RELATIONS BETWEEN INFLAMMATORY AND IMMUNE REACTIONS

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UDC 616.24-002-092:612.017.1]-092.9

KEY WORDS: inflammation; immunity; relations.

Changes in the morphogenesis of inflammation of the lungs under the influence of immunomodulators are independent of their modification of the humoral and cellular components of immunity [3].

The aim of this investigation was to continue the study of relations between inflammatory and immune reactions on other models of inflammation under conditions modifying it.

EXPERIMENTAL METHOD

Inflammation of the lungs was induced in 18 Wistar rats weighing 180 g by transthoracic injection of 1 ml of a living 24-h culture of *Staphylococcus aureus* 209 P, containing $20 \cdot 10^9$ bacterial cells, into the lung tissue. Phytohemagglutinin (PHA) in a dose of 5 mg was injected intramuscularly into 6 rats 4 days before infection and on alternate days throughout the period of inflammation; 6 rats were treated with cyclophosphamide (1 mg per animal) and 6 animals received no additional treatment. Inflammatation of the lungs was induced in another 6 rats by transthoracic injection of a killed culture (boiled for 40 min) of the same microorganism, in the same dose, and sterile physiological saline was injected transthoracically into the lungs of 6 control rats. All the animals were killed with a high dose of hexobarbital 5 days after injection of the *Staphylococcal* culture or physiological saline into the lungs. Sections through lung tissues were stained with hematoxylin and eosin, by Van Gieson's and the Gram–Weigert methods, by Feulgen's method for DNA, by Brachet's method for RNA, by the PAS reaction for glycoproteins (GP), and by the method of Hale and Mueller for glycosaminoglycans (GAG). The density of cellular infiltration was investigated (in conventional units) by the morphometric method of dot counting [1] and its character was studied by calculating (for 300 cells) the relative percentage of macrophages, polymorphonuclear leukocytes (polymorphs), lymphocytes, and fibroblasts. DNA was investigated cytochemically in polymorphs and lymphocytes of blood films by the same method using 2-wave cytophotometry [2] and concentrations of cationic proteins (with bromphenol blue at pH 8.2) and peroxidase activity (by the method of Graham and Knoll) also were studied. Titers of heterophilic agglutinins and antistaphylococcal antibodies were determined photometrically in the blood serum by a modified method [4]. The cellular immune response was assessed by the difference in volume (in ml) of the animals' hind limbs before and 48 h after injection of 0.1 ml of standard staphylococcal allergen into their foot pads. All the numerical data were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Inflammation of the lungs 5 days after injection of the living culture into them was characterized histologically by the development of catarrhal bronchitis with a spreading interstitial reaction, in the form of moderate edema and cellular infiltration of the alveolar septa. The onset of these processes was connected with the staphylococci because the animals' lungs were unchanged 5 days after physiological saline was injected into them. Under the influence of cyclophosphamide the severity and extent of inflammation were increased, as was confirmed by the development of lobular and confluent lobular bronchopneumonia. Administra-

I. M. Sechenov Research Institute of Physical Methods of Treatment and Medical Climatology, Yalta. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 466-468, April, 1987. Original article submitted April 22, 1986.

 0007-4888/87/0004-0530$12.50

TABLE 1. Changes in State of Blood Polymorphs and Lymphocytes during Inflammatory Changes in the Lungs (M ± m; c.u.)

Parameter	Cells	Material injected			
		living culture	killed culture	living culture + PHA	living culture + cyclophosphamide
DNA concentration	Lymphocytes	2.49±0.07	2.82±0.06	2.34±0.2	2.4±0.16
p			<0.02	>0.05	>0.05
Concentration of cationic proteins	Polymorphs	2.08±0.06	1.03±0.1	1.44±0.04	2.69±0.42
p			<0.001	<0.001	>0.05
Peroxidase activity	Polymorphs	1.8±0.16	1.43±0.15	1.4±0.08	1.61±0.06
p			>0.05	<0.05	>0.05

tion of PHA led to some decrease in the severity of bronchitis. The density of cellular inflammation of the bronchi in this case [0.38 ± 0.02 conventional unit (c.u.)] did not differ significantly from the control (0.36 ± 0.01 c.u.) whereas after injection of the living culture only, the value of this parameter was higher (0.41 ± 0.01 c.u., $p < 0.05$). However, when PHA was used the density of cellular infiltration around the vessels was increased (from 0.36 ± 0.02 c.u. in the control animals to 0.43 ± 0.01 c.u., $p < 0.05$). Under the influence of the living culture only, this parameter (0.41 ± 0.03 c.u.) did not differ significantly from the control. The use of PHA also led to an increase in the fraction of lymphocytes among infiltrating cells (27.66 ± 0.56%, p ± 0.001) and to a decrease in the fraction of fibroblasts (32.33 ± 2.31%, $p < 0.01$) compared with their values in animals receiving the living culture only (18.25 ± 0.63 and 42.75 ± 1.03%, respectively). The development of catarrhal bronchitis with an interstitial reaction also resulted from injection of the killed culture. In this case, however, (compared with injection of the living culture), cellular infiltration predominated significantly over edema (plasma exudation): the density index of cellular infiltration around the blood vessels rose from 0.4 ± 0.026 to 0.47 ± 0.01 c.u. ($p < 0.05$) in the groups compared. Numerous eosinophils appeared around the vessels and the fraction of fibroblasts was reduced (from 42.75 ± 1.03 to 37.57 ± 1.06 c.u., $p < 0.02$).

The bacterioscopic investigation showed that the largest concentrations of injected staphylococci were found in the lungs (foci of pneumonia) of the animals receiving cyclophosphamide. Smaller collections of staphylococci, but more widely dispersed throughout the tissue (alveoli, septa) were characteristic of the lungs of animals receiving PHA. Less frequent and numerically smaller concentrations of staphylococci were observed after injection of the killed culture. No flora was found in the lungs of animals receiving an injection of the living culture and physiological saline. In all the experimental animals (compared with the control) the concentrations of DNA (nuclei) and RNA (cytoplasm) were increased, though not significantly, as was shown histochemically in the epithelium of the bronchi and endothelium of the blood vessels and septal cells. In foci of edema (vessel walls, interalveolar septa) the GAG and GP concentrations were increased extracellularly under the influence of cyclophosphamide. Values of these parameters also were increased in the epithelium of the bronchi in cases when a killed culture of staphylococci and cyclophosphamide were given (compared with the control, and also when the living culture was used).

These differences found in the local lung changes after the various procedures corresponded to the specific character of the general changes in parameters of the state of the blood polymorphs and lymphocytes (Table 1). Compared with experiments in which the living staphylococcal culture was used, when a killed culture was injected the DNA concentration in the lymphocytes was increased and the concentration of cationic proteins in the polymorphs was reduced. The use of PHA simultaneously reduced the concentration of cationic proteins and peroxidase activity in the polymorphs. Cyclophosphamide caused no significant changes in the state of the blood cells tested (compared with injection of the living culture).

All the inflammatory processes in the lungs compared in this investigation thus have their own definite histological, bacterioscopic, histochemical, and cytochemical features, distinguishing one from another. Meanwhile the results of the immunological investigations showed that there was no statistically significant difference between the processes compared as regards the character of their characteristic humoral (antistaphylococcal antibodies, heterophilic agglutinins) and cell-mediated (skin tests) response. Even PHA and cyclophosphamide, due to the specific nature of the doses used and the duration of the experiment, did not lead to any significant change in the parameters of immunity during the development of inflamma-

tion under the influence of the living microflora only. However, the effect on inflammation due to other, nonimmunomodulating mechanisms of action was achieved.

In the more general sense, the difference in morphogenesis of inflammatory changes in the lungs (after injection of a killed and living microflora, after modification by PHA and cyclophosphamide) under conditions leading to similarity between the immune reactions developing in these cases, points to the absence of any close connection between the course of inflammation and the accompanying state of immunity. The absence of any direct connection between inflammation and immunity also was characteristic of other forms of inflammatory processes in the lungs: bronchiectasis and lung abscess [3].

Besides traditional ideas on the direct role of the state of immunity in the genesis of inflammation, the possibility has been demonstrated that changes in inflammatory and immune reactions may be unconnected with each other under the conditions of a pre-existing inflammatory process.

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RESTORATION OF HEMATOPOIESIS IN SUBCUTANEOUS BONE IMPLANTS IN AGING MICE

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UDC 616.71-089.843-07:616.71-018.46-003.971-053.9

KEY WORDS: bone marrow; bone; subcutaneous implantation; aging.

In heterotopic bone marrow transplants and also in the marrow of subcutaneously implanted bone, the hematopoietic cells proper are of recipient origin, whereas the stromal tissue, providing for restoration of hematophoiesis, belongs to the donor [4, 10]. This tissue chimerism enables the use of both experimental models — heterotopic bone marrow transplantation and subcutaneous bone implantation — to assess the microenvironmental functions of the stromal tissue in connection with its repopulation by the recipient's hematopoietic cells [3, 6, 10]. In comparative studies of the stromal tissue of the bone marrow microenvironment during heterotopic transplantation or in subcutaneous implants of the femur in young and old mice, contradictory results have been obtained [1, 9, 10], which can be explained by the particular features of the methods used for transplantation and for assessing restoration of hematopoiesis.

The investigation described below shows that removal of the contents of the medullary cavity before subcutaneous implantation of bone may reveal age differences in the ability of the stromal tissue of bone marrow to maintain restoration of hematopoiesis in the implanted bone.

EXPERIMENTAL METHOD

In experiments on recipient CBA mice aged 2-3 months, anesthetized with Ketalar (ketamine), one femur each from a young (2-3 months) and old (24-26 months) syngeneic donor was implanted subcutaneously on the lateral surface of the chest on each side. The contents of the medullary cavity of some bones were removed before implantation, and the bone marrow was destroyed with an injection needle, and repeatedly flushed out with cold McCoy's 5A nutrient

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 468-470, April, 1987. Original article submitted June 3, 1986.

0007-4888/87/0004-0532$12.50