Natural xenoantibodies – their relation to molecular landscape of environment. Presence and possible functions of xenoantibodies in mice sera

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Abstract

Samples of materials from mice habitat (air, feed, drinking water, litter) were investigated in order to estimate their bacterial content and number. The concentrations of bacteria in tested materials ranged from 6.1 × 10^1 to 3.1 × 10^5 cfu/appropriate unit. Species of Micrococcus, Staphylococcus, Bacillus and Pseudomonas genera dominated. The cell populations of Gram-positive bacteria overdominated Gram-negative ones. Extended in time investigations confirm the long-term stability of microflora composition of tested materials. Stable presence of bacterial components in animals habitat influences on mice sera antigen specifity composition. Concentrations of specific antibacterial antibodies in tested sera were higher in elder mice and their prevalence were dependent on antigen availability.

Key words: *bacterial immunostimulation, specific antibodies, natural xenoantibodies.*

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The investigations concerning the microbial state of food or human and animal habitats are mainly focused on evaluation of the degree of pollution and their sanitary state [1, 2]. On the other hand, epidemiological and experimental studies have led to the conclusion that certain microorganisms and their components modulate the immune system and in consequence influence favorable on animal/human health [3-5]. Although numerous studies on microflora of different environments have been carried out, very little attention has been paid to the relation between the distribution of these microorganisms and their immune sign in animal/human organisms. Therefore the aim of the study was to: (I) isolate and identify bacteria of laboratory animals habitat, (II) evaluate their distribution in feed, water, litter and nearest environment and (III) perform an examination of mice BALB/C sera with isolated bacteria for presence of specific antibodies.

Materials and Methods

Mice (their sera) and the inside of animal room (its microflora) of our Institute were the objects of investigation.

Bacteria isolation and identification

Ten grams samples of each solid materials (feed--LABOFEED H, wooden shaves, dust) were suspended in 100 ml of 0.9% NaCl. The concentrations of bacteria in suspensions and in tested water were determined by dilution plating methods. The 0.1 and 0.2 ml aliquots of sampled water and suspensions, their 10-fold serial dilutions in 0.9 NaCl up to 10^4 were spread on duplicate sets of nutrient agar (Biomed) plates. Air was sampled by exposition of open nutrient agar plates for 5; 10; 15 and 30 minutes. Plates were incubated (set 1) at 37°C for two days and next kept at room temperature for two weeks. Plates of set 2 were

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Table 1. The bacteria cells concentration in the habitat of laboratory animals

Source of samples	Sampling dates	Number of bacterial cells in 1 g (or ml or m³) of tested sample calculated after incubation of plates in:	
		20°C	37°C
air[1]	2001	1.32×10^3	1.18×10^3
drinking[2] water	1996	7.2×10^1	3.2×10^2
	2001	6.1×10^1	8.2×10^1
feed[3]	1996	1.3×10^5	7.8×10^4
	2001	8.2×10^4	3.1×10^5
litter[3] (wooden shavings)	1996	2.2×10^3	3.1×10^4
	2001	3.6×10^3	2.3×10^4
dust[3]	2001	4.7×10^4	7.1×10^3

Number of bacterial cells contained in: [1] – 1 m³, [2] – 1 ml, [3] – 1 g of tested material.

incubated solely at room temperature. During each incubation day plates were checked, arisen colonies counted and described. Sets of colonies initially differentiated on the basis of morphology (shape, color, consistence) were isolated and collected. Further differentiation and identification of isolates based on: (I) Gram staining, (II) growth on appropriate sets of prepared differentiative and selective media [6], (III) patterns of reactions with API and Lachema tests [7]. Characterized isolates were determined according to the Bergey,s Manual [8, 9].

Antigens preparation

Bacterial cells of dominated species of isolated genera were prepared in two forms, as the viable and fixed (heat-killed) cells suspended in borate buffer (pH 8.2) in concentration of 1×10^7 cfu/ml.

Mice and sera preparation

Sera were taken from two groups of mice (born in situ) in five years interval (1996 and 2001); 35 including 16 young – eight to ten weeks old, and 19 older- over 26 weeks old in first stage and 16 (12 young and 4 elders) in second stage.

Antibodies specifity assays

ELISA tests were carried out according Weeman and Schurs [10]. Borate (pH 8.2), PBS (pH 7.4) and citrate (pH 4.9) buffers were employed. Wells of plates (Nunc-Immuno, Maxi Sorp) were coated by all forms of prepared antigens: viable and fixed cells at concentration 5×10^5/well. Amount of 0.1 ml of tested and conjugated sera diluted in 1% BSA in PBS for each well were used. Standard of dilution of tested sera was 1:10 – 1:100. Peroxidase conjugated goat anti mouse (I) polyvalent immunoglobulins IgG+ IgA+ IgM (Sigma Immuno Chemicals), (II) IgG+IgM and (III) IgG (Jackson ImmunoResearch) were used in standard dilution 1:5000. Incubations of plates were performed for 2 h at 37°C or overnight at 4°C. All plates were read with Dynatech ELISA spectrofotometric reader at the wavelength 492 nm.

Results

The bacteria concentration and distribution in mice habitat is presented in table 1. It may be seen that the numbers of bacteria were basically stable for each of tested sources. The lower numbers of bacteria cells were found in drinking water – 6.1×10^1 cfu/ml, more numerous in bacteria cells concentration were feed and litter – 3.1×10^5 cfu/ml. There were not significant differences in bacteria concentration between samples taken in different time and growth temperature. Table 2 presents the degree of differentiation of isolated bacteria and the list of most frequent genera. More numerous in genera composition (6–5–4) were samples obtained from litter, air and dust. Number of isolated species from particular sources differ from 6 (feed) and 7 (litter, water) to 13 (air, dust). The dominating groups of cells and their systematic position are presented in table 3. As can be seen representatives of *Micrococcus*, *Staphylococcus* and *Bacillus* genera constituted majority of bacterial cells populations.

Table 4 shows the prevalence's and the levels of specific antibodies (in two groups of mice sera) which recognized antigens presented in mice habitat. Positive sera of younger mice were less numerous than in older group, e.g. 18.8% v. 47.4% for antigens of *Bacillus* and sera dilution 1/100. Percent of positive antigen – antibody reaction in less diluted (1:10) sera were more then three times higher in comparison with sera more diluted (1:100), e.g. 43.3 versus 6.3 and 84.2 v. 52.6 for reactions with antigens of *Micrococcus*. The results of sera specificity spectrum are

Table 2. Genera and species composition of the mice habitat bacterial microflora

Source of bacteria	Number of recognized:		List of isolated genera (number of species)
	genera	species	
air	5	13	*Micrococcus* (4), *Staphylococcus* (3), *Bacillus* (4), *Aeromonas* (1), *Serratia* (1)
drinking water	2	7	*Micrococcus* (3), *Staphylococcus* (4)
feed	2	6	*Staphylococcus* (3), *Bacillus* (3)
litter (wooden shavings)	6	7	*Micrococcus* (1), *Staphylococcus* (1), *Bacillus* (1), *Pseudomonas* (2), *Escherichia* (1), *Proteus* (1)
dust	4	13	*Micrococcus* (3), *Staphylococcus* (1), *Bacillus* (6), *Mycobacterium* (3)

Table 3. Genera and their cells contribution in total population of bacterial cells in the mice habitat

Genera	Species		Percent of bacterial cells population represented by genus in samples of:				
	number	dominants[1]	air	drinking water	feed	litter	dust
Micrococcus	5	*M. luteus* *M. varians* *M. roseus*	33.3	39.0	0.0	17.7	20.4
Staphylococcus	7	*S. auricularis* *S. cohnii* *S. varneri*	8.7	61.0	43.9	17.9	2.1
Bacillus	10	*B. megatherum* *B. coagulans* *B. cereus*	12.6	0.0	56.1	17.5	58.2
Pseudomonas	2	*P. putida* *P. cepacia*	0.0	0.0	0.0	11.8	0.0
other[2]	7	*E. coli* *P. vulgaris* *M. flavescens*	35.4	0.0	0.0	35.1	19.3

[1] *– species which were prevalent by number of cells and spread;* [2] *– represented by species of genera: Escherichia, Proteus (1) – litter, Aeromonas (1), Serratia (1) – air, Mycobacterium (3) – dust.*

Table 4. The presence of antibacterial xenoantibodies in laboratory mice sera

Microorganisms used as the antigens	Percent of sera contining specific antibodies against tested antigens			
	sera of young mice (<3 month) after dilution		sera of older mice (>6 month) after dilution	
	1:10	1:100	1:10	1:100
Micrococcus	43.8	6.3	84.2	52.6
Staphylococcus	87.5	25.0	94.6	89.5
Bacillus	56.3	18.8	78.9	47.4
Pseudomonas	37.5	0.0	63.2	21.1
Mycobacterium	–	–	55.5[1]	33.6[1]
Shigella[2]	0.0	0.0	0.0	0.0

[1] *– sera of nine mice were tested;* [2] *– control, bacteria were not present in tested environment.*

presented in table 5. As can be seen there were differences in sera spectrum specificities depending on (I) range of recognized classes of antibodies, (II) dilution of sera and (III) tested groups. Incubation of specific sera with goat anti-mouse IgGMA revealed that the higher number (12 of 35 and 3 of 16) of sera diluted 1:100 recognized three sources of available

Table 5. Diversity of distribution of antibacterial specifities in laboratory mice sera

Number of antigens recognized by tested sera	Number of sera (used in dilutions 1:10 and 1:100) containing specific antibodies against antigens of bacteria tested in:					
	stage 1		stage 2			
	recognized by goat anti-mouse Ig GMA				recognized by goat anti mouse IgG	
	1:10	1:100	1:10	1:100	1:10	1:100
0	1	6	1	7	4	15
1	8	8	2	2	3	1
2	8	5	3	3	3	0
3	13	12	4	3	3	0
4	3	3	3	1	2	0
5	1	1	2	0	1	0
6	1	0	1	0	0	0
number of tested sera	35	35	16	16	16	16

antigens, whereas 6 of 35 and 7 of 16 did not recognize presented antigens. Additionally, none of sera recognized all available antigens. Only one of the tested sera diluted 1:100 showed positive reaction with goat anti-mouse IgG.

Discussion

Bacteria and their components strongly influence on immune system [11,12] e.g. by antibodies generation or induction interleukins release [13]. Natural antibodies specific to toxins, bacteria, viruses are present in the sera of normal, nonimmunized humans or mice [14]. They are encoded by germline variable genes and their titers in mice range from 1/8 to 1/32 [14]. Most of sera of young and some of older mice presented in this work seems to posses natural antibodies; they showed positive reaction with ten times diluted sera and negative with hundred times diluted sera. Longer coexistence with antigens lead to higher than natural levels of specific antibodies and indicates that animals were stimulated by antigens (table 4). Natural antibodies are the essential part of the first line of defense and link innate and acquired immunity [14, 15]. Lack of natural antibodies may be the reason of existance of animals which did not posses in their sera any specifities for tested antigens (table 5). Explanation all of the relations between common nonpatogenic environmental bacteria and natural xenoantibodies seems to be open question for a long time.

References

1. Krysińska-Traczyk E, Skórska Cz, Milanowski J et al. (1996): Mikroflora powietrza fabryk mebli jako potencjalny czynnik zagrożenia zawodowego: stężenie i skład mikroflory oraz immunologiczna reaktywność pracowników na aeroalergeny drobnoustrojowe. Pneumonol Alergol Pol 64 (Supl. 1): 38-44.
2. Mackiewicz B, Prażmo Z, Milanowski J et al. (1996): Narażenie na pył organiczny i drobnoustroje jako czynnik wpływający na stan układu oddechowego pracowników stadniny koni. Pneumonol Alergol Pol 64 (Supl. 1): 19-24.
3. Cookson WO, Moffat MF (1997): Asthma: an epidemic in absence of infection? Science 275: 41-42.
4. Matricardi PM (1997): Infections preventing atopy: facts and new questions. Allergy 52: 879-882.
5. Holgate ST (2000): Science, medicine and the future. Allergic disorders. BMJ 320: 231-234.
6. Kędzia W. Diagnostyka mikrobiologiczna w medycynie. PZWL, Warszawa, 1990.
7. Code books, Catalogue analtique for Lachema Mikro-La-Tests and API-tests.
8. Bergey's manual of determinative bacteriology. Vol. 1. Williams & Wilkins, Baltimore, 1984.
9. Bergey's manual of determinative bacteriology. Vol. 2. Williams & Wilkins, Baltimore, 1986.
10. van Weemen BK, Schurs AH (1971): Immunoassay using antigen-enzyme conjugates. FEBS Lett 15: 232-236.
11. Bystron J, Hermanowá Z, Szotkovská J et al. (2004): Effect of Ribosomal Immunotherapy on the Clinical Condition and Plasma Levels of Cytokines IL-4, IL-5, IL-12 and IFNgamma and Total IgE in Patients with Seasonal Allergy during the Pollen Season. Clin Drug Investig 24: 761-764.
12. Holt PG, Sly PD, Björkstén B (1997): Atopic versus infectious diseases in childhood: a question of balance? Pediatr Allergy Immunol 8: 53-58.
13. Du Pan RM, Koechli B (1984): Interferon induction by the bacterial lysate Broncho-vaxom: a double blind clinical study in children. Kinderarzt 15: 646-651.
14. Ochsenbein AF, Zinkernagel R (2000): Natural antibodies and complement link and acquired immunity. Immunol Today 21: 624-630.
15. Manz RA, Hauser AE, Hiepe F, Radbruch A (2005): Maintenance of serum antibody levels. Annu Rev Immunol 23: 367-386.