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Efficacy of the Immunomodulator Glucasan® in Cells of the Immune System – including a Comparison with Other Commercial Products of Similar Application

(Version 1)

1. Summary

The immunomodulatory action and tumor growth inhibition of (1→3),(1→6) -beta-D-glucans has been known for a long time. It is extremely difficult to compare the efficacy of products provided by the various manufacturers on the basis of performance criteria and under realistic, practical conditions, as such a procedure cannot be standardized and will also be very expensive. For this reason, model experiments were carried out with phagocytes — cells belonging to the immune system which are of primary significance in warding off pathogens.

The experiments pursued the following objectives:

- Testing the efficacy of Glucasan® manufactured by Vitalize Health Products GmbH Wiesbaden, Germany, on essential immunological functions in phagocytes.
- Comparison of these actions with those elicited by eight other products available on the market claiming to "strengthen immunity".

The biological actions of the polysaccharide consisting exclusively of glucose depend, among other factors, on the branches of the macromolecular glucose chains and their molecular weight. Their structure is recognized by a pattern recognition receptor located on the surface of Langerhans cells of the skin, macrophages, granulocytes, and various other cells primarily belonging to the immune system. As a result, a cascade of reactions is induced inside the cell which lead to cell activation and the synthesis of various messenger substances (interleukins, chemokines). These signals are transmitted to other cells (e.g. keratinocytes, lymphocytes, antibody-producing plasma cells, liver, bone marrow) and account for an overall improved condition of the body's functions of defense. In human and porcine phagocytes, the lowest and highest rates of phagocytosis differ by more than one order in magnitude. These drastic differences in the phagocytosis rates can be evaluated as an indication of differences depending on the uptake of specific product molecules by the phagocytes and the subsequent radical formation and emission of photons (chemiluminescence) they are able to induce. In a special experimental design, the efficacy of the nine cell wall products examined was tested with regard to three relevant parameters of phagocyte function: the release of radicals, the

synthesis of the chemokine interleukin-8, and neopterin synthesis. Radicals are responsible for the inactivation and destruction of pathogens, whereas IL-8 shuttles the granulocytes from the bloodstream to the focus of infection, and neopterin is an indicator for the degree of activated immunity.

The single products were ranked according to the potency of their actions on these three parameters. The Vitalize product Glucasan® and another product were found to be at the top of the ranking list, three products ranked at the bottom, while the other products ranged at intermediate positions. The observed differences in efficacy depend on the bioavailability of the specific (1→3),(1→6)-beta-D-glucan which permits it to reach and bind to its target receptor on the phagocyte, and not on the potentially disparate (1→3),(1→6)-beta-D-glucan quantities in the cell wall products.

2. Structure and Function of the Immune System

The immune system has to fulfill a variety of functions. In the first place, it needs to protect the body from being infected by bacteria, viruses, fungi, and parasites. Other tasks consist in the establishment of immunity against tumors, destruction of tissues and compounds foreign or toxic to the body, participation in processes of healing (e.g. wound healing), development of symbioses with beneficial microorganisms populating exterior body surfaces or the interior excretory ducts and passages of the body (skin, gastrointestinal tract, respiratory organs, urinary tract collecting system, mammary gland). In addition, pathological alterations such as allergies and autoimmune diseases are also closely associated with the immune system.

The immune system is composed of two subsystems: the innate and the acquired immune system. Each subsystem consists of a set of typical cell types and humoral (non-cellular) factors. Both subsystems cooperate in a fine-tuned manner. An overview of the most important components of the immune system is shown in Table 1 (Janeway et al. 2002).

The cells belonging to the immune system have a short survival rate of several days. These cells and the humoral factors are therefore subject to a considerably higher turnover than the cells originating from other tissues of the body. Stress, malnutrition, intoxications, and diseases also exert a rapid and sustained action on the body's defenses.

Table 1. Components of the Innate and Acquired Immunity

	Immune System	
	innate	acquired
Heritability	yes	no
Cell Types	macrophages granulocytes Natural killer cells	T lymphocytes B lymphocytes (plasma cells)
Humoral Components	complement system interleukins interferons acute-phase proteins lysozyme, lactoferrin	antibodies
Recognition Protein	pattern recognition receptor	antibody
Specificity	spectrum of pathogens	antigen
Response	immediate	delayed
Allergy	no	yes

3. Actions of (1→3),(1→6)-beta-D-Glucan

The immunomodulatory and tumor-growth inhibitory action of (1→3), (1→6) -beta-D-glucan has been known for a long time. The purification and the molecular characterization of the specific receptor located on the surface of various immune cells and to which this biologically active compound binds, made it possible to understand the mechanism of action of (1→3), (1→6) -beta-D-glucan on a cellular level (Brown & Gordon, 2001; Willment et al. 2001).

Table 2. Actions of (1→3),(1→6)-beta-D-glucans

Actions on the innate and acquired immune system
• Binding to the pattern recognition receptor of phagocytes • Activation of phagocytosis (radical formation, biodegradation enzymes) • Neopterin discharge • Enhancement of cytokine production (IL-1, IL-6, IL-8, tumor necrosis factor α) • Increased leukocyte mobility • Activation of the complement system • Enhancement of antibody synthesis (IgA, IgG) • Increased cell formation in the bone marrow • Increased synthesis of acute-phase proteins in the liver • Inhibition of tumor cell growth
General actions in the body • Stabilization of the intestinal flora, suppression of the distribution of pathogenic microbes, fiber substance effects (improved intestinal motility) • Accelerated wound healing • Binding and elimination of heavy metals and toxins

(1→3),(1→6)-beta-D-glucans bind to a protein molecule (receptor) located on the external side of the cell membrane. Their binding triggers a cascade of reactions which lead to the activation of the cell and the synthesis of various messenger substances (interleukins, chemokines). These signals are transmitted within the immune system itself and to other tissues as well (e.g. liver, bone marrow, epidermal cells of the skin) thus improving the overall defense status in the body. The most relevant actions in this regard are summarized in Table 2 (Adam et al. 2001; Bohn & Miller 1995; Czop et al. 1978; Cheung et al. 2002; Lee et al. 2002; Janusz et al. 1987; Harler & Reichner 2001; Fleischer et al. 2000; Fleischer et al. 2001; Zhang et al 2001; Browder et al. 1988; Wakshull et al. 1999).

4. Parameter Selection for Model Experiments with Human and Porcine Phagocytes

The experiment conducted pursued the following objectives:

- Testing the efficacy of Glucasan® manufactured by Vitalize Health Products GmbH Wiesbaden, Germany, on essential immunological functions in phagocytes
- Comparison of these effects with those of eight other products which are commercially available and designed for similar application (strengthening the immune system).

The following parameters were selected:

4.1. Phagocytosis:

Phagocytosis represents one of the most important functions of the immune system. Phagocytes take up foreign particles (bacteria, immunocomplexes) and engulf them within specific intracellular compartments where specific degradation enzymes destroy these particles. Yeast cells and their cell wall constituents belong to the bodies and substances which undergo this process. Hence, at least two actions are induced by yeasts and the cell walls of yeast: 1. the uptake of yeast and yeast constituents by the phagocytes and their destruction by radicals, whereby radical whose formation can be followed by applying the chemiluminescence method, 2. binding of the yeast-specific (1→3),(1→6)-beta-D-glucan to a receptor located on the surface of the phagocyte, and the activation of phagocytosis with additional chemiluminescence. The measurement of chemiluminescence after the addition of yeast products encompasses both reactions. If the chemiluminescence of activated phagocytes is to be measured after its induction by means of additional, specific receptor binding, the method described in the following (see 4.2: Formation of Radicals) will be applicable.

4.2. Radical Formation

Before the lysosomal biodegradation enzymes can begin with the destruction of the structures vital to the pathogens, the bacterial constituents (proteins, nucleic acids, membrane lipids, polysaccharides) are destroyed and inactivated through the action

of radicals priming the site of attack for the enzymes. The radicals are created in the process of following chemical reactions which depend on the presence of oxygen.

The $O_2^{-\cdot}$ radical (superoxide radical) which is initially created by an enzymatic reaction is transformed into the highly reactive $OH^{\cdot}$ radical (hydroxyl radical), the most effective oxygen species contributing to the phagocytes' cell damaging activity.

$$NADPH + H^+ + 2O_2 \rightarrow 2\ O_2^{-\cdot} + NADP + 2H^+$$
$$2\ O_2^{-\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$$
$$O_2^{-\cdot} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + O_2$$

Other extremely reactive, chlorine-containing products are derived from H_2O_2 (hydrogen superoxide) involving myeloperoxidase, an enzyme which occurs in phagocytes:

$$H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HOCl$$
$$HOCl \rightarrow OCl^- + H^+$$
$$HOCl + Cl^- \rightarrow Cl_2 + OH^-$$

The hypochlorous acid HOCl, its anionic form $OCl^{-\cdot}$ and Cl_2 (chorine) constitute a potent cytotoxic mixture which chlorinates and oxidizes various molecular components of bacteria (proteins, lipids, nucleic acids), and primes them for enzymatic degradation.

Photons are emitted in the course of these reactions (chemiluminescence) which are captured and measured with the aid of sensitive analytical instruments. The enhancement of radical formation is a quality criterion for the product containing (1→3),(1→6)-beta-D-glucan.

4.3. Synthesis of the Chemokine Interleukin-8

This chemokine is a protein, which is released by activated phagocytes at the site of infection. It diffuses into the blood capillaries, where it mediates the migration of neutrophilic and basophilic granulocytes and T lymphocytes from the blood stream into the tissue containing the focus of infection. IL-8 hence functions as a signal

transmitter in phagocytosis (Janeway et al. 2002, p80). Figure 1 shows a schematic representation of this mechanism. Simultaneously, it stimulates the growth of new blood capillaries (angiogenesis) which are of great significance in supplying the regenerating tissues with oxygen and nutrients (for example, in the processes of wound healing).

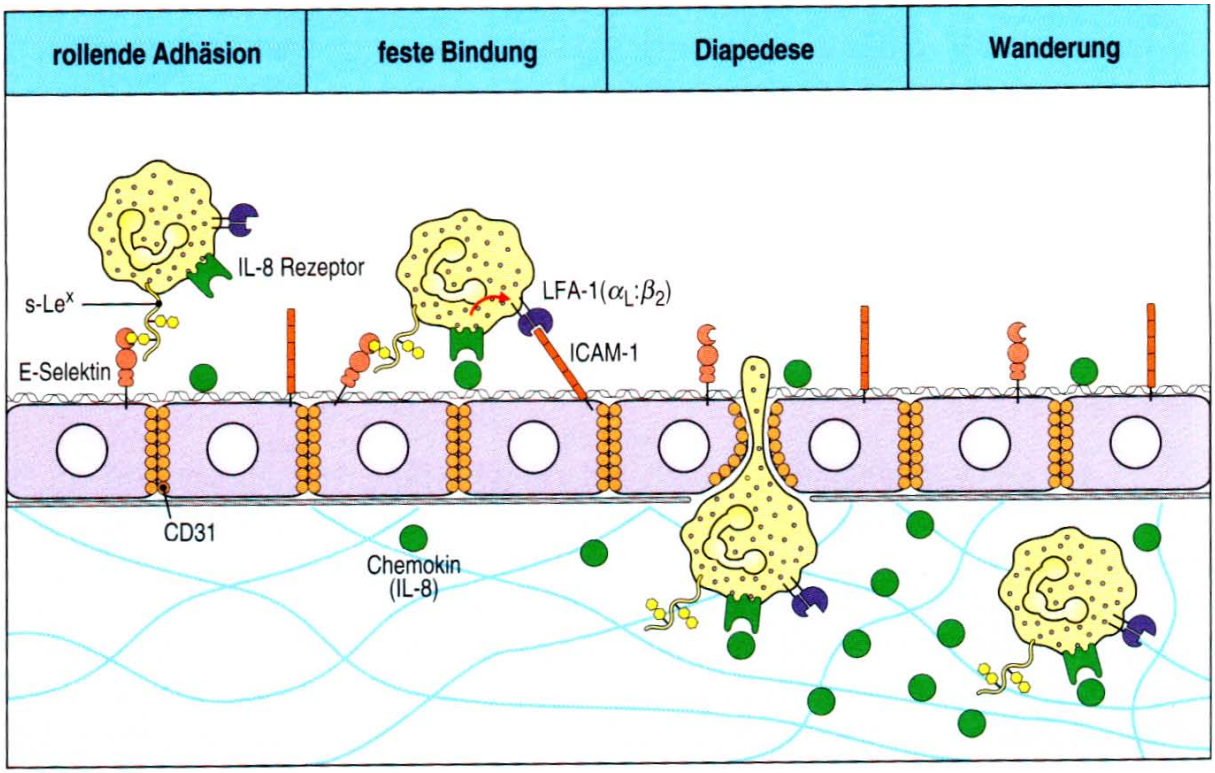


Fig. 1. Interleukin-8 induces the migration of neutrophilic granulocytes from the blood stream to the site of pathogenic invasion

4.4. Neopterin

Neopterin (6-D-erythro-trihydroxypropyl-pterin) is a substance of low molecular weight which is synthesized from its precursor guanosine triphosphate (GTP) through the action of GTP-cyclohydrolase I, a key enzyme in pteridine biosynthesis. 7,8-dihydro neopterin triphosphate is formed as an intermediate product. Cleavage of the phosphate group and a subsequent oxidation step then yields neopterin. The reaction steps are shown in Figure 2

Neopterin is released by the activated phagocytes. This is of relevance when the body needs to muster processes of defense. When T lymphocytes recognize foreign (e.g. bacteria), or modified autologous cell structures (tumor cells), they will produce

various mediator substances (lymphokines), including interferon-γ, to activate the phagocytes. Neopterin reflects the phagocytes' state of activity (Huber et al. 1984; Mora et al. 1997; Hoffmann et al. 1999).

The synthesis of neopterin is not only a secondary phenomenon among the immune reactions. Instead, it is also involved in various other mechanisms of defense. It enhances the actions of various reactive substances, such as hydrogen superoxide (H_2O_2),

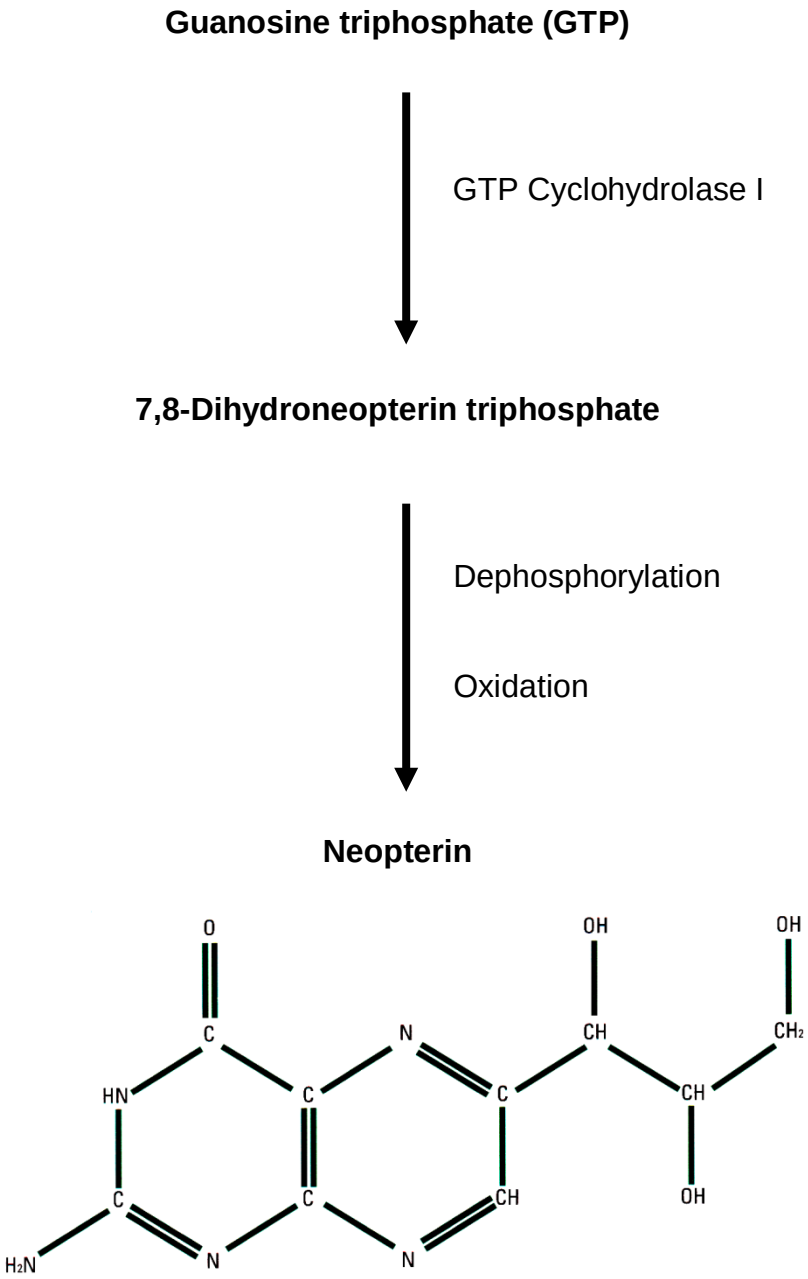


Fig. 2. Synthesis of neopterin from guanosine triphosphate

HOCl, peroxinitrite ($ONOO^-$, an effector molecule of nitrogen monoxide NO), chloramine, it enhances the expression of NF-kappaB (interleukin synthesis), and the gene of inducible NO synthase (formation of NO radicals, regulation of the blood supply in inflamed tissues). Besides, neopterin is involved in triggering programmed cell death (Baier-Bitterlich et al. 1995; Removal of damaged cells in infection, injury, and wound healing). An enhancement of neopterin release is consequently seen as a product quality criterion when such a reaction can be elicited by the active ingredient (1→3),(1→6)-beta-D-glucan.

5. Methods and Materials

5.1. Phagocytes

5.1.1. Blood Donors

All blood samples were obtained from the one and same person by puncturing the test subject's cubital vein. The blood donor was a 41-year-old male not suffering chronic or acute inflammatory disease, and not standing under the influence of any medication at the time of blood sampling. The porcine blood sample was also obtained by venepuncture.

5.1.2. Preparation of Cell Suspensions

The human and porcine blood samples were prepared according the micro-method described by Fotino et al. (1971). 0.5 ml of a 10% EDTA solution and 2 ml of a 6% dextran solution were added to 10 ml whole blood. This batch was incubated for 30 minutes at 37°C in a water bath. The leukocyte-rich plasma became visible in the upper part of the test tube after 30 minutes.

The plasma was separated and underlain with Ficoll-Paque. Afterwards, the samples were centrifuged at 1,800 rpm for 15 minutes at room temperature. The plasma gradient zones containing the lymphocytes/monocytes were withdrawn and the leukocyte layer was combined and thoroughly mixed with 1 ml PBS (phosphate-buffered saline).

The granulocytes/erythrocytes were washed by refilling the tubes with PBS and carrying out the subsequent centrifugation. The supernatant PBS solution was

discarded. Lysis of the erythrocytes proceeded by adding 4 x 0.5 ml water and 0.5 ml of a 4.5% NaCl solution, eliminating the erythrocytes by repeated washing and centrifugation. The sediments were taken up in 1 ml PBS.

Cells were counted by using a Neubauer's counting chamber. A cell count of 1 x 10^6 cells ml^{-1} was adjusted with PBS. 100 µl of this cell suspension, i.e. 1 x 10^5 cells, were applied in the measurements.

5.2. Determination of Phagocytosis by Chemiluminescence Measurement

Determination of phagocytosis by measuring chemiluminescence was done according to Heberer et al. (1982). For this purpose, a 6-channel Biolumat LB 9505 (Berthold Co.) was used, adjusted to a measurement range of λ = 320 - 650 nm. Calibration was performed with an ^{241}Am source. The overall measurement time of each analysis amounted to approx. 120 minutes. The areas under the intensity curves were integrated to quantify the analytical results.

1 ml barbiturate buffer (pH 7.3) was added to 20µl lucigenin solution and 100 µl of the cell suspension. Each of these three batches were placed in parallel position into two channels and adapted for approx. 10 minutes. The measurements were then started. After approx. 20 minutes, 100 µl of the suspension derived from the opsonized test substances or Zymosan were added. The overall time of measurement amounted to 120 minutes at a rate of 0.83 min^{-1}. Opsonization was done in human or porcine serum.

5.3. Radical Release in Activated Cells

20 µl lucigenin solution, 100 µl cell suspension, and 100 µl test solution/void reference solution were pipetted into 1 ml barbiturate buffer. The test solution had been previously made by mixing the test substances in a saturated solution of DMSO. Each of the batches were placed in parallel position into two channels and the measurement started. After 30 minutes of adaptation, 100 µl test solution was added and after further 30 minutes the Ca ionophore A23187 (Boehringer Mannheim, Fed. Rep. Germany; 1mmol dissolved in 1l DMSO). The overall measurement time amounted to approximately 120 minutes, at a rate of 0.83 min^{-1}. Phagocytosis is associated with an increase of the intracellular calcium concentration (Robinson et al.

1984; Azuma et al. 1986; Hamacchi et al. 1986). Bringing calcium ions into the phagocytes with a ionophore permits an activation of the reactions taking place during phagocytosis without having to apply pathogens. This, in turn, permits studying the effects of ß-glucan mediated by the specific receptor, and avoids the influence of processes which otherwise would be induced when the yeast cell wall product is phagocytized.

5.4. Release of Neopterin and Interleukin-8 in Activated Cells

The method is as described under 5.3., with the exception that the lucigenin solution is not used. After stimulation (120 minutes), the solutions were spun in a centrifuge and the clear supernatant was frozen at –20 °C.

The quantitative analysis of neopterin was performed by HPLC/ESI-MS on a RP-18 column with positive ionization in the selected ion mode. The detection limit of the method is 2 ng/ml.

IL-8 determination was carried out in a high-sensitivity enzyme immunoassay (Biosource, Camarillo, CA, USA) according to manufacturer's instructions. The detection limit of this method is at 0.01 pg/ml.

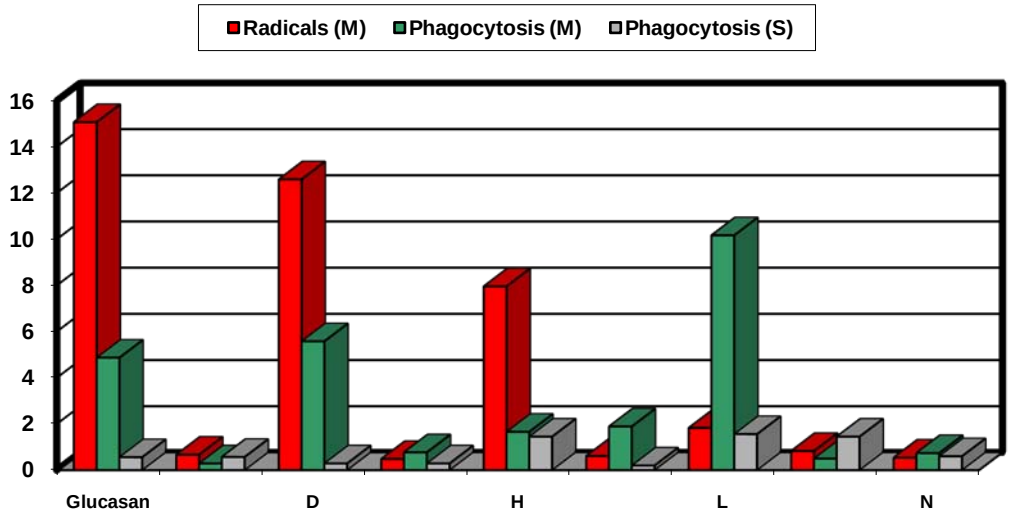
Withdrawal and preparation of blood samples, and the measurements described in this Section, were performed by Dr. rer. nat. Michael Rothe, FILT GmbH Berlin. FILT GmbH Berlin works in compliance with GLP guidelines (Good Laboratory Practice).

6. Results and Discussion

6.1. Phagocytosis and Radical Formation

The rate of phagocytosis was examined in human (Fig. 3 middle columns) and porcine phagocytes (Fig. 3, right columns). Product L produced the highest rate in human phagocytes, followed by the Product D and the Vitalize Product. Values applying to the Products F and N were at about 15% relative to the Vitalize Product, all other products yielded values below 10%. Porcine phagocytes displayed markedly lower values as compared to human phagocytes (except Products B and M), which made evaluation and comparison rather difficult. Some were in the range of the detection limit of the method. The Vitalize Product comes right after the Products L, M, and H.

The Vitalize Product produced the highest increases of radical formation in activated



phagocytes (100%, left column in Fig. 3), followed by the Products D (84%), H (53%) and L (12%), whereas the activation rate of the other products was below 5%.

Fig. 3. Radical formation and phagocytosis in human and porcine phagocytes (relative units).

6.2. Synthesis of Interleukin-8

Fig. 4 shows the influence of the various products on the synthesis of the chemokine interleukin-8. The Vitalize Product exerted the strongest influence (100%), followed by Product H with 71%, Product L with 64%, Product D with 58%, and Product M with 10%. The remaining Products B, I and N did not produce any effect at all.

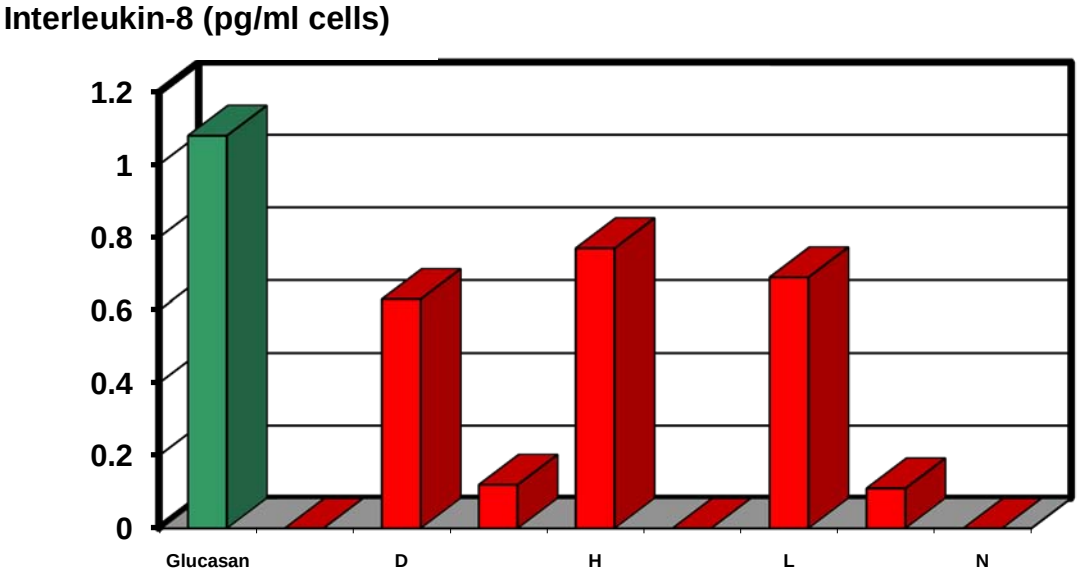


Fig. 4. Interleukin-8 synthesis in phagocytes

6.3. Synthesis of Neopterin

Fig. 5 demonstrates the results of the test series performed to examine the influence of the various products on the release of phagocytic neopterin. If the amount produced by the Vitalize Product is set to 100%, then the Products L and M yielded values of 174% and 126%, respectively. The Products N (=108%) and F (=104%) did not differ significantly from the Vitalize Product in this regard. Product D (93%), Product H (76%), Product B (21%) and Product I (8%) followed in rank.

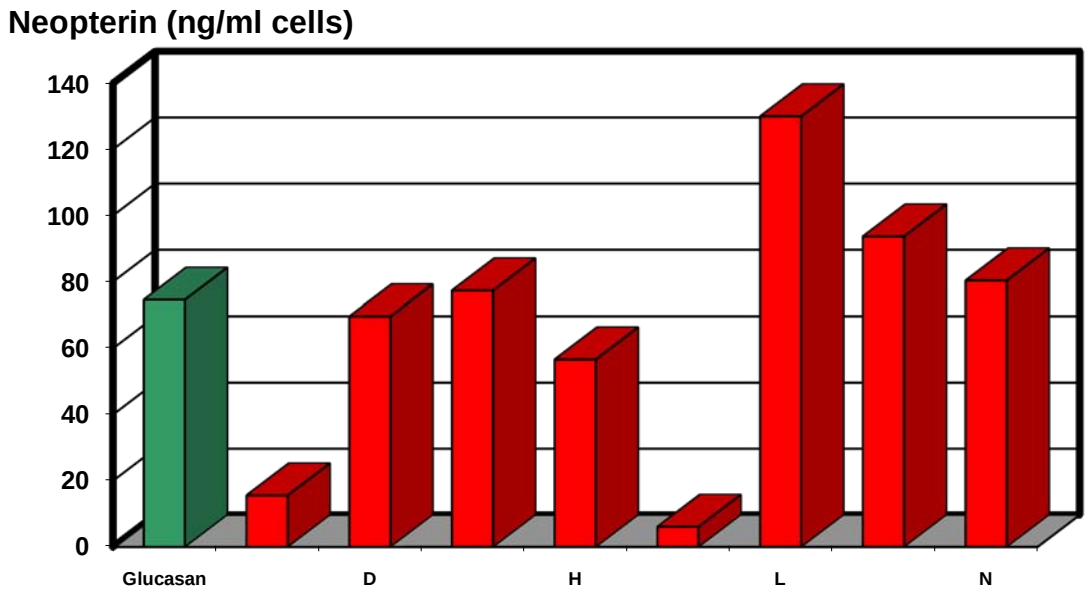


Fig. 5. Synthesis of Neopterin in Phagocytes

6.4. Experimentally Determined Rank of the Examined Products According to Their Efficacy in a Phagocytotic System

6.4.1. Rate of Phagocytosis

The lowest and highest phagocytosis rates measured in human and porcine phagocytes differ by more than one order of magnitude (see Fig. 4). There is cluster formation regarding the potency of examined products which is rather obvious. It shows that Product L and the Vitalize Product both rank high in the two test systems (Product L on rank 1, Vitalize Product on rank 3 and 4). Products B and F take on the last places 8 and 9. For a better overview of the results, the values obtained in the human and porcine phagocytosis tests were ranked, and the mean rank value was calculated for each product examined. The following order resulted for the mean

values (the product with the highest phagocytosis rate has the lowest rank number): Product L < Vitalize Product < Product H < Product D < Product M < Product N < Product I < Product F < Product B. The drastic differences in the phagocytosis rates visible in Figure 4 can be interpreted as an indication to differences in the uptake of the individual products into the phagocytes, having consequential effects for the subsequently occurring radical formation and photon emission (chemiluminescence).

6.4.2. Radical Release and the Synthesis of Interleukin-8 and Neopterin

The products were ranked, in order to compare the products with respect to their activity-enhancing effects in radical formation and the synthesis of interleukin-8 and neopterin as the relevant parameters of phagocytotic function. Rank 1 was assigned to the product showing the strongest effect, whereas Rank 9 was assigned to product showing the weakest effects. The mean value of all three ranking positions was then calculated.

Table 3. Ranking positions of products of similar application according to the respective manufacturers (strengthening of the immune system, immunomodulation). The numerical values represent the mean values (Mean) of the ranking positions obtained for the potency of the respective products with regard to radical formation (RF), interleukin-8 synthesis (IL8), and neopterin synthesis (Neopterin).

Product	Mean	(RF,IL8,Neopterin)
Vitalize	1.7	(1,1,3)
L	2.7	(4,3,1)
H	4.0	(3,2,7)
D	4.0	(2,4,6)
M	4.3	(5,6,2)
F	5.7	(9,5,3)
N	6.3	(8,8,3)
B	7.3	(6,8,8)
I	8.0	(7,8,9)

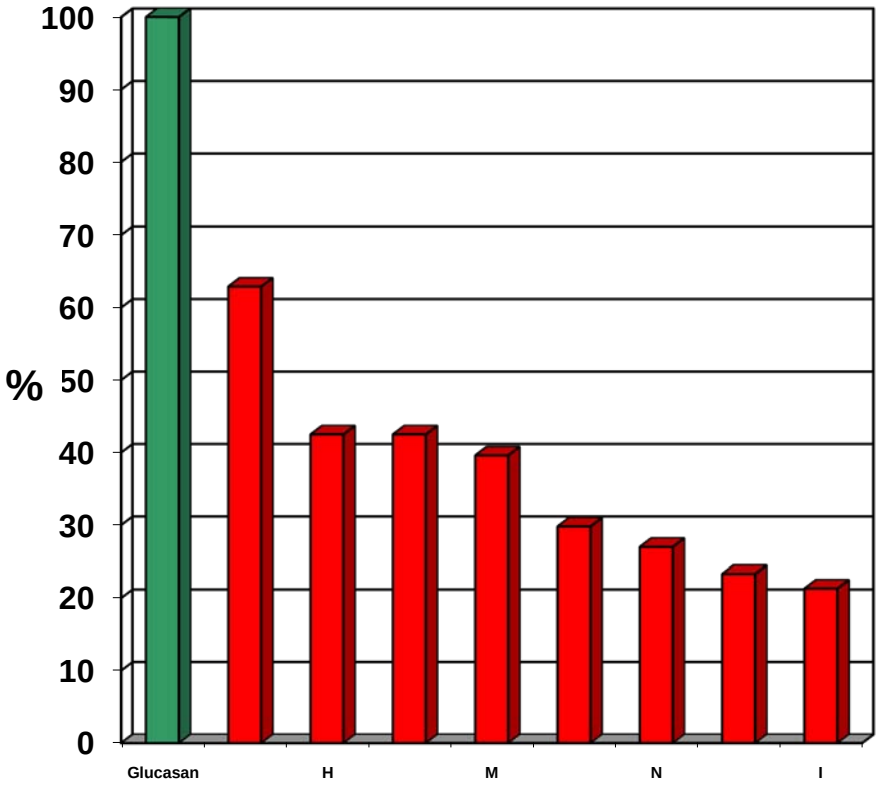


Fig. 6. The numerical values represent the inverse mean values (1/mean) of the ranking positions of each of the products, based on the potency elicited in radical formation, interleukin-8 synthesis, and neopterin synthesis (see Table 3). Values are reported as percentages.

Both the Table and Figure 6 show that the products can be allocated to three groups: the Vitalize Product and Product L lead in the rank list, the Products I, B and N are at the bottom, while Products H, D, M and F hold intermediate positions.

As compared to the determination of the phagocytosis rate, which measures the uptake and destruction of the products within the phagocytes, the analysis of radical formation, IL-8 and neopterin synthesis monitors the individual, relevant reactions taking place in phagocytes which have been transformed into an activated state by means of an ionophore-mediated incorporation of calcium ions. In this case, the non-opsonized product remains outside the phagocytes. This permits an examination of the intracellular effects induced by the specific receptor binding of (1→3),(1→6)-ß-D-glucans. The various effects of the individual products thus reflect the capability of the (1→3),(1→6)-ß-D-glucan available in the respective product of making contact

with its target receptor (Müller et al. 1996, 2000). The assumption seems plausible that products with a high amount of (1→3),(1→6) -ß-D-glucan should display a high degree of efficacy. Information on the ß-glucan contents had not been available for most of the products provided. According to their manufacturers, two products were reported to have a ß-glucan content of >80%. These products ranged at an intermediate and bottom position in the efficacy spectrum. It may be assumed that the ß-glucan content of most products lies in a range between 15 and 30%. The observed differences in efficacy varying by more than one order of magnitude make it appear rather unlikely that these heterogeneous ß-glucan contents were responsible for this effect. Accordingly, it is postulated that the differences in ß-glucan – receptor interactions refer to dissimilar structural arrangements of the (1→3),(1→6) -ß-D-glucan molecules in the products. Such disparity obviously depends on the yeast cell type and its preparation. The (1→3),(1→6) -ß-D-glucan polymers in the intact cell walls of yeasts build a layer which accounts for the mechanical stability of the cell wall. In the course of cell preparation, the cell wall components (proteins, lipids) are usually destroyed and leached out, the wall structure is loosened up allowing water to infiltrate, which ultimately causes the cell wall material to swell. The dry powder of the Vitalize Product, for example, is capable of taking up an amount of water which is 8 or 9 times greater than its net weight. This is a condition for the ß-D-glucan molecules to couple to their target receptor located on the outside of the phagocytic membrane. ß-D-glucan molecules in products which are merely classifiable as dried yeast are incapable of reacting with the receptor. Electron microscopic images of products supplied by various manufacturers were made at the Food Chemistry Institute of the Technical University Berlin (Prof. L.-G. Fleischer) and revealed remarkable differences in this regard. It is also imaginable that the structure of the ß-D-glucans has been altered as a consequence of rigorous preparation methods, to an extent that makes receptor recognition impossible.

It is known that mannoproteins also exert an activating influence on phagocytes. However, we only possess insufficient information as to their contents in the various products used. Mannoproteins are able to modify the effects of the ß-D-glucans. But the overall contents should be less important than the spatial configuration of the molecules which ultimately accounts for receptor binding as to this group of

biologically active molecules as well. This is without relevance for the ranking system here determined.

6.5. Biological Significance

There are two directions in which the interpretation of the results shown here and their significance for immune resistance goes: 1. towards the significance of the general phagocytosis, 2. to the significance of the results concerning the radical formation, IL-8- and neopterin synthesis on activated phagocytes.

The answer to the first topic regarding the phagocytosis of yeast cell wall products depends on the fundamental question whether a possibility results for contact with phagocytes and thereby their phagocytosis for the yeast absorbed or, rather, their cell wall products in the intestine.

Bhogal and collaborators discovered (Bhogal et al. 1987), that neutrophil granulocytes can penetrate through the epithelium layer of the intestinal mucosal barrier in the free lumen. If the pathogenic E. coli germ were in the intenstinal lumen, then this would be phagocyted and lysozyme, lactoferrin and cationic peptide would be released from the phagocytes. The phagocytosis of the exciters was morphologically confirmed by these authors using electronmicrographs. The function of neutrophile granulocytes as effector cells was, above all, accepted to date. In the last few years it has been proven that neutrophile can also form different cytokines, like Interleukin-1, Il-3, Il-8, Il-12, tumor necrosis factor α, transforming growth factor ß (TGF ß) (Bliss et al. 2000), (Baumann et al. 2002). These interleukines and further mediators can affect other immune cells. The conception is not wrong that these mediators can modulate the local immune system of the intestine wall. Provided naturally that the cell wall product is actually phagocyted. As the results represented here show, large differences are to be expected with the individual products when this method takes effect.

In the case of the second effect direction - radical formation, Il-8 and neopterin synthesis - the situation is different. The cell wall product must win gain cells in the

intestine-associated lymphatic tissue (GALT). Beier and Gebert (1998) were able to show that yeast particles are already channelled over the M-cells within one hour.
In the Peyer plaques they can come into contact with different immune cells. The phagocytosis of the cell wall product is not necessary. By binding the ß-glucan of the cell wall product to the receptor in the phagocytes (macrophage, granulocyte) a condition of increased reaction readiness is triggered, which after contact between cells and a pathogen (e.g. E. coli) leads to its accelerated admission and phagocytosis. To this extent, the measurement taken here reflects the situation in vivo to certain degrees. At the start of the test the phagocytes and the cell wall product are mixed with one another.

Only after addition of the Ionophors and influx of calcium ions into the cells does radical formation, Il-8 and neopterin synthesis begin. Their extent is different and depends on the activity condition of the cells triggered by the ß-glucan reaction. Immune cells of the intestine-associated lymphatic tissue (GALT), in particular lymphocytes and granulocytes can leave the intestine wall and spread out over the blood path throughout the body. Through this and over mediators, the increased reaction readiness induced in the intestine wall also becomes effective in other body regions (see table 2). To that extent, the result of these measurements is to be estimated as an indicator for the ability of the respective cell wall product to put the phagocytes a condition of increased reaction readiness (alert stand-by) in the case of a conflict with pathogens. The determined hierarchical order of the nine examined products speaks to their quality as nutritional supplements for the support of immune resistance in humans and animals.

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