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Immunoblot analysis of components of barley recognized by IgE antibodies in sera from pig farm workers

Components of the crude extract of barley, recognized by IgE antibodies in sera from 132 pig farm-workers in southern Taiwan, were investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Among the sera tested, 43 (32.6%) showed IgE reactivity to 15 components of barley with molecular mass ranging from 148 000 to 14 000 daltons (Da). Heterogeneity in the IgE-binding patterns of the samples tested was observed. However, the major antigen appears to be the component with about 37 kDa, in 32 (74.4%) of the 43 immunoblot-positive serum samples. Furthermore, components with about 55 and 67 kDa which reacted with 26 (60.5%) and 18 (41.9%) of the positive samples, respectively. These might also be considered as important allergens of the flour made from entire seeds of barley. The results suggest that barley may be considered to play a role in the IgE-mediated occupational respiratory disorders and the 37 kDa component has also been shown to be the major allergen.

Occupational respiratory disorders are common among workers exposed to cereal flour [1, 2]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using sera from allergic workers have been used as a tool to screen out allergens [3, 4]. Reports about allergic bakers and farmers pointed out that α -amylase, inhibitors of α -amylase and protease are major allergens [5, 6]. Here we studied the percentages of IgE-reactive components by using sera from randomly selected workers on pig farms; most of them had been exposed for over 10 years to feed dust that was partly made from entire seeds of barley.

Flour of barley was collected directly from a feed factory, extracted with pH 7.0, 0.5 M phosphate-buffered saline, for 18 h at 4°C; the protein part was concentrated with ammonium sulfate, and dried by lyophilization. The protein extracts were analyzed by the methods used in our clinical routine [7]; after reducing with 2% SDS at 100°C

for 5 min, the antigens were separated on 12.5% SDS-PAGE in the Protein II system (Bio-Rad), then transferred onto PVDF membrane in 500 mA for 1 h. After transfer, the membrane sheet was cut into 0.4 × 12 cm strips for incubation overnight with all the sera. The alkaline phosphatase-conjugated anti-human IgE ϵ secondary antibody was reacted at room temperature; next, substrate was added for color reaction. The relation of appearance rate of each component and the exposure groups was analyzed with SPSS Software.

The sera were collected from 133 workers from six different types of pig farms in southern Taiwan. Twenty-seven workers (20.4%) had allergic problems of biological-related causes, and six complained of being hypersensitive to feed dust. At least fifteen components of barley with a molecular mass ranging from over 94 kDa to 14 kDa components were recognized by their reactivity to IgE antibodies. Heterogeneity in the IgE-binding patterns of the samples tested was observed. The major antigen appears to be the component with a molecular mass of about 37 kDa, in 32 (74.4%) of the 43 immunoblot-positive serum samples. Components with a molecular mass of about 55 kDa and 69 kDa reacted with 26 (60.5%) and 18 (41.9%) of the positive samples, respectively. The positive reactions on immunoblots were sig-

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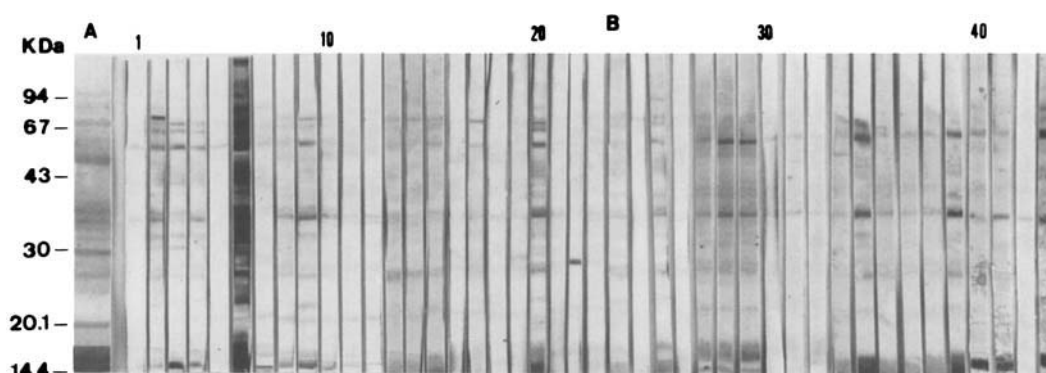


Figure 1. Identification of IgE-binding components by alkaline-phosphatase conjugated anti-human IgE antibodies after SDS-PAGE of the crude extract of barley with sera from 43 positive reactive sera collected from pig farms.

Table 1. Results of specific IgE χ^2 analysis by exposure groups in 133 sera from 6 pig farms

Exposure groups (workers)	Component (kDa)	Specific positive IgE reactive rates (%)														
		1	2	3	4	5	6	7 ^{a)}	8	9*	10	11 ^{a)}	12	13	14	15
I (38)		1.5	1.5	1.5	1.5	1.5	2.3	2.3	3.0	5.2	1.5	0.8	1.5	0.8	0.0	0.8
II (38)		2.3	1.5	1.5	1.5	4.5	6.0	11.3	3.8	11.3	3.8	2.3	6.7	1.5	1.5	1.5
III (25)		0.8	0.8	0.8	1.5	2.3	3.0	4.5	2.3	5.3	1.5	3.0	3.0	3.0	2.3	1.5
IV (32)		1.5	0.8	0.8	0.8	3.4	2.3	2.3	1.5	3.0	1.5	0.0	1.5	0.8	0.8	0.0
Total (133)		6.0	3.8	4.5	5.3	10.5	13.5	20.3	10.5	24.8	8.3	6.0	12.8	6.0	4.5	3.8

a) $P < 0.05$

nificantly associated with those workers having asthma, and the two major components which showed positive reactions with IgE were significantly associated with the exposure groups by χ^2 analysis.

The concentrations of respirable dust from personal samplers were between 0.014 mg/m³ and 1.450 mg/m³, but the prevalence of respiratory disorders was over twenty percent, and about one tenth of the workers had bad lung functions. It was postulated that a small amount of a low concentration, below the threshold limit value (TLV), can induce occupational asthma. It is important to determine the concentration of allergy-causing components in the air of workplaces. For the identification of these allergens, isoelectric gel analysis will be performed in a future study.

Results obtained in this study suggest that barley may be considered to play a role in the IgE-mediated occupational respiratory disorders on pig farms. Furthermore, the 37 kDa component appearing in barley powder

seems to be the major allergen, and showed significant association with exposure groups.

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References

- [1] Garcia-Casado, G., Armentia, A., Sanchez-Monge, R., Malpica, J. M., Salcedo, G., *Clin. Exp. Allergy* 1996, 26, 428–435.
- [2] Houba, R., Van Run, P., Heedrik, D., Doekes, G., *Clin. Exp. Allergy* 1996, 26, 154–163.
- [3] Armentia, A., Sanchez-Monge, R., Gomez, L., Barber, D., Salcedo, G., *Clin. Exp. Allergy* 1993, 23, 410–415.
- [4] Franken, J., Stephan, U., Neuber, K., Bujanowski-Weber, J., Ulmer, W. T., Konig, W., *Int. Arch. Allergy Appl. Immunol.* 1991, 96, 76–83.
- [5] Stacie, M., Jones, M. D., Chiara, F., Magnolfi, M. D., Sara, K., Cooke, S. B., Hugh, A., Sampson, M. D., *J. Allergy Clin. Immunol.* 1995, 96, 341–351.
- [6] Reisbig, R. R., Bruland, Q., *Biochem. Biophys. Res. Commun.* 1983, 114, 190–196.
- [7] Shen, H. D., Choo, K. B., Wang, S. R., Lin, W. L., Chang, Z. N., Han, S. H., *J. Allergy Clin. Immunol.* 1991, 88, 802–807.