Evaluation August 2014

Samples:

1. Immu-911
2. C-FX
3. Bio Immune
4. Ginseng
5. Immunity Plus
6. Fucoidan
7. Glucan #300

We performed several tests focused on possible effects on various segments of the immune system. First, we incubated our samples with two human cancer cell lines (breast cancer line ZR-75-1 and lung cancer line H209) under different conditions. When incubated with fetal calf serum, the results would show in the samples somehow inhibited the growth and proliferation of cancer cells. Our experiments clearly showed that none of the samples inhibited proliferation of cells. The second part of these experiments evaluated the effects on the same cancer cell lines cultivated in medium without fetal calf serum. Under these conditions, cancer cells do not proliferate, so any significant changes in proliferation would mean that the samples stimulate the cell proliferation. Due to the completely negative effects, data are not shown in graph.

Next, we focused on phagocytosis. In tests of natural immunomodulators, phagocytosis is usually the test of choice, as if the sample does not stimulate phagocytosis, it might have little effects on additional facets of the defense reactions. We employed synthetic hydroxyethyl methacrylate particles known for minimal nonspecific adhesion to the membrane of phagocytosing cells and measured the effects of injection of mice with different doses of tested material on the phagocytic activity of peripheral blood neutrophils. Data summarized in Table 1 showed that the tested samples had almost no activity (with exception of limited activity of Bio Immune, but only at doses which are not feasible to use). Compared to that, Glucan #300 showed clear dose-dependent stimulation of phagocytosis.

Table 1 Effects on phagocytosis

Dose (μg)	**25**	**50**	**100**	**200**	**400**	**800**
Immune-911	30.8	32.5	33.6	29.7	34.6	36.0
C-FX	27.9	31.5	32.6	30.8	32.9	33.3
Bio Immune	31.2	33.5	34.7	35.1	36.2	36.3
Gingseng	29.6	29.8	30.7	31.2	33.0	31.4
Immunity Plus	28.8	32.8	33.1	32.9	34.1	33.8

Fucoidani	29.7	30.7	32.5	35.1	32.7	33.8
Glucan #300	44.1	48.8	55.7	56.1	55.9	60.9

Control values (PBS) were 31.3 ± 2.7.

Phagocytosis results in internalization of the prey, but represents only one of the several subsequent steps, leading to burst of metabolic activity and final killing and/or destruction of the ingested material. Another part of the internalization process is the subsequent burst of metabolic activity. Part of it is the production of active oxidative species, necessary for killing and destruction of bacteria. Production of active oxygen molecules is necessary for the destruction of invading microorganisms. For example, all glucans were repeatedly shown to stimulate oxidative burst. Therefore, we evaluated the effects of our glucans on production of superoxide anion. Data summarized in Table 2 show that most of the tested samples have very little activity, statistically significant activity was shown only in case of Immunity Plus and Fucoidani.

Table 2 Effects on production of superoxide anion

	HL-60 cells (nmol/2.5x10^5 cells)
Immune-911	0.42
C-FX	0.37
Bio Immune	0.43
Gingseng	0.37
Immunity Plus	0.54
Fucoidan	0.55
Glucan #300	1.81
PBS	0.35

Immunomodulators are well known to stimulate production and secretion of various cytokines, with a wide range from IL-1, IL-2, and IL-6 to TNFα, and IFNγ. To compare the effects of our group of samples, we measured the production of IL-2 by splenocytes and IFN-γ in the blood. Under normal circumstations, splenocytes do not produce IL-2, so the basal levels are almost zero. Because of that, the stimulation by

tested samples can be considered statistically significant. However, we have to keep on mind that the actual amount of secreted cytokines is very low and in real situation represents no real effects. Particularly important is the comparison to the effects of glucan (Tables 3 and 4).

Table 3 Effect of individual samples on IFNγ secretion

Sample	IFNγ (pg/ml)
Immune-911	26.3
C-FX	13.9
Bio Immune	22.8
Gingseng	8.9
Immunity Plus	27.8
Fucoidan	15.8
Glucan #300	201.2
PBS	2.1

Table 4 Effects on production of IL-2

Dose (μg)	25	50	100	200	400	800
Immune-911	8.3	11.9	16.9	33.8	29.9	44.9
C-FX	2.2	8.1	6.9	18.9	20.1	19.0
Bio Immune	10.2	28.8	33.3	40.2	44.6	46.5
Gingseng	1.1	9.8	10.9	12.8	16.7	20.7
Immunity Plus	47.1	56.5	89.3	102.4	111.7	120.5
Fucoidan	7.8	18.2	20.8	28.9	37.4	48.5
Glucan #300	109.8	201.2	1 047.6	1 122.8	1 109.9	1 226.6

pg/ml

Conclusion

From our experiments we can concluded that none of the tested samples have any significant activity towards stimulation of the immune system. As both the cellular (phagocytosis and superoxide anion production) and the humoral (production of two cytokines) branches were not significantly stimulated, we cannot recommend these samples to be considered as immunostimulants. Their very low activity can be clearly seen when compared to the effects of Glucan #300.

Readers seeking more details on used techniques and on direct comparisons of various natural immunomodulators should read these recent comparative studies:

Vetvicka, V., Vetvickova, J.: β1,3-Glucan: Silver bullet or hot air? Open Glycoscience, 3:1-6,2010.

Vetvicka, V., Vetvickova, J.: Comparison of immunological effects of commercially available β-glucans. Applied Sci. Rep., 1, 2014, doi:10.7243/2054-9903-1-2

Vetvicka, V., Vetvickova, J.: Natural immunomodulators and their stimulation of immune reaction: True or false? Anticancer Res. 34:2275-2282, 2014.

Evaluation November 2014

Samples:

1. Imortalium
2. Ultimate Killer Biotic FX
3. Glucan #300

We performed several tests focused on possible effects on various segments of the immune system. First, we incubated our samples with two human cancer cell lines (breast cancer line ZR-75-1 and lung cancer line H209) under different conditions. When incubated with fetal calf serum, the results would show in the samples somehow inhibited the growth and proliferation of cancer cells. Our experiments clearly showed that none of the samples inhibited proliferation of cells. The second part of these experiments evaluated the effects on the same cancer cell lines cultivated in medium without fetal calf serum. Under these conditions, cancer cells do not proliferate, so any significant changes in proliferation would mean that the samples stimulate the cell proliferation. Due to the completely negative effects, data are not shown in graph.

Next, we focused on phagocytosis. In tests of natural immunomodulators, phagocytosis is usually the test of choice, as if the sample does not stimulate phagocytosis, it might have little effects on additional facets of the defense reactions. We employed synthetic hydroxyethyl methacrylate particles known for minimal nonspecific adhesion to the membrane of phagocytosing cells and measured the effects of injection of mice with different doses of tested material on the phagocytic activity of peripheral blood neutrophils. Data summarized in Table 1 showed that the tested samples had limited activity and mostly at too high doses. Compared to that, Glucan #300 showed clear dose-dependent stimulation of phagocytosis.

Table 1 Effects on phagocytosis

Dose (μg)	**25**	**50**	**100**	**200**	**400**	**800**
Imortalium	30.1	32.0	32.4	31.9	35.6	37.7
Ultimate Killer Biotic FX	30.9	31.2	35.5	36.8	38.1	39.3
Glucan #300	41.1	48.3	53.2	54.4	53.9	61.2

Control values (PBS) were 30.6 ± 2.5.

Phagocytosis results in internalization of the prey, but represents only one of the several subsequent steps, leading to burst of metabolic activity and final killing and/or destruction of the ingested material. Another part of the internalization process is the subsequent burst of metabolic activity. Part of it is the production of active oxidative

species, necessary for killing and destruction of bacteria. Production of active oxygen molecules is necessary for the destruction of invading microorganisms. For example, all glucans were repeatedly shown to stimulate oxidative burst. Therefore, we evaluated the effects of our glucans on production of superoxide anion. Data summarized in Table 2 show that the tested samples have very little activity, but clearly lower than Glucan #300.

Table 2 Effects on production of superoxide anion

	HL-60 cells (nmol/2.5x10^5 cells)
Imortalium	0.45
Ultimate Killer Biotic FX	0.56
Glucan #300	1.72
PBS	0.36

Immunomodulators are well known to stimulate production and secretion of various cytokines, with a wide range from IL-1, IL-2, and IL-6 to TNFα, and IFNγ. To compare the effects of our group of samples, we measured the production of IL-2 by splenocytes and IFN-γ in the blood. Under normal circumstations, splenocytes do not produce IL-2, so the basal levels are almost zero. Because of that, the stimulation by tested samples can be considered statistically significant. Particularly important is the comparison to the effects of glucan (Tables 3 and 4).

Table 3 Effect of individual samples on IFNγ secretion

Sample	**IFNγ**
Imortalium	28.9
Ultimate Killer Biotic FX	58.9
Glucan #300	189.8
PBS	2.6

Data in pg/ml.

Table 4 Effects on production of IL-2

Dose (μg)	25	50	100	200	400	800
Imortalium	8.4	12.7	28.7	26.5	30.2	47.2
Ultimate Killer Biotic FX	9.9	34.3	67.9	101.1	189.2	190.7
Glucan #300	101.1	188.3	997.6	1 101.3	993.9	1 106.0

Data in pg/ml.

Conclusion

From our experiments we can concluded that none of the tested samples have strong stimulatory activity towards the immune system. As both the cellular (phagocytosis and superoxide anion production) and the humoral (production of two cytokines) branches were not strongly stimulated and the action is marginal, we cannot recommend these samples to be considered as potent immunostimulants. Their very low activity can be clearly seen when compared to the effects of Glucan #300.

For more details on used techniques and on direct comparisons of various natural immunomodulators, please read these recent comparative studies:

Vetvicka, V., Vetvickova, J.: β1,3-Glucan: Silver bullet or hot air? Open Glycoscience, 3:1-6,2010.

Vetvicka, V., Vetvickova, J.: Comparison of immunological effects of commercially available β-glucans. Applied Sci. Rep., 1, 2014, doi:10.7243/2054-9903-1-2

Vetvicka, V., Vetvickova, J.: Natural immunomodulators and their stimulation of immune reaction: True or false? Anticancer Res. 34:2275-2282, 2014.

Dose (mg)	25	50	100	200	400	800
Phagocytosis						
Immune-911	30.8	32.5	33.6	29.7	34.6	36
C-FX	27.9	31.5	32.6	30.8	32.9	33.3
Bio Immune	31.2	33.5	34.7	35.1	36.2	36.3
Gingseng	29.6	29.8	30.7	31.2	33	31.4
Immunity Plus	28.8	32.8	33.1	32.9	34.1	33.8
Fucoidan	29.7	30.7	32.5	35.1	32.7	33.8
Imortalium	30.1	32	32.4	31.9	35.6	37.7
Ultimate Killer Biotic FX	30.9	31.2	35.5	36.8	38.1	39.3
Glucan #300	44.1	48.8	55.7	56.1	55.9	60.9
PBS	31.1	31.1	31.1	31.1	31.1	31.1
IL-2						
Immune-911	8.3	11.9	16.9	33.8	29.9	44.9
C-FX	2.2	8.1	6.9	18.9	20.1	19
Bio Immune	10.2	28.8	33.3	40.2	44.6	46.5
Gingseng	1.1	9.8	10.9	12.8	16.7	20.7
Immunity Plus	47.1	56.5	89.3	102.4	111.7	120.5
Fucoidan	7.8	18.2	20.8	28.9	37.4	48.5
Imortalium	8.4	12.7	28.7	26.5	30.2	47.2
Ultimate Killer Biotic FX	9.9	34.3	67.9	101.1	189.2	190.7
Glucan #300	109.8	201.2	1047.6	1122.8	1109.9	1226.6
IFN (pg/ml)						
Immune-911	26.3					
C-FX	13.9					
Bio Immune	22.8					
Gingseng	8.9					
Immunity Plus	27.8					
Fucoidan	15.8					
Imortalium	28.9					
Ultimate Killer Biotic FX	58.9					
Glucan #300	189.8					
PBS	2.6					
Superoxide Anion						
Immune-911	8.3					
C-FX	2.2					
Bio Immune	10.2					
Gingseng	1.1					
Immunity Plus	47.1					
Fucoidan	7.8					
Imortalium	0.45					
Ultimate Killer Biotic FX	0.56					
Glucan #300	109.8					
PBS	0.36					