

# Immunomorphological Peculiarities of Spleen According to Microbial State and Age of Body

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## **Abstract**

The aim of study is immunomorphologic investigation of regular (conventional) and Germfree (gnotobionts) white rats during aging. The results of study showed that generally spleen lymphoid tissue of gnotobionts unlike conventional ones don't reduce during aging. In follicles, despite of simplifying antigenic loading, has been increased (in comparison with the control) phagocytic activity, enhancing with the aging process. Has been indicated aging increasing of T-lymphocytes' number on the background of unchanged number (comparing with the control, not depending on age) of B-cells. So, Immunopoiesic potential of gnotobionts' spleen depends not so as on the influence of "external" antigenic stimulus as on the influence of "internal" (processes of depo and hemokatharsis) factors, creating "local" conditions for antigenic stimulation of organ.

**Keywords:** *gnotobionts, spleen, immunomorphology, aging*

**A**s it is known, the spleen represents the organ of lympho-, myelo- and erythropoiesis at the same time, that is realized by appropriate structures [1,2,3]. According to recent data [4] except of important immune functions, the spleen fulfils the function of depo of platelets, red cells and granulocytes as well. Degradation - hemokatharsis - of dead platelets and red cells undergoes within this organ too. Realization of immune processes, storage (white pulp) and processes of hemokatharsis (red pulp) accomplishes due to characteristic cytoarchitectonic of this interesting organ and particularly the vascular system of special structure. The central arterioles surrounded by periarteriolar lymphoid "sheaths" ("cuffs") end to capillaries, freely joining to strands of red pulp strands. Therefore, circulating cells, reaching the strands and sustaining there are "recognized" by macrophages, while the part of platelets and red cells, avoiding phagocytosis returns to blood circulation through venous sinusoids. The lymphoid tissue of spleen that, as it was said, is gathered around the central arterioles in the kind of "sheaths", consists of adjacent T-zone and peripheral B-zone.

Lymphocytes create primary or non-antigen-contacting ("nonstimulating") lymphoid follicles with germinative centers containing "immune memory" cells. So the spleen lymphoid tissue under the antigenic stimulation and in is characterized by special structure that might be expressed differently in the organisms of various age. Thereby everything above-mentioned stimulate the interest of versatile morphological study of immunogenesis of this important organ in norm and especially in "ideal norm" (with minimal antigenic stimuli) using the Germfree (gnotobiont) organisms during the postnatal ontogenesis. It should be mentioned, that non-microbial (with chemically "clean" non-antigenic diet) animals are unchangeable objects for study of histophysiology of immunocompetentive organs.

The aim of given paper is study of influence of microbe state and age on spleen lymphopoietic potential. For this purpose immunomorphologically was investigated the spleen of regular (conventional) and non-microbe (gnotobiont) white rats of different ages. Morphometry, cell accounting and data processing were performed by mean of morphometric [5] methods.

The investigation shows, that as the absolute as well as relative mass of adult (6-10months) and old (24-32 months) gnotobiont's spleen doesn't differ essentially (*Tab.1*) from the analogous findings of control group. So, this parameter doesn't depend on microbe state and age of body.

As to the relative areas, engaged by the different structural components of spleen, (according to the same table) the findings of adult gnotobionts and conventional animals differ significantly. Comparing the areas of lymphoid and connective tissues of rats' spleen has been revealed that the dynamic differs as follows: mainly, in old gnotobionts' spleen, the lymphoid tissue takes twice as more area (in comparison with its control), while connective tissue takes approximately 2,5 as less. This fact indicates to the high lymphopoiesic activity of old gnotobionts' spleen. The results of cell counting in periarterial zone and follicles, proving the same, show (*Tab.2*) that the amount of blasts, big and small lymphocytes in gnotobionts increases with the aging process on the background of small lymphocytes' decrease in B zones.

As to the influence of microbic state, despite of aging group, the same table shows that only in B-zones (comparing with control) of gnotobionts' has been revealed the high level of reticular cells, middle-sized lymphocytes and macrophages (indicating to the active phagocytosis) on the background of common lack of the other studying cells.

Immunomorphological studies of gnotobionts' and conventional rats' spleens (by method of Kuns, PAP method) showed (*Tab.3*), that on the background of common lack of the rest other cells in gnotobionts (comparing with control, despite of aging group), together with the aging process, the number of Ig - positive blasts and T-lymphocytes increase, though in comparison with gnotobionts' parameters keeps low level. As to the amount of B-lymphocytes, according to the table, it doesn't depend on the influence of microbic factor and age.

Data \ Experiment		Adult (6-10 month)		Old (24-32 months)	
		Gnotobiont	Conventional	Gnotobiont	Conventional
Spleen mass (index AE spleen mass/body mass)	X+ Sx	600,1±51,4 (0,25)	552,4±56,2 (0,23)	659,3±44,2 (0,22)	578,21±46,2 (0,17)
	t		0,62 (p>0,005)		1,3 (p>0,05)
Area of lymphoid tissue (white pulp)	X+ Sx	17,9±0,92	19,4±0,98	18,31±0,94	8,7±0,43
	t		1,1 (p>0,05)		3,3 (p<0,001)
Area of red pulp	X+ Sx	75,22±4,1	73,2±3,9	69,4±3,1	63,1±3,2
	t		0,4 (p>0,05)		1,4 (p>0,05)
Area of connective tissue (capsule + trabecules)	X+ Sx	6,88±0,32	7,4±0,36	12,3±0,58	28,2±1,3
	t		1,1 (p>0,05)		11,2 (p<0,001)

**Tab.1** Gnotobionts' and conventional rats' masses (mg) and findings of structural components (% area of whole slice) of relative areas.

Data	Experiment	Adult (6-10 month)		old (24-32 months)	
		gnotobiont	conventional	gnotobiont	conventional
Periarterial Zone					
reticular	X+ Sx	3,42±0,18	2,85±0,15	7,65±0,43	8,61±0,44
	t		2,4(p>0,05)		1,6(p>0,05)
blasts	X+ Sx	0,75±0,04	1,74±0,09	0,54±0,03	0,51±0,03
	t		10,1(p<0,001)		0,7(p>0,05)
big lymphocytes	X+ Sx	2,4±0,12	3,86±0,21	1,94±0,1	1,45±0,08
	t		6,1(p<0,001)		3,8(p<0,001)
middle-sized lymphocytes	X+ Sx	20,7±1,1	14,8±1,2	18,2±0,9	24,41±1,22
	t		2,5(p<0,02)		4,1(p<0,001)
small lymphocytes	X+ Sx	39,6±2,6	43,6±2,24	20,68±1,6	13,85±0,71
	t		1,2(p>0,05)		3,9(p<0,001)
Follicles					
reticular	X+ Sx	6,1±0,4	4,4±0,19	1,47±0,9	7,35±0,41
	t		3,4(P<0,001)		7,4(p<0,001)
blasts	X+ Sx	2,9±0,17	3,52±0,21	1,2±0,08	1,74±0,09
	t		2,3(P<0,05)		4,5(p<0,001)
big lymphocytes	X+ Sx	16,5±1,1	33,7±1,48	1,85±0,61	21,4±1,1
	t		9,3(P<0,001)		8,4(p<0,001)
middle-sized lymphocytes	X+ Sx	36,2±1,8	29,1±1,49	38,9±1,9	31,5±1,6
	t		16,6(P<0,001)		3,0(p<0,001)
small lymphocytes	X+ Sx	29,8±2,2	22,4±1,1	12,5±0,72	15,8±0,83
	t		3,0(P<0,001)		3,0(p<0,001)
plasmocytes	X+ Sx	1,1±0,07	1,4±0,07	4,76±0,28	6,4±0,31
	t		3,1(P<0,001)		3,8(p<0,001)
macrophages	X+ Sx	5,3±0,27	3,8±0,21	9,84±0,6	7,74±0,31
	t		4,3(P<0,001)		3,1(p<0,001)

**Tab.2** Content of cells in periarterial zone and follicles of gnotobionts' and conventional rats spleen (%).

Data \ Experiment		Adult (6-10 month)		Old (24-32 months)	
		Gnotobiont	Conventional	Gnotobiont	Conventional
Ig + blasts	X+ Sx	0,76±0,05	1,55±0,08	0,52±0,03	0,32±0,02
	t		8,4(P<0,001)		5,6(p<0,001)
Ig + plasmocytes	X+ Sx	2,16±0,14	3,61±0,2	2,3±0,16	4,55±0,24
	t		5,9(P<0,001)		7,8(p<0,001)
Ig +lymphocytes	X+ Sx	5,9±0,4	6,44±0,31	2,45±0,16	2,71±0,14
	t		1,1(p>0,05)		1,2(p>0,05)
Ig + cells generally	X+ Sx	8,82±0,43	11,6±0,57	5,27±0,26	7,58±0,38
	t		3,9(P<0,001)		5,0(p<0,001)
T – lymphocytes	X+ Sx	28,4±2,1	35,2±3,2	22,6±2,3	17,2±1,2
	t		1,7(p>0,05)		2,1(p>0,05)
B – lymphocytes generally	X+ Sx	38,8±3,6	46,1±4,8	35,8±3,2	28,6±3,2
	t		1,2(p>0,05)		1,6(p>0,05)
absolute amount of cariocytes (x10 <sup>-7</sup> )	X+ Sx	22,6±1,1	27,8±1,3	24,4±1,2	28,8±1,4
	t		3,05(P<0,001)		2,4(p<0,02)
absolute amount of Ig + cells (x10 <sup>-6</sup> )	X+ Sx	19,9±0,9	32,2±1,5	12,9±0,6	21,8±1,1
	t		7,0(P<0,001)		7,12(p<0,001)

**Tab.3** Content of immunocompetent cells in gnotobionts' and conventional rats spleen (%).

So, gnotobionts' spleen lymphoid tissue (comparing with control group) is less exposed to the aging reduction and its relative area in old gnotobionts' spleen in average is twice as much, in comparison with control group of the same age. Substitution of connective tissue in gnotobionts' spleen is less expressive as well. As to the cytoarchitectonic of spleen, despite of limited antigenic loading, has been revealed the strengthening of phagocytotic activity in follicles (comparing with control), which increases together with the aging.

It must be emphasized about the aging increase of T-lymphocytes' amount in gnotobionts' spleen on the background of B-lymphocytes (in comparison with the control) equal amount. So, immunopoiesic potential of gnotobionts' spleen depends not so as on microbe stimulus, as on "local" antigenic (products of lysis of red cells and trombocytes) loading of this organ, that might be connected with the strengthening process of hemocatheresis.

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## **Иммунорфологические особенности селезенки в зависимости от микробного статуса и возраста организма**

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### **Р Е З Ю М Е**

Целью работы являлось иммунорфологическое изучение селезенки обычных (конвенциональные) и безмикробных (гнотобионты) белых крыс в возрастном аспекте. Результаты исследования показали, что в целом лимфоидная ткань селезенки гнотобионтов, в отличие от обычных, не редуцируется с возрастом. Несмотря на упрощение антигенной нагрузки, в фолликулах повышается количество В-клеток (сравнении с контролем, независимо от возраста). Следовательно, иммунопотенциальный потенциал селезенки гнотобионтов зависит не столько от влияния внешних антигенных стимулов, сколько от действия внутренних (процессы депонирования и гемокатереза) факторов, создающих местные условия антигенного стимулирования органа.

**Ключевые слова:** *гнотобионты, селезенка, иммунорфология, старение*