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Immunological and Central Nervous System Changes in Mice Suffering from *Staphyloccocus aureus* and Treated with *Saccharomyces cerevisiae* var. *vini* Living Cells

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ABSTRACT. The influence of *Saccharomyces cerevisiae* var. *vini* viable cells on the *Staphylococcus aureus*-suffering mice was ascertained: (*1*) effect of *S. aureus* living cells on antibacterial and antitoxic antibodies, (*2*) effect of *S. aureus* on the number of neurons and macroglial cells in different areas of mouse hippocampus, (*3*) effect of *S. cerevisiae* var. *vini* viable cells on the above-mentioned changes of immune and nervous system. Treatment with *S. cerevisiae* var. *vini* provokes immune stimulation and change of the total number of macroglial elements in the CA1 field of hippocampus.

Abbreviations

CNS	central nervous system	MDP	muramyl dipeptide(s)
Dg	dentate gyrus	*Scv*	*Saccharomyces cerevisiae* var. *vini*
LPS	lipopolysaccharide(s)	SEB	staphylococcal enterotoxin B

Host defense presents one of the most critical physiological challenges for an animal. The ability to prevail against infection with pathogenic microorganisms requires (*1*) that animals possess a sensory system capable of detecting pathogens (Besedovsky and Del Rey 1996), (*2*) that the animals are able to remove or inactivate the pathogens once detected. In vertebrates, specialized immune cells (macrophages, granulocytes and certain T cells) and blood-borne molecules (complement) directly destroy pathogens. The efforts are supported by endocrine, metabolic and cardiovascular changes that are coordinated by autonomous and endocrine areas of brain.

Brain mediates host defense mechanisms – components of the "acute phase response" to infection include fever, somnolence and elevations of plasma corticosteroids, as well as behavioral alterations such as social withdrawal and hypophagia. In this way, the immune system is able to recruit a wide variety of body-wide defense mechanisms in response to infection (Goehler *et al*. 2001).

T-cell-dependent immune sensory mechanisms, many immune cells, including dendritic cells and macrophages, express specific receptors for bacterial cell-wall constituents or viral proteins (Medzitov and Janeway 1997). LPS is believed to be the important marker for G^--bacteria (*see*, *e.g.*, Schwarzová and Čižnár 2004) whereas peptidoglycan by-products such as MDP serve as salient stimuli from G^+-bacteria (Pabst *et al*. 1999).

Whereas both types of immune-sensory transduction mechanisms lead to production of mediators capable of signaling to the brain, most of them are derived from T-cell-independent stimuli such as LPS and MDP. However, it was shown that T-cell activation by superantigen derived from G^+-bacteria, SEB, activates the hypothalamus–pituitary–adrenal axis and initiates anxiety-like behavior in mice (Shurinn *et al*. 1997). Those findings raise interesting issues regarding the specificity of neuronal responses to immune stimuli. In this context it is especially interesting to study the influence of these bacteria on the structural and functional peculiarities of limbic regions of brain responsible for emotion and behavior. Furthermore, it looks extremely interesting to learn about the effects of *Staphylococcus aureus* living cells on immune and nervous (limbic) system and to show the existence or absence of common features between influence of toxin and living cell of the same origin.

Many scientists have shown the unique therapeutic properties of probiotic – *Saccharomyces cerevisiae*. It has been used for treatment of different types of diarrhea diseases (Broussard and Surawicz 2004; Korulog and Koturoglu 2005) but to our knowledge, there have been no reports about the influence of *S. cerevisiae* viable cells on *S. aureus in vivo*.

We acertained the influence of *S. aureus* living cells on (*i*) antibacterial and antitoxic antibodies of mice, (*ii*) the number of neurons and macroglial cells in the different areas of mouse hippocampus, and (*iii*) to clarify, if *Scv* has some influence on possible above mentioned changes.

MATERIAL AND METHODS

Microorganisms and cultivation. A strain of *Scv* was kindly supplied by E. Kirtadze (*Institute of Biotechnology and Biochemistry, Georgian Academy of Sciences*, Tbilisi). *S. aureus* clinical strain belongs to the microorganism bank of the *Biopharm-L* pharmaceutical company (Georgia). The yeast strain was recovered from storage and grown at ambient temperature for 2 d on YPD agar slopes or broth, which contained (in %) yeast extract (*Difco*) 1, bacto-peptone (*Difco*) 2, dextrose 2, with or without agar 1.5. *S. aureus* was grown for 1 d at 37 °C on meat-peptone agar slants or broth, prepared as follows: 1 L meat extract, and (in %) bactopeptone (*Difco*) 1, NaCl 0.5, glucose (*Difco*) 1.5, with or without agar 1.5.

Animals. Four-week-old male mice (20–25 g mass) were housed in flexible plastic cages and handled according to the rules for animal welfare of the *Council of European Communities* (86/609/EEC). Three groups of mice were used: (*1*) intact, (*2*) infected, and (*3*) infected, treated with living cells of *Scv* (5 animals in each groups).

Infection and treatment. S. aureus viable bacterial suspension was made by 0.5 McFarland standard for injection. LD_{50} was determined experimentally to identify the dose causing infection; it was 1.5 mL of this standard suspension for each animal.

To initiate infection mice of 2nd and 3rd groups were challenged hypodermically with 1.5 mL of staphylococcal suspension. After 3 d post infection mice of the 3rd group were treated with 0.5 mL of *Scv* suspension (daily dose) made by McFarland standard. The body temperature was measured daily using standard bio-telemetry procedures.

Immunology. The passive hemagglutination test (Solovjov *et al*. 1980) for detection of antibacterial and antitoxic antibodies was used 10 d after treatment; blood was collected from the intra-orbital vein. The antibacterial and antitoxic erythrocyte diagnostics were prepared in the *Laboratory of Immunology, G. Eliava Institute of Microbiology, Virology and Bacteriophages, Georgian Academy of Sciences*.

Histology. The brains of mice were removed from the skull 10 d after treatment, under intraperitoneal injection of 4 % 2,2,2-trichloroethane-1,1-diol ("chloral hydrate"; 60 mg/kg). For histological investigation the brains were lodged in 4 % formaldehyde for 6 d at room temperature; the brains were then embedded in paraffin using a standard procedure. The paraffin-embedded material was cut in the microtome and serial sections (2 sections in each series) of 25-μm thickness were made. The first section from each series was stained with cresyl violet (Nissl method); the 2nd section was silver-impregnated by Gallyas silver impregnation method (Gallyas *et al*. 1980).

Cell counting on cresyl-violet-stained sections. The determination of the number of neurons and macroglial cells was made in CA1, CA3 and Dg of mice. The borders of areas were identified according to the *Atlas of Mouse Brain* by Williams (1999).

Cells were counted using the stereological method of West (1991). In each case 8–10 sections stained with cresyl violet were used. The preparations were examined under light microscope (*Leica* DM, LA) at 5 × 20 and 5 × 40 magnification. The size of the morphometric grid was 625 μm^2. In each case the total number of cells was put in the formula

$$N_{tot} = \Sigma Q \times 1/t,$$

where N_{tot} is the total number of neurons, t probability of use of samples, and Q sum of neurons in the section.

Silver impregnation of degenerated neurons. To detect the degenerated neurons silver impregnation was used (Gallyas *et al*. 1980). A modification of scale (Freund 1991) was used for semiquantitative analysis of cell death in silver-stained sections.

Statistical analysis: Data were analyzed by statistical software MINITAB Realize 13.1. In all tests a p value <0.05 was taken as an indication of statistical significance.

RESULTS

Immunology. Changes obtained using the passive hemagglutination test are shown in Table I. In comparing with control animals, the titer of antitoxic and antibacterial antibodies was increased in both infected and treated animals. Except for the titer of antitoxic antibodies at infected mice all changes are statistically relevant.

The animals in the acute phase of infection do not eat. Besides, body temperature was raised (Table II).

Table I. Passive hemagglutination test response (titer of antibodies, means ± SD)

Antibodies	Animals				
	intact	infected	*p*	treated	*p*
Antitoxic	2 ± 0.1	37 ± 2	0.13	46 ± 0.1	0.001
Antibacterial	2 ± 0.1	53 ± 18	0.04	53 ± 18	0.04

Table II. Changes of animal body temperature (°C, means ± SD)

Temperature	°C	*p*
Control	37 ± 0.2	0.007
At acute phase of infection	40 ± 0.9	0.007
During treatment	39 ± 0.6	0.007
At the end of treatment	38 ± 0.2	0.074

Changes of neuron cell number (thionine-stained preparations) and quantitative changes of macroglia. By a histological study it was shown that the total number of neurons in the hippocampus (CA1, CA3, Dg) is changed compared with control material, but these changes are not statistically significant (Table III).

Table III. Number of neurons and neuroglia (means ± SD)

Number of	Animals				
	intact	infected	*p*	treated	*p*
Neurons					
CA1	2810 ± 480	3160 ± 843	0.6	4400 ± 1160	0.2
CA3	1110 ± 129	1310 ± 535	0.6	1700 ± 352	0.1
Dg	4910 ± 22.0	3280 ± 688	0.4	3060 ± 954	0.3
Neuroglia					
CA1	761 ± 181	649 ± 157	0.5	1710 ± 392	0.05
CA3	438 ± 53.2	401 ± 192	0.8	893 ± 282	0.1
Dg	724 ± 168	680 ± 237	0.8	1630 ± 531	0.1

The statistically significant changes of the total number of macroglial cells were detected only in CA1 of animals treated with *Scv.*

Semiquantitative analysis of silver-impregnated degenerative cells. In the group of infected animals as in the group of the treated ones, the degenerative cells corresponded to state II (10–50 %) in all fields of the hippocampus.

DISCUSSION

Because of a long association with human food and drink, the *Saccharomyces* genus has been considered to be a harmless saprophyte. Very few yeasts have been studied as possible biotherapeutics and *S. boulardii* is one of the first and currently the only one commercialized in human medicine (Brunnel and Patte 1972). Other *Saccharomyces* species or members of other yeast genera probably have probiotic activity similar to that of *S. boulardii* or even better. Many literature data reporting on various mechanisms of action of *S. boulardii in vivo* indicate that the host protection by yeast viable cells can be explained by immunomodulation, reduction of toxin production or action and competition for adhesion sites or nutrients in the presence of this species (Broussard and Surawich 2004; Rodrigues *et al*. 2000).

In our study, yeast identified as *Scv* (ambient and agroindustrial strain isolated in Georgia) was selected because it has been known to be a relatively safe microorganism and is genetically very close to *S. boulardii*. However, the staphylococcal infection was chosen because *S. aureus* is an important human pathogen, the pathogenesis of which was not diminished by the application of antibiotics; there are no literature data about the influence of *S. cerevisiae* viable cells on immune and CNS by *S. aureus* infection *in vivo*.

Changes of antibacterial and antitoxic antibody titer enables us to detect the development of infection and influence of yeast living cells on the immune system of *S. aureus*-suffering animals. The titer of both antibodies was higher in the sera from infected animals comparing to the intact ones but results obtained from *Scv*-treated group were higher than in infected animals. So, protective efficacy of yeast viable cells explain the induction of immune response against *S. aureus* infection. This effect may be of interest for improving the resistance to bacterial infection.

For the influence of *Scv* on the CNS we decided to estimate the total number of neurons and glial elements (macroglia) in the different subdivisions of hippocampus. The cell loss is the specific process for many diseases provoked by different factors. No less important than quantitative analysis of cells is the investigation of reaction of macroglia – one of the general characteristics of all the most important processes occurring in the CNS as a result of some diseases or the influence of different foreign factors.

According to our results, a matched dose of *S. aureus* provoked changes in the number of neurons or microglia of mouse hippocampus, but the obtained results were not statistically significant. Recent data of other authors show that the SEB causes some significant changes in the CNS: it induces fever, brain c-Fos expression (Elmquest *et al*. 1996; Goehler *et al*. 2001), and augmentation of appetitive neophobia (Shurin *et al*. 1997). Such data increase our interest in the elucidating the mechanism of action of *S. aureus*, as well as clarifying the general difference of the effect of its viable cells and its toxin on the CNS.

Surprisingly, it was found that the number of macroglia was truly increased in the CA1 in the *Scv*-treated group. Because (as mentioned *above*), *S. aureus* itself do not provoke any true modification in the cytoarchitecture of the studied areas, we can speculate that the alteration in the quantity of glial cells is the result of the influence of *Scv* viable cells itself. We cannot explain the mechanism by which *Scv* provoked such quantitative changes or why such changes do not take place in other regions of the hippocampus. Besides many literature data, which considered the unique therapeutic properties of probiotic *S. cerevisiae* and *S. boulardii*, recent investigations show their pathogenesis. It was shown that intravenous administration of clinical and nonclinical strains of *S. cerevisiae* in the C5-deficient mice provoked an increase of glial cells in the brain (parenchyma). Other authors determined that long-time treatment with *S. boulardii* by itraperitoneal and oral administration may induce *S. boulardii* infection, which includes cardial dysfunction and inflammatory processes (Eng *et al*. 1984; Aucott *et al.* 1990). It is possible that the modification in the number of macroglia is one of the characteristic signs of influence of *Saccharomyces* yeasts on brain cytoarchitecture. At the same time, a lot of experimental data indicate the special vulnerability of CA1 compared with other regions of the hippocampus, which could be explained by its particular neurochemical peculiarities and connections. The same takes place in our material also.

The presence of true quantitative changes of glial elements provoked by the treatment of mice with *Scv* put in doubt the use (without additional investigation of its mechanism) of living cells of *S. cerevisiae* in medicine.

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