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SHORT COMMUNICATION

IMMUNOADJUVANT ACTIVITIES OF FUNGAL CELL WALLS

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The chemical compositions and structures of fungal cell walls are quite different from those of bacterial cell walls. So for comparison, during studies on the immunoadjuvant activities of the cell walls of various gram-positive bacteria (Kotani et al., 1975), the immunoadjuvancies of fungal cell walls were examined. Crystalline ovalbumin was used as a test pro-

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tein antigen and administered with a variety of fungal cell wall preparations to guinea-pigs as water-in-oil emulsions, as in a previous study (Kotani et al., 1975).

Fourteen specimens of fungal cell walls from seven species were assayed for immunoadjuvancy. These specimens were prepared as described in the respective references listed in Table 1. The methods used for immunization of guinea-pigs and assays of the development of delayed-type hypersensitivity and stimulation of circulating antibody levels were essentially as described in an accompanying paper (Kotani et al., 1975). In brief, induction of delayed-type hypersensitivity to ovalbumin was examined by the corneal test, and serum antibody levels were determined by the quantitative precipitin reaction.

The results of adjuvant assays are summarized in Table 1. Some specimens of fungal cell walls showed definite adjuvant activity, but as a whole, fungal cell walls seemed to be less potent than bacterial cell walls in stimulating either humoral or cellular immune responses. It seems significant that the cell walls from the yeast-forms of *Candida albicans* (6713) and *Histoplasma capsulatum* (G184B) showed immunoadjuvant activity, at least for induction of delayed-type hypersensitivity but those from the mycelial forms of these fungi did not show any activity for either induction of delayed-type hypersensitivity or stimulation of antibody production. Zymosan preparations from Fleishmann and Oriental yeasts also differed from each other in their immunoadjuvant activities: Fleishmann yeast zymosan was active in development of a positive corneal response and stimulation of circulating antibody levels, while Oriental yeast zymosan showed no adjuvancy. The cell walls from *Geotrichum candidum* (4028) also exhibited weak but definite activity in both induction of delayed-type hypersensitivity and stimulation of antibody production. *Saccharomyces cerevisiae* cell walls from both strain Hansen 0209 and Press yeast (Toyo Brewery Co., Shizuoka), including a highly purified preparation of β–1, 3-glucan isolated from yeast cell walls, were highly effective in stimulating antibody production, but their adjuvant activities to induce delayed-type hypersensitivity, though detectable, were weak. The cell walls from *Paracoccidioides* (*Blastomyces*) *brasiliensis* (7913, yeast- and mycelial forms), *Trichophyton mentagrophytes var. asteroides* and *Epidermophyton floccosum* (TEF–30) proved to be adjuvant-inactive, at least under the present experimental conditions.

Although there are several reports on the activity of yeast cell walls to stimulate humoral immune responses (Suzuki et al., 1971; Mifuchi, Shimizu and Seike, 1972b; Hosoi et al., 1972; Shimizu, Mifuchi and Nakano, 1973; Hosoi et al., 1973; Nagakawa et al., 1974), little attention has been paid to the immunoadjuvant activities of cell walls of fungi other than *Saccharomyces cerevisiae*. So far as we know, no data are available on which to evaluate the activity of fungal cell walls to induce cell-mediated immune responses to a protein antigen. Thus it is very interesting that this work showed that some fungal cell walls had definite activity for induction of delayed-type hypersensitivity in terms of development of a positive corneal response. The different biological activities of the cell walls from different fungal species and of the same species at different phases may reflect differences in the chemical characteristics of the cell wall preparations. However, the exact nature of the chemical differences which cause these differences in the biological activities of cell wall preparations are unknown.

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TABLE 1. *Immunoadjuvant activities of various fungal cell walls*

No. of experimental group	Test material	Dose (μg)	Corneal response (48 hr) Mean (Range)	Antibody level (Ratio)[a] Mean±S.E.[c]	IgG_2 Mean (Range)	Reference
3	*Saccharomyces cerevisiae* (Hansen 0209)	100	0.8 (0 -1.0)	1.8 ±0.12**	1.2 (0 -2.0)	Mifuchi, Shimizu and Seike, 1972a
23		200	0.2 (0 -1.0)	3.0 ±0.67*	0.4 (0 -1.0)	
23	*Saccharomyces cerevisiae* (Press yeast, Toyo Brewery)	200	1.0 (0 -2.0)	4.0 ±0.20**	0.9 (0 -2.0)	Shimizu, Mifuchi and Nakano, 1973
4	β-1, 3 glucan from *Saccharomyces cerevisiae* walls	200	1.5 (0 -3.0)	4.0 ±0.65**	1.4 (0 -3.0)	Misaki et al., 1968
	Zymosan					
3	from Fleishmann yeast	100	2.0 (1.0-3.0)	1.9 ±0.15**	0.7 (0 -2.0)	Kwapinski, 1965
3	from Fleishmann yeast	200	2.0 (2.0-2.0)	1.4 ±0.19	0.1 (0 -0.5)	
3	from Oriental yeast	200	0.25(0 -1.0)	1.3 ±0.30	0	
	Candida albicans (6713)					
3	Yeast-form	200	2.0 (2.0-2.0)	1.8 ±0.45	1.1 (0 -2.0)	Yamaguchi, 1974
3	Mycelial form	200	0	0.81±0.12	0.2 (0 -0.5)	
	Paracoccidioides brasiliensis (7193)					
6	Yeast-form	200	0	1.2 ±0.37	0.6 (0 -1.5)	Kanetsuna et al., 1969
24	Mycelial form	200	0	0.65±0.11	0	
	Histoplasma capsulatum (G184B)					
5	Yeast-form	200	2.0 (1.0-3.0)	1.6 ±0.22	1.5 (0 -2.5)	Kanetsuna et al., 1974
5	Mycelial form	200	0.5 (0 -2.0)	0.91±0.22	0.5 (0 -2.5)	
7	*Trichophyton mentagrophytes var. asteroides*	200	0.2 (0 -1.0)	1.1 ±0.32	0	Noguchi et al., 1971
1	*Epidermophyton floccosum* (TEF-30)	100	0.1 (0 -0.5)	0.53±0.14	ND[d]	Nozawa, Kitazima and Ito, 1973
2	*Geotrichum candidum* (4028)	100	1.5 (1.0-3.0)	6.7 ±3.22	1.5 (0.5-3.0)	Matsuoka, 1969
3		200	1.0 (0 -2.0)	1.8 ±0.24**	1.4 (1.0-3.0)	
1	FICA-type control	—	0.6 (0 -1.0)	[219±52][b]	0	
2	FICA-type control	—	0	[45±23]	0	
3	FICA-type control	—	0.4 (0 -1.0)	[67± 8]	0.3 (0 -1.0)	
4	FICA-type control	—	0	[106± 7]	0.6 (0 -2.0)	
5	FICA-type control	—	0.2 (0 -1.0)	[212±55]	0.8 (0 -2.0)	
6	FICA-type control	—	0	[167±16]	0.4 (0 -1.0)	
7	FICA-type control	—	0	[82±31]	ND	
23	FICA-type control	—	0.3 (0 -1.0)	[106±18]	0	
24	FICA-type control	—	0	[228±11]	ND	

[a] Ratio of antibody nitrogen (μg/ml serum specimen) in the test group to that in the respective control group.
[b] μg Antibody nitrogen/ml serum specimen.
[c] The difference between the test and respective control groups was significant at a level of 5% (*) or 1% (**), by the "Student" t-test.
[d] Not determined.

REFERENCES

Harada, T., K. Nomoto, R. Koyanagi, H. Yamada, K. Takeya, Y. Nagakawa, and M. Hosoi. 1974. Effect of adjuvants derived from microorganism. I. Strain difference in adjuvant effects. Proc. 4th Annu. Meet. Jap. Soc. Immunol. 186–188. [In Japanese]

Hosoi, M., Y. Nagakawa, H. Oshikata, and K. Nomoto. 1972. Effects of yeast cell walls on the functions of lymphoid system. Proc. 2nd Annu. Meet. Jap. Soc. Immunol. 48–50. [In Japanese]

Hosoi, M. Y. Nagakawa, H. Oshikata, and K. Nomoto. 1973. Effects of yeast cell walls on the functions of lymphoid system. II. Comparison of stimulatory effects of various adjuvants on antibody production. Proc. 3rd Annu. Meet. Jap. Soc. Immunol. 278–281. [In Japanese]

Kanetsuna, F., L. M. Carbonell, R. E. Moreno, and J. Rodriguez. 1969. Cell wall composition of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. J. Bacteriol. 97: 1036–1041.

Kanetsuna, F., L. M. Carbonell, F. Gil, and I. Azuma. 1974. Chemical and ultrastructural studies on the cell walls of the yeast-like and mycelial forms of *Histoplasma capsulatum*. Mycopath. Mycol. Appl. 54: 1–13.

Kotani, S., T. Narita, D. E. S. Stewart-Tull, T. Shimono, Y. Watanabe, K. Kato, and S. Iwata. 1975. Immunoadjuvant activities of cell walls and their water-soluble fractions prepared from various gram-positive bacteria. Biken J. 18: 77–92.

Kwapinski, J. B. 1965. Complement and properdin assays. p. 260–275. *In* Methods in Serological Research. John Wiley and Sons, New York.

Matsuoka, T. 1969. Effect of snail enzyme on the cell walls of *Geotrichum candidum*. Shikoku Acta Medica. 25: 416–426. [In Japanese]

Mifuchi, I., T. Shimizu, and I. Seike. 1972a. Antitumor action of yeast cell wall and the reticuloendothelial system in mice. Medicine and Biology 84: 345–348. [In Japanese]

Mifuchi, I., T. Shimizu, and I. Seike. 1972b. Antitumor action of yeast cell wall and its adjuvant activity in mice. Medicine and Biology 85: 177–180. [In Japanese]

Misaki, A., J. Johnson, Jr., S. Kirkwood, J. V. Scaletti, and F. Smith. 1968. Structure of cell wall glucan of yeast (*Saccharomyces cerevisiae*). Carbohydr. Res. 6: 150–164.

Nagakawa, Y., H. Oshikata, M. Hosoi, and K. Nomoto. 1974. Studies on the adjuvant effect of yeast cell walls. Proc. 4th Annu. Meet. Jap. Soc. Immunol. 496.

Noguchi, T., Y. Kitajima, Y. Nozawa, and Y. Ito. 1971. Isolation, composition and structure of cell walls of *Trichophyton mentagrophytes*. Arch. Biochem. Biophys. 146: 506–512.

Nozawa, Y., Y. Kitajima, and Y. Ito. 1973. Chemical and ultrastructural studies of isolated cell walls of *Epidermophyton floccosum*. Biochim. Biophys. Acta 307: 92–103.

Shimizu, T., I. Mifuchi, and M. Nakano. 1973. Adjuvant activity of yeast cell wall. Medicine and Biology 87: 281–284. [In Japanese]

Suzuki, S., M. Suzuki, T. Matsumoto, and Y. Okawa. 1971. Growth inhibition of Sarcoma-180 solid tumor by the cells of regional lymph node and spleen from mice administered with yeast polysaccharides. Gann 62: 343–352.

Yamaguchi, H. 1974. Effect of biotin insufficiency on composition and ultrastructure of cell walls of *Candida albicans* in relation to its mycelial morphogenesis. J. Gen. Appl. Microbiol. 20: 217–228.