ensure similar genetic backgrounds. Before infection, all animals were housed under specific-pathogen-free conditions.

Experimental infection with *C. neoformans*. *C. neoformans* (ATCC 24067) was cultured on agar slants containing 2·1 % YM broth for 4 days at 27 °C. Fungi were enumerated with a haemocytometer, and the viable number determined in c.f.u. For pulmonary infection, wild-type C57BL/6 and $MPO^{-/-}$ mice were intranasally challenged with 0·04 ml fungal suspension. Thirty minutes later, the lungs of two wild-type and two $MPO^{-/-}$ mice were removed aseptically and homogenized in 1 ml sterile saline to determine the initial number of organisms. At various time points after the challenge, selected organs (lungs, brain, spleen) were harvested aseptically and homogenized in sterile saline in the presence of protease inhibitors (Complete Protease Inhibitor Cocktail Tablets; Roche). Appropriate dilutions of the homogenates were plated in duplicate onto trypticase soy agar plates (Eiken Chemical). After 2 days incubation at 37 °C, the number of viable organisms was determined in c.f.u. Data were recorded as the mean log(c.f.u.) per organ. The remaining homogenates of the lungs and brain were centrifuged at 19 000 *g* for 10 min to remove cell debris and filtered with 0·45 μm pore-size filters (MILLEX-HV; Millipore). The final supernatants were frozen at −20 °C until assayed for cytokines. For intravenous infection, mice were warmed under a heat lamp to vasodilate the tail vein, and fungi were injected in a 0·1 ml volume through a 27-gauge needle. The spleen was removed and homogenized in 1 ml sterile saline and the aliquots were diluted and plated onto the agar plates.

Quantification of cytokine and chemokine levels. The lung and brain supernatants were analysed using the Bio-Plex system and a Luminex 100TM analyser (Bio-Rad) according to the manufacturer's instructions. Results were expressed as the mean ± SD. The detection limit of the assay was 1·95 pg ml^{-1} for all cytokines and chemokines, as stated by the manufacturer.

Preparation of sections and slides. Five wild-type and five $MPO^{-/-}$ mice were sacrificed for histopathologic examination at the same time points as those for c.f.u. measurement. The whole lungs and brain were removed and fixed in a buffered 4 % paraformaldehyde solution, dehydrated in ethanol, and embedded in paraffin for sectioning. The sections of these organs were processed for haematoxylin and eosin (H&E) and Grocott staining using standard protocols. Five well-separated cross sections of each organ were obtained and all available fields were observed by light microscopy.

Statistical analysis. Survival curves were analysed by the Kaplan–Meier log-rank test. Differences in the number of c.f.u. were examined by the Mann–Whitney *U* test. $P<0·05$ was considered significant.

RESULTS AND DISCUSSION

To examine the contribution of MPO to immunological defence against pulmonary infection with *C. neoformans*, wild-type and $MPO^{-/-}$ mice were infected intranasally or intravenously with $3·6\times10^6$ or $8·7\times10^5$ viable yeast, respectively. After 6 months intranasal infection, there was a 94 % survival rate among infected wild-type mice (Fig. 1a), and all of the intravenously infected wild-type mice remained viable after 3 months (Fig. 1b). Thus, the dose of *C. neoformans* induced minimal mortality in C57BL/6 control mice. In contrast, there was rapid death in $MPO^{-/-}$ mice infected with *C. neoformans*, with a 100 % death rate before 1 month and 4 months of intravenous and intranasal infection, respectively (Fig. 1a, b). The difference in survival rates between wild-type and $MPO^{-/-}$ mice was highly significant ($P<0·001$).

We reasoned that the most likely explanation for the increased mortality in $MPO^{-/-}$ mice following intranasal inoculation of *C. neoformans* was an impaired clearance of the initial inoculum from the airway. To assess this possibility, we compared the lung fungal burden in $MPO^{-/-}$ mice to that of wild-type controls (Fig. 2). Nearly half of the inoculum, $1·7\times10^6$ fungi, was recovered from the lung of wild-type and $MPO^{-/-}$ mice 30 min after infection. The number of *C. neoformans* in the lungs increased simultaneously for the first 7 days in both groups and peaked on day 19, with a slightly but significantly higher number of c.f.u. in the $MPO^{-/-}$ mice. In the wild-type mice, a gradual elimination of the fungi occurred between days 19 and 34, but the $MPO^{-/-}$ mice failed to eliminate *C. neoformans* from the lung. Consequently, by day 60, $MPO^{-/-}$ mice contained nearly 200-fold more fungi than the infected control group ($P<0·01$).

Minimal inflammation was observed histopathologically on day 7, irrespective of genotype, and similar degrees of inflammation were observed on day 19 in both groups (Fig. 3). The lungs of infected wild-type mice showed only localized areas of leukocyte infiltration at days 34 and 60, whereas $MPO^{-/-}$ mice at day 34 exhibited extensive areas of inflammatory infiltration, and all of the airway spaces were filled with the inflammatory cells at day 60. Thus, the lung c.f.u. and the development of pulmonary inflammation correlated with the increased mortality in $MPO^{-/-}$ mice.

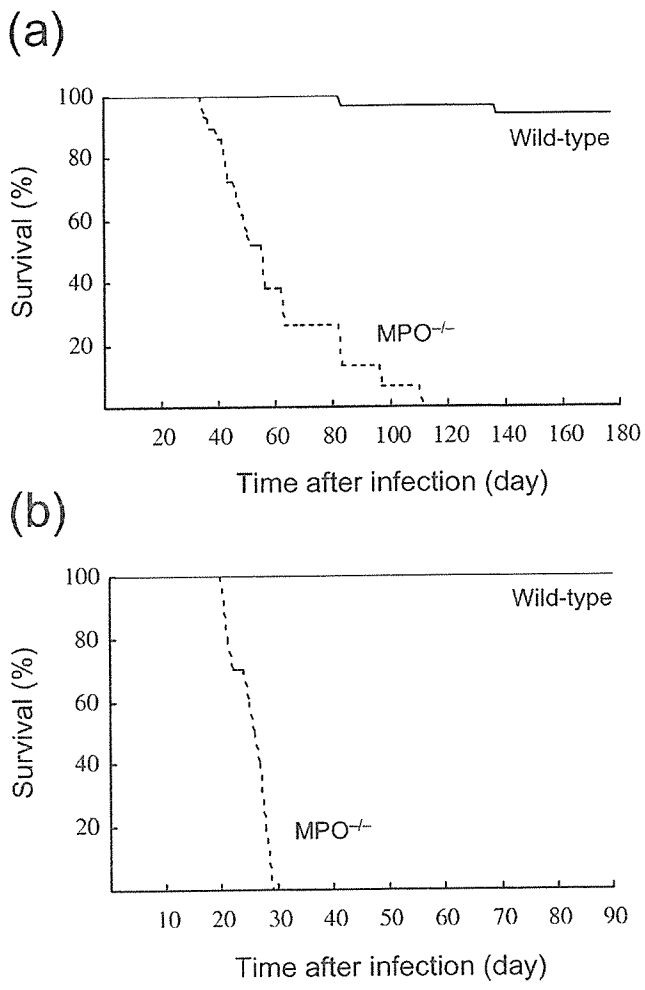

Fig. 1. Survival of wild-type and $MPO^{-/-}$ mice following infection with *C. neoformans*. (a) Wild-type ($n=32$) and $MPO^{-/-}$ ($n=29$) mice were intranasally infected with $3{\cdot}6\times10^6$ c.f.u. *C. neoformans*, and survival was observed over the course of 180 days. (b) Wild-type ($n=10$) and $MPO^{-/-}$ ($n=10$) mice were intravenously infected with $8{\cdot}7\times10^5$ c.f.u. *C. neoformans*. $P<0{\cdot}001$ ($MPO^{-/-}$ versus wild-type mice) for both intranasal and intravenous infections. The data are representative of two independent experiments.

Our previous report also demonstrated that the numbers of *C. neoformans* in the lungs of wild-type and $MPO^{-/-}$ mice 48 h after infection do not differ significantly (Aratani *et al.*, 2000). Taken together, these results strongly suggest that the MPO-dependent oxidative system plays an important role in the *in vivo* host defence against *C. neoformans*, although this system is inefficient in the early period after the infection.

Since Th1 cytokines have been shown to be critical for effective host defences in murine models of cryptococcosis (Decken *et al.*, 1998; Hoag *et al.*, 1997; Kawakami *et al.*, 1996) and there is accumulating evidence that neutrophils play an important role in modulating the balance of Th1 and Th2 responses (Mednick *et al.*, 2003; Tateda *et al.*, 2001), we suspected that the higher fungal burden observed in the infected $MPO^{-/-}$ mice could be caused by a reduction in Th1 cell activity. Interestingly, levels of interleukin (IL)-2, IL-12p70 and interferon (IFN)-γ in the lungs of $MPO^{-/-}$ mice at day 7 of infection were significantly lower than those in infected wild-type mice (Fig. 4), suggesting a role for neutrophil-derived hypohalous acids in driving Th1-type host responses.

Previous studies have shown that the constitutive production of IL-4 in transgenic mice results in an increased susceptibility to infection with *Leishmania major* (Leal *et al.*, 1993), and that resistance to infection occurs when the endogenously synthesized IL-4 is neutralized in the susceptible mice (Chatelain *et al.*, 1992). Consistently, in our study, the level of IL-4 was significantly higher in the $MPO^{-/-}$ mice at day 7 compared to the wild-type controls (Fig. 4), although there was no significant difference between the mice with different genotypes in the levels of IL-5 and IL-10 (data not shown). The levels of these cytokines were not significantly different between the mice with different genotypes before infection and at day 34 of infection (Fig. 4). These results indicate that MPO deficiency affects Th1 and Th2 immune response in the early stage of pulmonary cryptococcus infection.

In murine models, production of IL-1 in the airways is required for full neutrophil migratory responses to LPS or diesel exhaust particles (Ulich *et al.*, 1991; Yang *et al.*, 1997). It is of interest that the lung concentration of IL-1β was significantly higher in the $MPO^{-/-}$ mice than in the wild-type at days 7 and 34 post-infection, and that IL-1α was also significantly higher in the $MPO^{-/-}$ mice at day 34 (Fig. 4), since these data suggest that higher concentrations of these pro-inflammatory cytokines enhanced the pulmonary inflammation of the $MPO^{-/-}$ mice. Overexpression of IL-1β in the lung epithelium leads to pulmonary inflammation, with an increase in the level of keratinocyte-derived cytokine (KC) (Lappalainen *et al.*, 2005). Indeed, in our model, we have observed a time-dependent increase in the level of KC in the lung (Fig. 4). However, there was no difference in the level between the wild-type and $MPO^{-/-}$ mice, suggesting that this chemotactic mediator for neutrophils is not a limiting factor for the higher inflammation observed in the $MPO^{-/-}$ mice. It is well accepted that leukocyte migration from the vasculature occurs by a multistep process, and that this process is dictated by the sequential activation of adhesive proteins and their ligands on both leukocytes and endothelial cells (Wagner & Roth, 2000). Exposure of endothelial cell monolayers or neutrophils *in vitro* to IL-1 causes expression of selectins and integrins (Schleimer & Rutledge, 1986; Scholz *et al.*, 1996). In addition, IL-1 treatment *in vivo* induces intercellular adhesion molecule-1 (ICAM-1) in lung (Komatsu *et al.*, 1997). Taken together, it is possible that the elevated IL-1α/β levels in $MPO^{-/-}$ mice could facilitate the transmigration of circulating neutrophils into tissues.

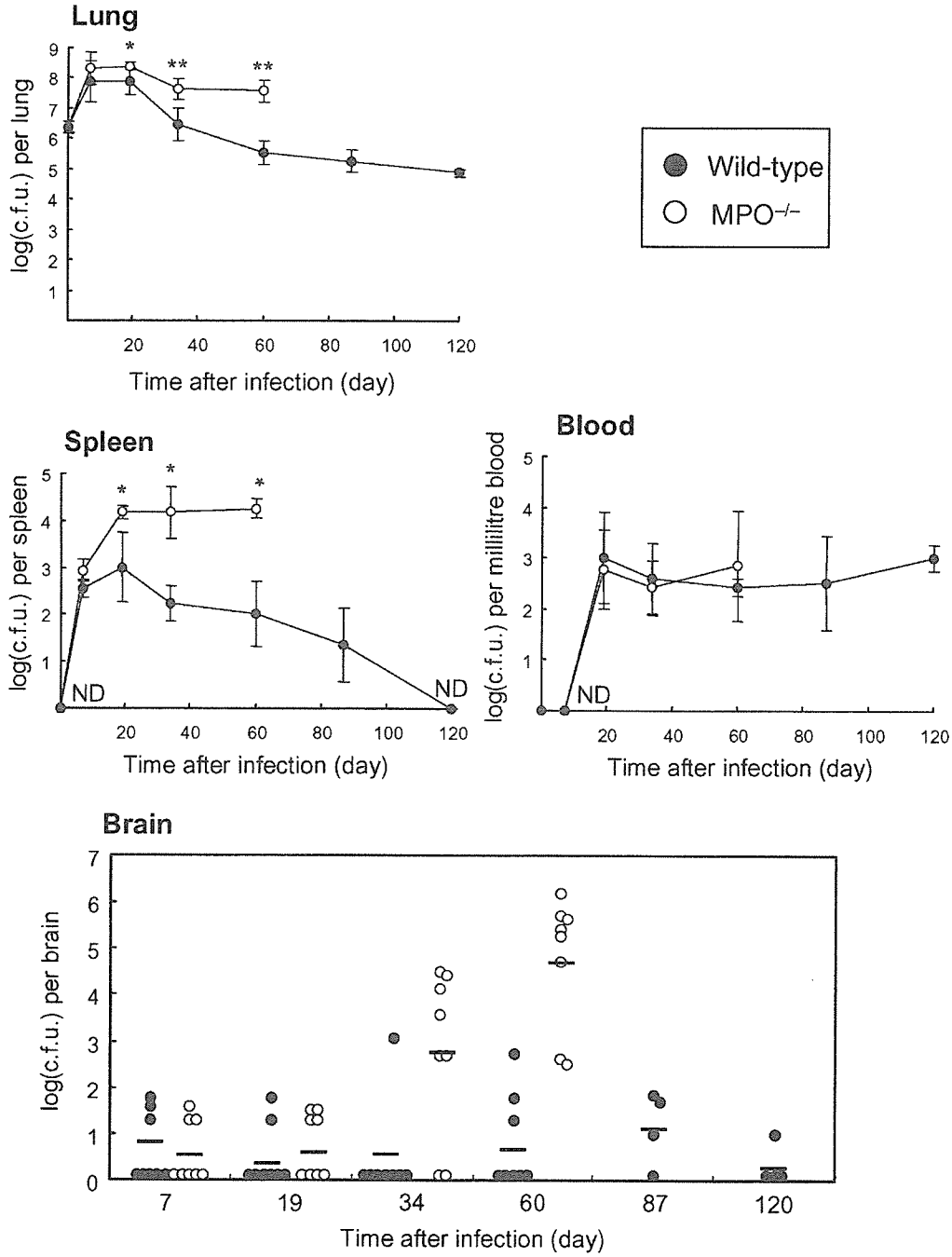

Fig. 2. Culture of *C. neoformans* from various organs after intranasal infection of mice. Wild-type and $MPO^{-/-}$ mice were inoculated intranasally with $3{\cdot}6 \times 10^6$ c.f.u. *C. neoformans* per mouse and analysed on days 0, 7, 19, 34, 60, 87 and 120. Aliquots of homogenized organs were plated on agar plates, and total c.f.u. per organ was determined. Five mice were used for each group. Results represent mean log(c.f.u.) per organ ± SD. ND, not detectable (below 5 c.f.u. per organ). *, $P<0{\cdot}05$; **, $P<0{\cdot}01$ compared to wild-type infected mice. The data are representative of two independent experiments.

The recovery of *C. neoformans* from the brain of infected wild-type and $MPO^{-/-}$ mice was compared (Fig. 2). Whereas cryptococci were nearly undetectable in the brain of wild-type mice at all time points, significant numbers of fungi were found in the $MPO^{-/-}$ mice on days 34 and 60 post-infection. We carried out histological analysis of the brain in five control and five $MPO^{-/-}$ mice on day 60 after infection. One $MPO^{-/-}$ mouse showed a cellular infiltrate including neutrophils in the meningeal area (Fig. 5a, b). Another mouse showed cyst-like cavities in the medulla

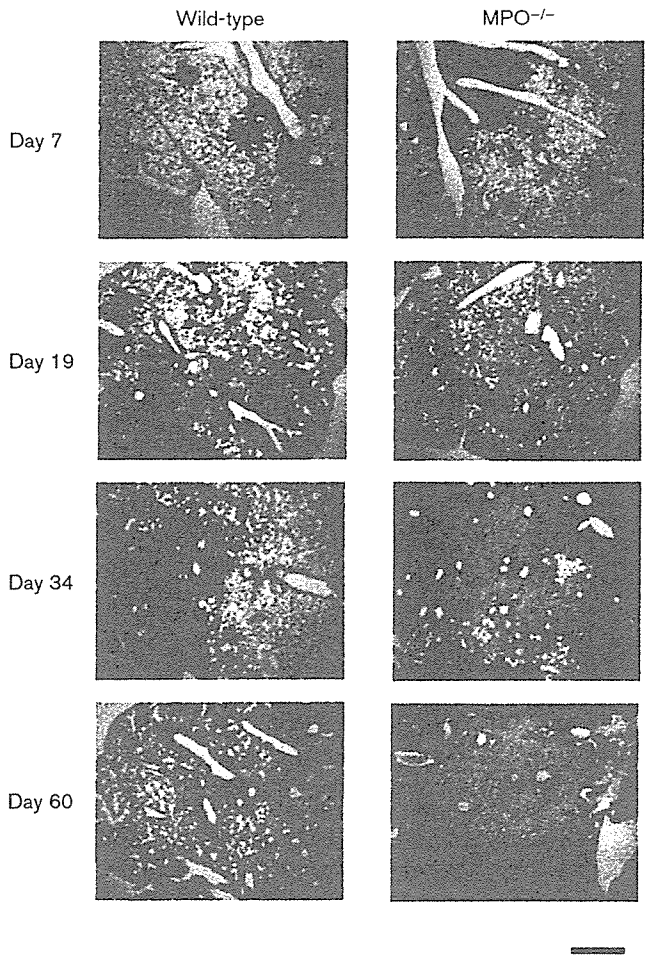

Fig. 3. Lung sections taken from wild-type and $MPO^{-/-}$ mice 7, 19, 34 and 60 days after intranasal challenge with $3{\cdot}6 \times 10^6$ c.f.u. *C. neoformans*. Sections were stained with H&E. Three mice in each experimental group were examined and a representative result is shown. Bar, 1 mm.

oblongata (Fig. 5d), in which no cellular infiltrate was observed. Numerous cryptococci were observed in the meningeal area (Fig. 5c) and within huge cysts (Fig. 5e) in the brain and medulla oblongata. However, no obvious inflammation was found in the brain of three other $MPO^{-/-}$ mice. In contrast, no pathological change was observed in all five of the wild-type mice (Fig. 5f). Since dissemination of the fungi into the cerebrum is often fatal in humans, we considered the possibility that the large fungal burden was the cause of death of the $MPO^{-/-}$ mice. However, this process was unlikely to be the main cause of death, because no wild-type mice infected intravenously ($8{\cdot}7 \times 10^5$ c.f.u.) died, despite the fact that $> 1 \times 10^6$ c.f.u. fungi had invaded the brain at day 20 of infection (data not shown). In the $MPO^{-/-}$ mice given higher doses of *C. neoformans*, the more severe pneumonia as well as the higher lung fungal burden may be the cause of death, since the difference in survival of the mice with different genotypes correlated well with the differences in the levels of lung fungal burden (Figs 1 and 2) and in the severity of pneumonia (Fig. 3).

Of note, the $MPO^{-/-}$ mice showed a dramatic increase in KC production in the brain at day 34 post-infection, which was significantly higher than that in wild-type mice (Fig. 6), suggesting that this chemokine at least might partly contribute to the occasional infiltration of neutrophils into the brain, as observed in Fig. 5. Since *C. neoformans* induces IL-8 production by human microglia (Lipovsky *et al.*, 1998), the higher KC level in $MPO^{-/-}$ mice could result from the higher fungal burden in the brain. In an animal model in which IL-8 was delivered intracerebrally, neutrophils rapidly invaded the blood–brain barrier (Bell *et al.*, 1996). In one of five mice, we observed a slight but consistent accumulation of inflammatory cells, and a subset of these cells could be morphologically identified as neutrophils (Fig. 5). This accumulation of neutrophils suggests that a higher brain fungal burden occasionally stimulates a neutrophil mobilization into the brain across the blood–brain barrier in response to KC. In mice of both genotypes, the brain concentrations of Th1-associated (IL-2, IL-12p70, IFN-γ), Th2-associated (IL-4, IL-5, IL-10) and pro-inflammatory (IL-1α, IL-1β) cytokines did not change during the course of infection (data not shown).

Although the fungal burdens in the spleen were similar in both groups for the first 7 days, those of $MPO^{-/-}$ mice on and after day 19 were significantly higher ($P < 0{\cdot}05$) than those of wild-type mice (Fig. 2). However, the blood fungal burden of the mice was equivalent to that in the wild-type (Fig. 2), suggesting that the higher distributions of fungi from the lungs to the spleen were not due to an increased systemic dissemination from the lungs, but rather due to a decreased local resistance in the spleen. To more rigorously examine the role of MPO during systemic infection without an ongoing localized pulmonary infection, an intravenous infection experiment was performed. Wild-type and $MPO^{-/-}$ mice were infected intravenously with $8{\cdot}7 \times 10^5$ viable yeast. To determine fungal dissemination kinetics from the bloodstream, wild-type and $MPO^{-/-}$ mice were analysed on days 1 and 5 post-infection. The fungal burden in the spleen of wild-type mice on day 5 was equivalent to that on day 1. In contrast, the burden in $MPO^{-/-}$ mice on day 5 was significantly higher than that on day 1 (Fig. 7), indicating that the control of fungal growth within the spleen was dependent on MPO production. Since intravenous infection experiments result in a systemic infection of blood-borne fungi in the absence of an ongoing pulmonary infection, the results indicate that MPO was important for host defence against *C. neoformans* not only in the lungs but also in peripheral organs.

C. neoformans is acquired via the respiratory tract and occasionally disseminates to the central nervous system in some immunocompromised patients (Lee *et al.*, 1996). So far, MPO deficiency is not known to be associated with human cryptococcosis. Since mouse neutrophils are devoid

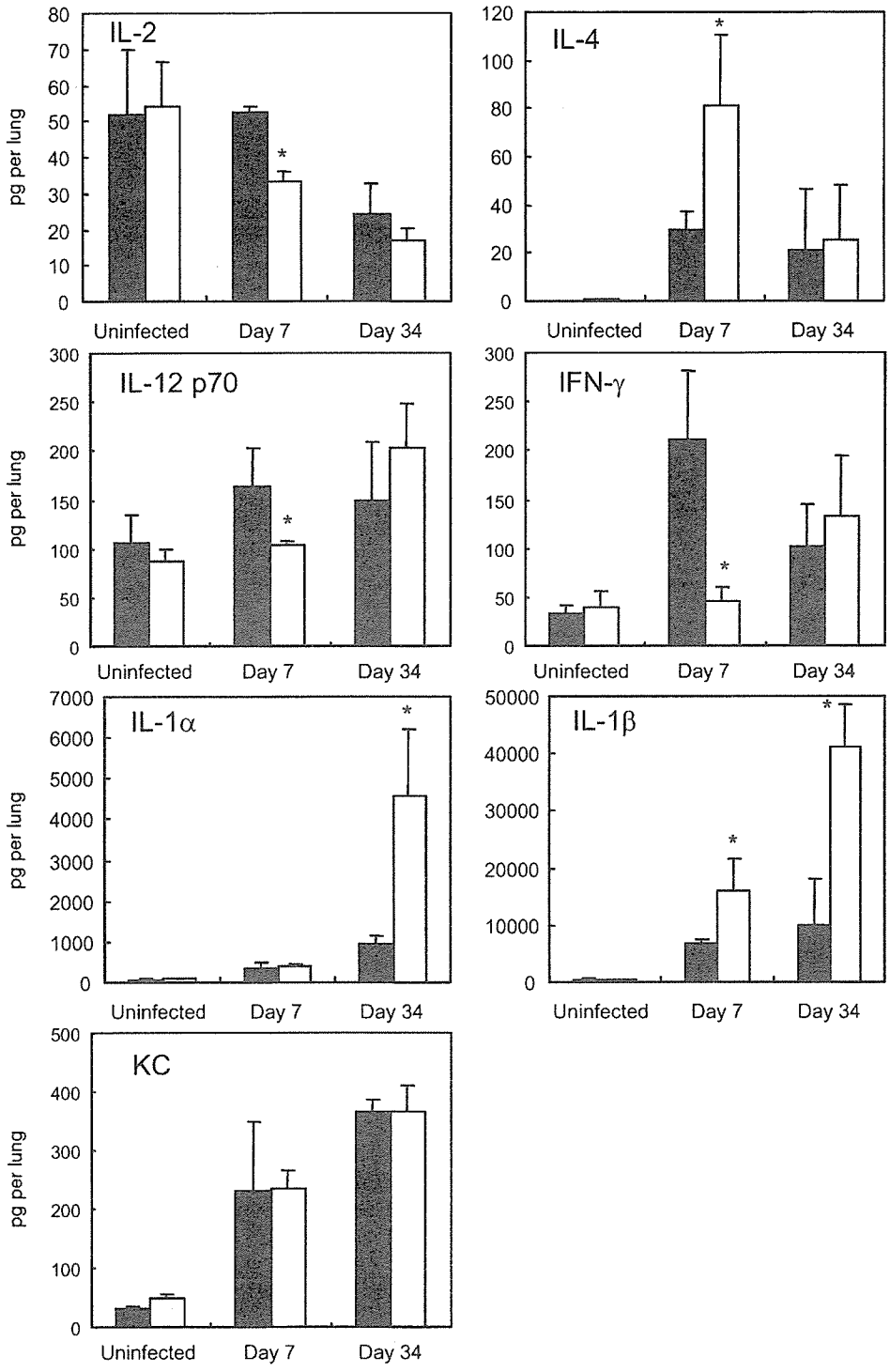

Fig. 4. Cytokine and chemokine levels in the lungs of wild-type and $MPO^{-/-}$ mice after infection with *C. neoformans*. Wild-type (black bars) and $MPO^{-/-}$ mice (open bars) were intranasally infected with $3{\cdot}6 \times 10^6$ c.f.u. *C. neoformans*. Mice were killed before infection, or at 7 or 34 days post-infection, and the lungs were harvested and homogenized. Cytokines and chemokines were measured in triplicate using the Bio-Plex system. Data are expressed as pg cytokine per lung. Results are presented as the mean ± SD of four infected or uninfected mice. *, $P < 0{\cdot}05$ compared to wild-type infected mice.

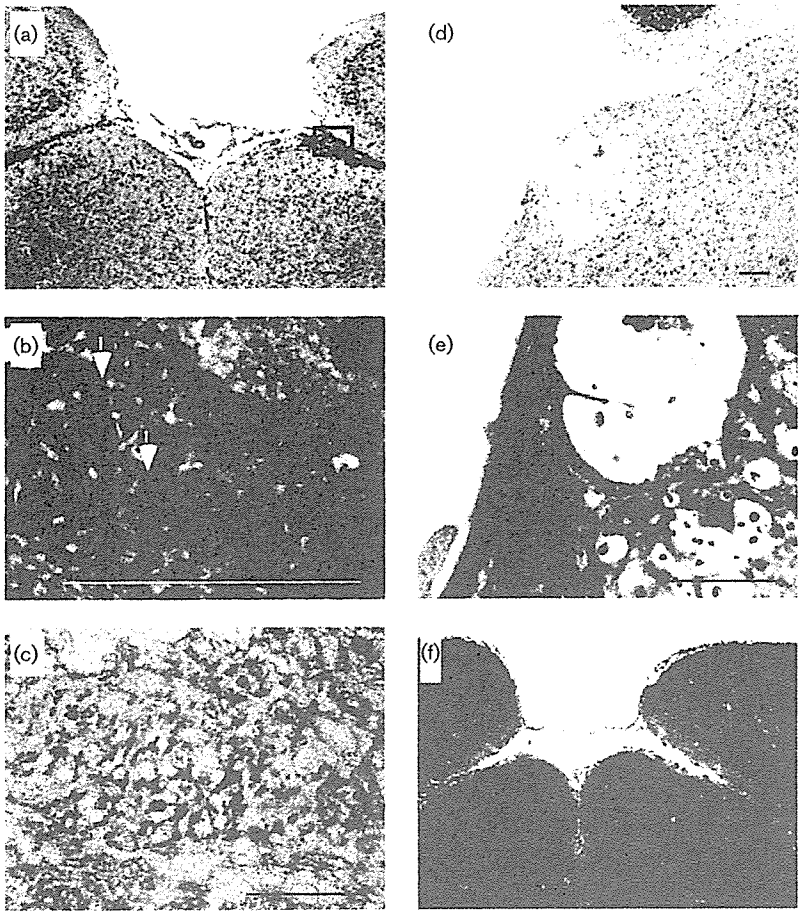

Fig. 5. Histopathology of brain of MPO$^{-/-}$ mice 60 days after *C. neoformans* infection. Brain sections of wild-type (f) and MPO$^{-/-}$ mice (a–e) 60 days after intranasal challenge with $3{\cdot}6 \times 10^6$ c.f.u. are shown. *C. neoformans* were stained with H&E (a, b, d and f) or Grocott methamine silver (c and e). Panel (b) is a higher magnification of the cellular infiltrate observed in the box shown on panel (a). Arrows indicate neutrophils. Bars, 1 mm.

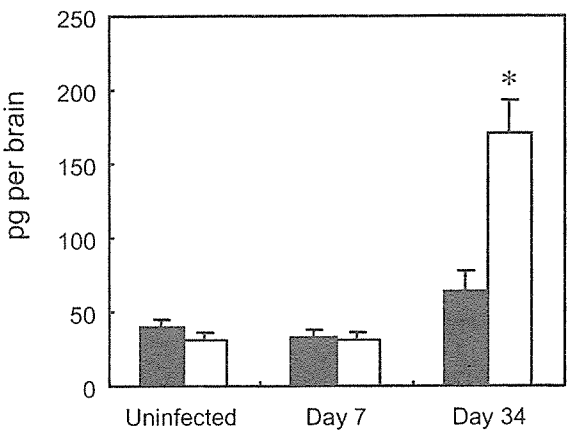

Fig. 6. KC levels in the brains of wild-type and MPO$^{-/-}$ mice after infection with *C. neoformans*. Wild-type (black bars) and MPO$^{-/-}$ mice (open bars) were intranasally infected with $3{\cdot}6 \times 10^6$ c.f.u. *C. neoformans*. Mice were killed before infection, or at 7 or 34 days post-infection. The KC levels of brain homogenates were measured in triplicate using the Bio-Plex system. Data are expressed as pg KC per organ. Results are presented as the mean ± SD of four infected or uninfected mice. *, $P<0{\cdot}05$ compared to wild-type infected mice.

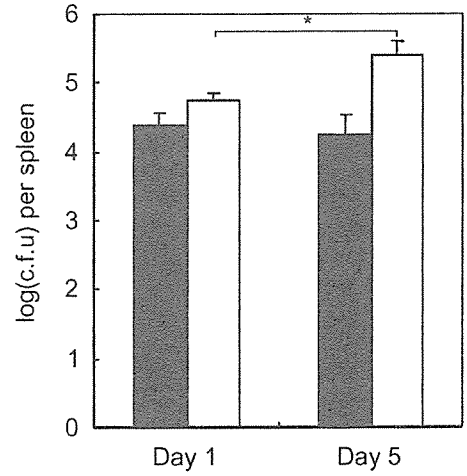

Fig. 7. Cultures from spleen after intravenous infection with *C. neoformans*. Wild-type (black bars) and MPO$^{-/-}$ mice (open bars) were inoculated intravenously with $8{\cdot}7 \times 10^5$ c.f.u. *C. neoformans* per mouse and analysed on days 1 and 5. Aliquots of homogenized organs were plated on agar plates, and total c.f.u. per organ was determined. Five mice were used for each group. Results represent mean log(c.f.u.) per organ ± SD of the means for five animals. *, $P<0{\cdot}05$.

of defensins (Eisenhauer & Lehrer, 1992), prominent non-oxidative contributors to human anticryptococcal defence (Mambula *et al.*, 2000), impaired oxidative systems in mice may permit a more severe infection than in humans. Whereas cell-mediated immunity, non-oxidative systems and reactive nitrogen intermediates (Lovchik *et al.*, 1997) likely contribute to microbial clearance, our data strongly suggest that impairment of the MPO-mediated antimicrobial system could be one of the risk factors for the infection.

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●症 例

インフルエンザウイルス感染を契機に発症した致死的侵襲性肺アスペルギルス症の1例

杉野 圭史[1)] 本間 栄[1)] 高谷 久史[1)] 宮本 篤[1)] 坂本 晋[1)]
川畑 雅照[1)] 岸 一馬[1)] 坪井 永保[1)] 元井 紀子[2)] 吉村 邦彦[1)]

要旨：症例は56歳男性．特発性肺線維症（IPF）に対し無治療で経過観察中，発熱，咳嗽が出現し，画像上新たな浸潤影が認められた．IPFの急性増悪が疑われ，ステロイド薬が投与された．その後，浸潤影に対するTBLBの検体からアスペルギルス菌糸が多数認められたため，侵襲性肺アスペルギルス症（IPA）と診断され，直ちに抗真菌薬が開始された．しかしながら，症状・検査所見の改善は得られず，第21病日に死亡した．剖検肺では，両肺に膿瘍が形成され，内部に多数のアスペルギルス菌糸とそれらの血管内および肺胞腔内への侵襲が認められた．さらに肺の随伴所見として，広範囲のびまん性肺胞傷害およびUIP所見を認めた．本症例では，明らかな全身性免疫不全は合併しておらず，血清中のB型インフルエンザウイルス抗体が高値であったことから，既存のIPFに加えてインフルエンザウイルス感染を契機に末梢血リンパ球数の低下や気道線毛系の障害がもたらされた結果，IPAを発症したものと考えられた．

キーワード：侵襲性肺アスペルギルス症，インフルエンザ，間質性肺炎，リンパ球減少，非免疫不全宿主
Invasive pulmonary aspergillosis, Influenza, Interstitial pneumonia, Lymphocytopenia, Nonimmunocompromised host

緒 言

侵襲性肺アスペルギルス症（IPA）は，一般に高度の免疫不全に合併する日和見感染症である[1)]が，稀に明らかな免疫不全を伴わない宿主にも発症することが報告されている[2)]．今回，B型インフルエンザウイルス感染を契機に細胞性免疫の低下や気道線毛系の障害がもたらされた結果，IPAを発症し，びまん性肺胞傷害（DAD）による呼吸不全で死亡した，特発性肺線維症/usual interstitial pneumonia（IPF/UIP）の1剖検例を報告する．

症 例

症例：56歳，男性．

主訴：発熱，呼吸困難．

既往歴：2000年10月食道癌に対し，内視鏡的粘膜切除術を施行．その際，胸部画像上，間質性陰影を指摘された．精査の結果，IPFと臨床診断され，無治療で経過を観察されていた．2001年1月と4月，食道癌に対してCDDP+5FUによる抗癌剤治療を施行．2002年10月上縦隔に放射線合計66.6Gy照射．

家族歴：特記すべきことなし．

喫煙歴：20本/日×32年間（20歳～52歳）．

職業歴：特記すべきことなし．

現病歴：2005年2月上旬より38℃を超える発熱，咳嗽が出現．徐々に呼吸困難も自覚されたため，近医を受診したところ，肺炎の診断で2月22日に入院となった．入院後，1週間にわたりSBT/ABPCの点滴静注で加療されるも症状の改善はなく，胸部X線上の浸潤影が悪化したため，同月28日にTBLBを施行された．画像上，基礎疾患であるIPFの急性増悪も疑われたため，翌29日よりメチルプレドニゾロン1g/日による3日間のステロイドパルス療法が行われたが，さらに病状が悪化し，3月4日に当院へ転院となった．

入院時身体所見：身長175cm．体重55kg．体温36.8℃．血圧120/80mmHg．脈拍90/分・整．眼瞼結膜貧血なし．眼球結膜黄染なし．表在リンパ節触知せず．両側背下部にfine cracklesと両側背部全体にcoarse cracklesを聴取．心音純，心雑音なし．腹部平坦かつ軟，肝脾触知せず．浮腫なし．

検査所見（Table 1）：入院時の血液検査では白血球数18,600/μl，CRP 8.9mg/dl，血沈47mm/hrと炎症反応

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[2)]同 病理部

（受付日平成17年7月12日）