Comparative Characteristics of the Morphology of Rabbit Lymph Nodes after Implantation of Porous Titanium with Composite Nanocoating and Hydroxyapatite Particles Embedded into Pores

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We studied changes of microarchitectonics of lymph nodes in mature rabbits in response to introduction of bioimplants based on porous titanium with carbon-containing nanocoating or nanocoating with hydroxyapatite embedded into pores. Moderate local reactivity of the lymphoid system was observed in animals with implants coated with carbon-containing films within 30 days after surgery. In animals with nanocoated implants containing hydroxyapatite, the immune response was more pronounced. Introduction of implants led to activation of cellular and humoral mechanisms of immunity in animals of both groups.

Key Words: *porous titanium; implants; coating; lymph nodes*

Original methods of applying carbon-containing nanocoating (CCN) with thickness of ≈20 nm were developed at the Institute of Metal Physics, Ural Division of the Russian Academy of Sciences, [3] for improving biocompatibility of porous titanium (PT) with the bone tissue [1]. The criterion for bio-inactivity of the implant is the reaction of the immune system, one of the striking manifestations of which, of course, is the change in cellular composition and structure of lymph nodes.

The purpose of this study was to examine changes in the microarchitectonics of lymph nodes in response to introduction of bioimplants based on PT with CCN containing the potential osteo-inducer, hydroxyapatite (HA), embedded into the pores.

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MATERIALS AND METHODS

The work was performed on 11 adult rabbits. The implants were introduced into tibial and femoral condyles under general anesthesia. The operations were performed by D. G. Bliznets, A. I. Isaikin, PhD, and E. B. Makarova, PhD. Implants were pre-saturated with adherent autologous bone marrow cells. The cells were multiplied by culturing for 14 days in complete culture medium (McCoy, 5A, 20% FCS, L-glutamine, gentamicin, heparin in 24-well plates at 37°C and 4% CO_2 content). In group 1, implants made of PT, coated with CCN were installed in 5 rabbits. In group 2, PT coated with CCN with HA particles $(Ca_{10}PO_4)_6(OH)_2$ embedded into the pores, were implanted to 3 rabbits. The time of observation after surgery was 1 month. The reference group included intact animals (n=3). Lymph nodes were fixed in 10% formalin, dehydrated in ascending ethanol concentrations and embedded in paraffin; serial 5-6-μ sections were prepared, stained with hematoxylin and eosin and by van Gieson. Microscopy was performed using AxioStarPLUS Carl Zeiss micro-

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scope with magnification up to 1000. Morphometric studies were carried out using a Video ResT-Master Morfologia 4.0 hardware-software complex. The ratios of areas of structural elements in lymph nodes were studied. The mean number of cells in the field of view and their percentage were also determined. The area of the field of view was 0.025 mm^2. To detect intergroups differences, nonparametric Mann–Whitney test was used. Statistical hypothesis considered to be confirmed at a significance level of $p \leq 0.05$.

RESULTS

According to histological examination, the cortical substance of regional lymph nodes in group 1 rabbits had uneven thickness due to well-defined follicles of different sizes. The mantle zone was narrow, germinal zones were somewhat expanded in some animals and blurred in others. Subcapsular and trabecular sinuses contained a moderate amount of lymphocytes. In some animals, the paracortical zone was locally widened. In the medulla, sinuses were moderately dilated and the number of lymphoid cells decreased (Fig. 1). In the lymph nodes, perivascular edema was detected. In group 2 animals, the cortex of regional lymph nodes was enlarged. In most animals, it was due to an increase in the area of follicles and expansion of trabecular sinuses containing large amounts of cells. The paracortical zone was diffusely enlarged. The mantle zone was thinned; germinal zones were widened and thickened (Fig. 2). In medullary cords, infiltration with plasma cells, eosinophils, and macrophages was clearly seen. The medullary sinuses were expanded due to edema with a large number of lymphocytes.

In group 2, similar but less pronounced changes were found in the contralateral lymph nodes. In animals of both groups, neither morphological signs of acute inflammation (increased count of polymorphonuclear leukocytes), nor chronic inflammation or rejection (epithelioid cells and foreign body giant multinucleated cells) were found. No morphological and oncogenic effects of implants on the lymphoid tissue, namely, tissue and/or cellular atypia, were revealed.

To objectify the results of histological studies, morphometric measurements were performed. In group 2, the cortical plateau (T-zone) in the regional lymph nodes occupied 1.5 times greater area than in group 1 rabbits. Despite the lower numerical density of lymphocytes, the absolute content of the cells in this zone tended to increase due to the greater area of the cortical plateau. A possible cause for reduction of cell density was edema of lymphoid tissue. The relative content of lymphoblasts in group 2 was 4.4 times higher than in group 1 (Table 1). Their absolute number increased, and the number of mature lymphocytes decreased. In group 2 animals, the content of eosinophils and macrophages was increased. In the group with HA, the area of paracortical zones was enlarged to 192%, but the differences were not significant because of great error of the mean. The increase in the number of lymphoblasts, macrophages, and plasma cells in this group should be noted.

The number of follicles and their size, the number of follicles with light central zone and without it, the area of germinal zones and medulla, numerical density of lymphocytes per section area in germinal centers and medullary cords in animals of both experimental groups did not differ significantly. However, the differ-

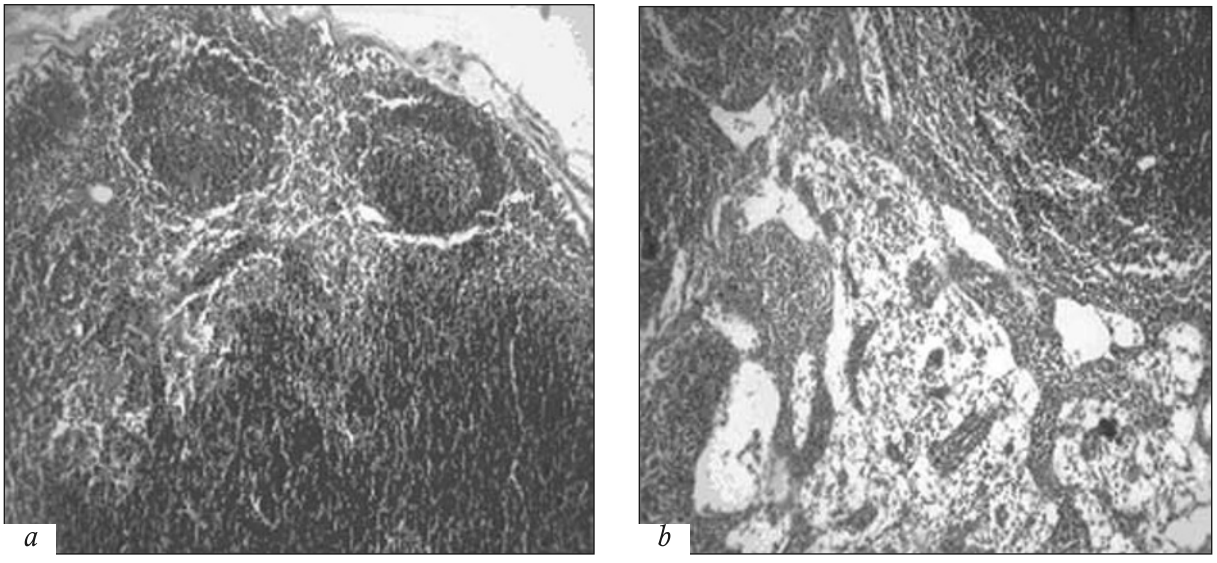

Fig. 1. Lymph node of a rabbit from group 1. Staining with hematoxylin and eosin, ×100. *a*) cortex, paracortical hyperplasia; *b)* medulla with dilated sinuses.

TABLE 1. Cell Composition of Regional Lymph Nodes in Rabbits One Month after Implantation (%; *M*±*m*)

Zone of lymph node		Group		
		control	1	2
Cortical plateau	Lymphoblasts	1.5±0.3	0.95±0.12	4.27±0.63*
	Immunoblasts	0±0	0±0	0.47±0.18
	Prolymphocytes	1.0±0.2	2.20±0.22	2.86±0.18
	Lymphocytes	96.50±0.90	96.45±0.42	89.47±1.68*+
	Plasma cells	0.20±0.00	0.10±0.06	1.07±0.27
	Neutrophils	0±0	0±0	0.13±0.06
	Eosinophils	0±0	0.1±0.1	0.67±0.24+
	Macrophages	0.8±0.4	0.20±0.14	4.27±0.63*+
Germinal zone	Lymphoblasts	36.2±3.4	25.10±3.91	50.93±3.30*+
	Immunoblasts	0±0	0±0	0±0
	Prolymphocytes	20.2±0.6	11.35±1.81	23.2±2.42
	Lymphocytes	43.6±2.8	63.55±5.48	25.87±5.74*+
	Plasma cells	0±0	0±0	0±0
	Neutrophils	0±0	0±0	0±0
	Eosinophils	0±0	0±0	0±0
	Macrophages	0±0	0±0	0±0
Paracortical zone	Lymphoblasts	0.90±0.14	1.40±0.32	7.40±1.13*+
	Immunoblasts	0±0	0.05±0.05	0.80±0.18
	Prolymphocytes	0.90±0.14	2.60±0.43	7.73±1.75
	Lymphocytes	97.2±0.85	95.00±0.76	80.2±4.5*+
	Plasma cells	0.30±0.14	0.25±0.09	1.27±0.37*+
	Neutrophils	0.10±0.14	0.10±0.06	0.27±0.18
	Eosinophils	0.10±0.14	0.30±0.13	1.20±0.64
	Macrophages	0.5±0.7	0.3±0.1	1.13±0.65*
Medulla	Lymphoblasts	0.30±0.14	11.35±6.77	13.80±4.32
	Immunoblasts	0.30±0.14	0.35±0.21	1.67±0.53
	Prolymphocytes	0.8±0.3	6.20±3.55	6.67±1.39
	Lymphocytes	93.9±0.7	64.85±10.87	54.33±12.99
	Plasma cells	2.7±0.7	13.45±7.22	12.73±4.05
	Neutrophils	0.10±0.14	0.30±0.13	0.40±0.06
	Eosinophils	0±0	1.70±0.29	7.67±3.43*+
	Macrophages	1.90±0.14	1.80±0.36	2.73±0.64

Note. $p \leq 0.05$ compared to: *group 1; +control.

ence in cellular composition of the germinal zones was significant in these groups. In group 2, the number of lymphoblasts and centroblasts exceeded that in group 1, to 2 times. In group 2, a significant increase in relative and absolute number of eosinophils in the medullary cords was revealed. In animals of both groups, some differences were also found in the contralateral lymph nodes. Thus, the density of lymphocytes per section area in the cortical plateau and paracortical zone significantly decreased and the relative content of

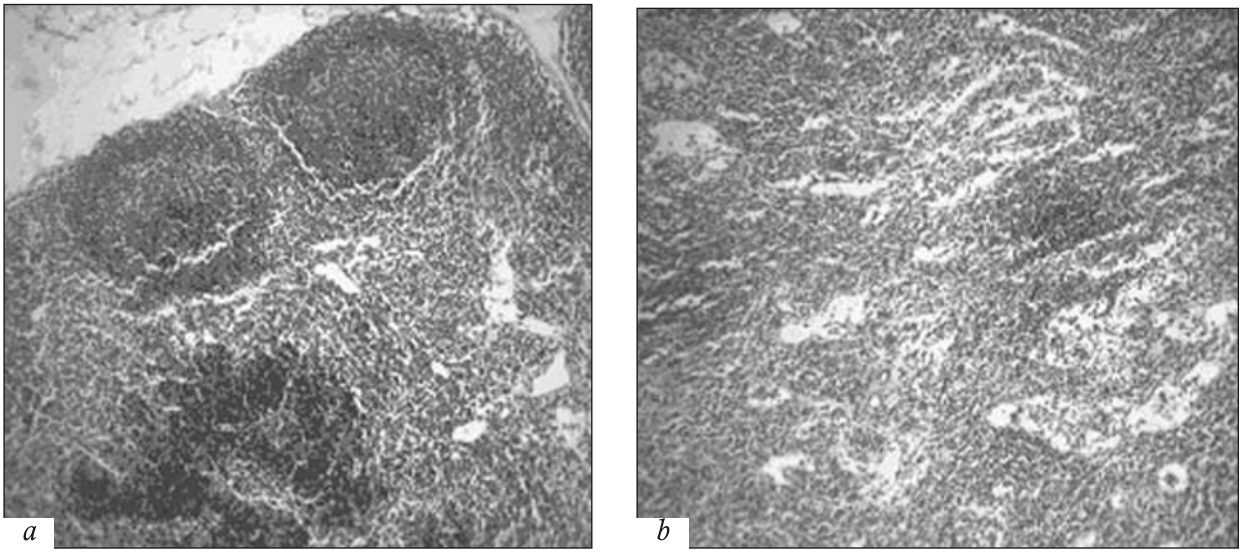

Fig. 2. Lymph node of a rabbit from group 2. Staining with hematoxylin and eosin, ×100. *a*) cortex, hyperplasia of germinal centers causing follicular hyperplasia; *b*) medulla (increased cellularity of sinuses).

lymphoblasts increased 2-fold in HA group compared to CCN group. In animals with HA, eosinophilia of the medulla was found.

According to published data, a significant increase in the area of cortical plateau in regional lymph nodes, paracortical zone, nodules with germinal centers, medullary cords, and medullary sinuses was revealed 30 days after introduction of titanium nickelide implants [2]. Introduction of the PT implants with CCN (group 1) induced a less pronounced response of regional lymph nodes. Therefore, the purpose of coating of metal structures with films insulating them from the body tissues, i.e. the increase of bioinertness of the metal, was achieved. The results with embedding of HA in pores of the implants are controversial. Most authors report the absence of immunological reactions in the organism in response to HA. However, it is known that the rate of degradation of synthetic HA at pH 7.0 is very low. Also, there is no doubt about the fact that the introduced HA was resorbed by macrophages, which in turn results in the development of inflammatory reactions, increased secretion of cytokines and proteolytic enzymes in the area of crystal phagocytosis. Giant cells and lymphocytes are also present in this zone [1]. Hence, changes observed 1 month after surgery probably are the reaction of lymphoid organs to the events inevitably accompanying biodegradation of synthetic HA.

Thus, moderate local reactivity of lymphoid system was registered in animals with PT implants coated with CCN. In the group of animals with HA embedded into pores, morphological changes in the lymph nodes were more pronounced. The response to introduction of the implants in both groups was complex, *i.e.* included cellular and humoral reactions.

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