

To elucidate the role of (1→3)-β-D-glucan in pulmonary aspergilloma, serum concentrations of (1→3)-β-D-glucan were measured repeatedly for as long as 10 months in eight patients. In four patients with inactive disease, concentrations of (1→3)-β-D-glucan were in the normal range. The concentrations of (1→3)-β-D-glucan increased in two patients, although the disease was inactive. This increase might show the earliest stage of the invasive process of the disease. In two other patients with active disease, (1→3)-β-D-glucan increased. Other parameters, such as galactomannan, immunodiffusion and a radio-allergosorbent test, as well as inflammatory markers such as C-reactive protein and the leukocyte count, did not show any consistent tendency in regard to the activity of the disease. Thus, a (1→3)-β-D-glucan assay may add valuable data for evaluating the disease activity and understanding the disease process of pulmonary aspergilloma.

Keywords: Inflammatory markers

(1→3)-β-D-glucan in patients with pulmonary aspergilloma

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Introduction

In a previous study, we measured serum (1→3)-β-D-glucan in patients with pulmonary aspergillosis and found that (1→3)-β-D-glucan increased in some cases of pulmonary aspergilloma, in which the fungus was considered not to have invaded the surrounding tissue or blood stream [1].

In the present study, to elucidate the role of (1→3)-β-D-glucan in pulmonary aspergilloma, serum concentrations of (1→3)-β-D-glucan were measured repeatedly for as long as 10 months in eight patients.

Materials and methods

Subjects

Eight patients with pulmonary aspergilloma were enrolled in the study (Table 1). Of the eight patients, the disease was inactive in six. In patient 7, hemoptysis was frequently observed throughout the follow-up period, and in patient 8, the fungus ball steadily increased in size. The disease was judged to be active in these two patients. Itraconazole (ITCZ) at 100–200 mg/day, was administered throughout the follow-up period to all but patient 7.

Clinical evaluation

The patient's physical condition, a chest X-ray and blood tests (leukocyte count, C-reactive protein) were examined once a month in each patient to evaluate the activity of the disease.

Measurement of (1→3)-β-D-glucan

The concentrations of (1→3)-β-D-glucan were measured following the method described by Obayashi *et*

al. [2,3] and Morita *et al.* [4]. With this method, the cut-off value for (1→3)-β-D-glucan was set at 20 pg/ml [5].

Measurement of other *Aspergillus*-related substances

Anti-*Aspergillus* immunoglobulin (Ig)E antibody was measured by a radio-allergosorbent test (>0.34 U/ml was positive). The presence of *Aspergillus*-specific antibodies in sera was examined by immunodiffusion. The *Aspergillus*-specific cell-surface component galactomannan was assayed with Pastorex Aspergillus (Sanofi Diagnostics Pasteur, Genk, Belgium). The sensitivity of the test was 15 ng/ml of galactomannan [6].

Results and discussion

On the basis of the concentration of (1→3)-β-D-glucan and the clinical activity of pulmonary aspergilloma, the patients were classified into three groups: group 1, normal (1→3)-β-D-glucan with inactive disease; group 2, increased (1→3)-β-D-glucan with inactive disease; and group 3, increased (1→3)-β-D-glucan with active disease.

Group 1 consisted of patients 1–4 (Table 2). In this group, the values of galactomannan, immunodiffusion, radio-allergosorbent test and C-reactive protein were variable in each patient, and there were no significant correlations among these values.

Patients 5 and 6 were classified in group 2 (Table 3). In this group, (1→3)-β-D-glucan exceeded 20 pg/ml. However, there were no obvious signs of active disease. Other parameters, such as galactomannan, immunodiffusion, the radio-allergosorbent test and

Table 1. Details of all study patients

Patient	Age (years)	Sex	Diagnosis	Treatment	Initiation of treatment
1	60	Male	Aspergilloma	ITCZ at 150 mg/day	21 Nov 1995
2	78	Male	Aspergilloma	ITCZ at 200 mg/day	14 Apr 1995
3	78	Male	Aspergilloma	ITCZ at 100 mg/day	11 Jan 1994
4	67	Female	Aspergilloma	ITCZ at 150 mg/day	05 Dec 1994
5	67	Male	Aspergilloma	ITCZ at 200 mg/day	28 Feb 1995
6	67	Male	Aspergilloma	ITCZ at 100 mg/day	28 Sep 1994
7	79	Male	Aspergilloma		
8	83	Male	Aspergilloma	ITCZ at 150 mg/day	23 Aug 1995

ITCZ, itraconazole.

Table 2. Follow-up study of group 1

	Leukocytes (count/ μ l)	CRP (mg/dl)	RAST (U/ml)	Immuno-diffusion	Galactomannan	Glucan (pg/ml)
Patient 1						
08 Nov 1995	7400	8.7	4.2	+	-	13.4
11 Nov 1995	6900	4.2	ND	ND	ND	4.9
04 Dec 1995	6600	5.0	4.12	+	-	6.3
22 Jan 1996	8300	4.9	3.42	+	-	11.7
19 Feb 1996	6400	5.1	3.99	+	-	13.6
18 Mar 1996	7500	5.0	2.96	+	-	13.6
Patient 2						
13 Jun 1995	4700	0.8	0.37	+	-	9.0
07 Aug 1995	4900	0.9	<0.34	+	-	19.3
04 Sep 1995	4500	1.1	<0.34	+	-	15.3
03 Oct 1995	5200	1.1	0.37	+	-	5.2
30 Oct 1995	4800	0.6	0.46	+	-	14.3
27 Nov 1995	5400	0.4	0.69	+	-	4.5
26 Dec 1995	7700	0.4	0.50	+	-	11.2
22 Jan 1996	5500	0.9	0.49	+	-	12.0
19 Feb 1996	6800	0.6	0.53	+	-	14.7
18 Mar 1996	5800	1.4	0.56	+	-	3.0
Patient 3						
16 May 1995	7000	0.4	1.46	+	ND	6.8
20 Jun 1995	5300	0.6	1.54	ND	-	5.6
11 Jul 1995	6500	1.0	1.21	ND	-	0.0
08 Aug 1995	5600	0.7	1.28	+	+	1.3
12 Sep 1995	6600	0.5	1.37	ND	-	0.8
20 Oct 1995	5900	0.4	1.58	ND	-	1.6
14 Nov 1995	7100	0.6	2.16	+	-	4.2
12 Dec 1995	5500	1.2	2.22	ND	-	6.0
16 Jan 1996	5300	0.4	1.85	ND	-	19.2
13 Feb 1996	5400	0.9	2.06	+	+	ND
12 Mar 1996	5300	0.7	1.69	ND	-	15.7
Patient 4						
22 May 1995	6300	0.0	0.50	+	+	12.2
19 Jun 1995	7100	0.1	0.48	+	+	6.1
17 Jul 1995	7200	0.0	0.54	+	+	12.0
14 Aug 1995	7100	0.0	ND	ND	ND	8.8
25 Sep 1995	7100	0.0	0.43	+	+	4.6
23 Oct 1995	6900	0.1	0.35	+	+/-	7.4
20 Nov 1995	8700	1.2	0.82	+	+	5.8
18 Dec 1995	6400	0.2	0.77	+	+	14.9
08 Jan 1996	5700	0.1	0.79	+	+	6.5
11 Mar 1996	6100	0.1	0.69	+	+	6.7

CRP, C-reactive protein; RAST, radio-allergosorbent test; ND, not determined.

C-reactive protein, were dissociated from each other. Thus the reason why there appeared to be no obvious signs of disease may be that *Aspergillus* had just started to invade the surrounding tissue and had not progressed enough to be detectable by conventional methods. However, further follow-up studies are required to verify this hypothesis.

Group 3 comprised patients 7 and 8 (Table 4). Patient 7 had complained of frequent hemoptum since

1979, when he was diagnosed as suffering from pulmonary aspergilloma. In this case, values of (1 \rightarrow 3)- β -D-glucan and radio-allergosorbent test were elevated. Galactomannan was seldom detected, however. In patient 8, the fungus ball showed steady growth until 1995, when the patient was enrolled in the study. In this case, to elucidate the day-to-day variation, the concentrations of (1 \rightarrow 3)- β -D-glucan were measured daily for 2 weeks. The size of the fungus ball remained

Table 3. Follow-up study of Group 2

	Leukocytes (count/ μ l)	CRP (mg/dl)	RAST (U/ml)	Immuno- diffusion	Galacto- mannan	Glucan (pg/ml)
Patient 5						
22 Jun 1995	5800	1.6	<0.34	+	-	7.2
17 Aug 1995	4900	3.1	<0.34	+	-	18.8
14 Sep 1995	ND	ND	<0.34	+	-	22.5
09 Nov 1995	7300	2.6	ND	ND	ND	22.4
07 Dec 1995	6000	2.9	ND	+	-	17.1
08 Feb 1996	6700	2.7	ND	+	-	21.3
27 Feb 1996	7400	3.0	ND	ND	ND	ND
14 Mar 1996	ND	ND	ND	ND	ND	21.6
Patient 6						
18 May 1995	9500	12.7	1.77	-	-	21.0
15 Jun 1995	8100	3.7	1.44	ND	-	13.8
14 Jul 1995	9300	3.6	1.45	-	-	10.8
25 Aug 1995	7700	5.5	0.89	ND	-	26.0
22 Sep 1995	6500	3.8	1.16	ND	-	27.4
20 Oct 1995	9100	3.0	1.60	-	-	28.7
24 Nov 1995	7800	2.7	1.70	ND	-	37.3
22 Dec 1995	8200	5.0	2.10	ND	-	26.8
19 Jan 1996	5000	2.5	1.91	-	-	22.5
16 Feb 1996	4600	6.4	2.13	ND	-	14.2
15 Mar 1996	7600	4.2	1.85	ND	-	24.6

CRP, C-reactive protein; RAST, radio-allergosorbent test; ND, not determined.

Table 4. Follow-up study of Group 3

	Leukocytes (count/ μ l)	CRP (mg/dl)	RAST (U/ml)	Immuno- diffusion	Galacto- mannan	Glucan (pg/ml)
Patient 7						
26 May 1995	5500	2.6	10.12	ND	-	60.1
30 Jun 1995	6200	2.4	9.38	ND	-	59.3
28 Jun 1995	6500	3.0	11.20	+	+	55.0
25 Aug 1995	6900	3.1	12.95	ND	+	52.1
22 Sep 1995	5500	2.4	12.37	ND	-	54.1
20 Oct 1995	6200	4.3	12.71	+	-	56.9
14 Nov 1995	5500	3.5	20.92	ND	-	41.8
19 Dec 1995	6700	3.3	18.45	ND	-	33.6
16 Jan 1996	6500	3.1	15.81	ND	-	24.6
20 Feb 1996	10 900	2.4	21.60	ND	-	47.6
Patient 8						
31 Jul 1995	5800	0.2	<0.34	-	-	ND
23 Aug 1995	ND	ND	ND	ND	ND	42.0
24 Aug 1995	ND	ND	ND	ND	ND	28.2
25 Aug 1995	ND	ND	ND	ND	ND	32.4
28 Aug 1995	ND	ND	ND	ND	ND	32.4
29 Aug 1995	ND	ND	ND	ND	ND	35.1
30 Aug 1995	5200	0.1	ND	ND	ND	36.0
31 Aug 1995	ND	ND	ND	ND	ND	34.5
04 Sep 1995	ND	ND	ND	ND	ND	16.1
05 Sep 1995	ND	ND	ND	ND	ND	11.1
07 Sep 1995	5100	0.2	ND	ND	ND	11.0

CRP, C-reactive protein; RAST, radio-allergosorbent test; ND, not determined.

unchanged and (1 \rightarrow 3)- β -D-glucan values were normalized during the period.

In contrast to the prevalent notion, increased (1 \rightarrow 3)- β -D-glucan values in this group might imply that *Aspergillus* in the lesion of aspergilloma can invade surrounding tissue and/or the bloodstream, though the invasion is radiologically undetectable. The prompt decline in serum (1 \rightarrow 3)- β -D-glucan in patient 8 may support this concept.

In summary, the data presented here suggest that the (1 \rightarrow 3)- β -D-glucan assay may add valuable data for evaluating the disease activity as well as for understanding the disease process of pulmonary aspergilloma.

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