BARRIER-FIXING FUNCTION IN GERMFREE ANIMALS

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UDC 612.42.017.1-082:576.85.083.5

The state of the barrier-fixing function was studied in germfree and ordinary guinea pigs and rats. After contamination with Escherichia coli 055 (in doses of $5 \cdot 10^8$ and $10 \cdot 10^{10}$ bacterial cells for subcutaneous and oral administration, respectively) only transient bacteriemia was observed in the ordinary animals in the early stages after infection. Meanwhile the bacteriemia in the germfree animals increased progressively to cause death of the animals in the course of 2-3 days. A reduced fixing and bactericidal power of the regional lymphatic system and of the deep structures of the monocytic-phagocytic system was discovered in the germfree animals. Experimental confirmation of the role of antibodies in the manifestation of the barrier-fixing function was obtained with respect to E. coli. The experiments show the important role of the microbial factor in the formation of the barrier-fixing function of the body.

KEY WORDS: barrier-fixing function; monocytic-phagocytic system; bacteriemia; germfree animals.

The ability of the various tissue structures, especially of lymphoid tissue, to fix a microbial agent, to which the name barrier-fixing function of the body has been applied [1-3, 6], plays an important role in the resistance of the body to infection. However, the role of the microbial factor in the formation of the barrier-fixing function has not yet been adequately studied.

The object of this investigation was to study the activity of the superficial and deep barrier systems in germfree animals by comparison with ordinary animals.

EXPERIMENTAL METHOD

Germfree and ordinary guinea pigs (aged 3-4 weeks) and Wistar rats (aged 3-4 months) were used. The germfree guinea pigs were obtained by hysterotomy by the writer's own method, using an operating isolator [5]. The germfree rats were obtained from "IFFA-CREDO" (France) and reared in transparent plastic gnotobiological isolators of the writer's own design and polyvinyl chloride film isolators made by the firm of "Celster-Isotechnie" (France). The French isolators are equipped with a special sluice system (of the DPTE type), with an interleaving connecting device, ensuring the rapid insertion of sterile materials into the chamber of the isolator.

To feed the germfree guinea pigs a semiliquid diet of type L-477 was used, and the germfree rats were given a diet of type L-474E12. These diets have proved very satisfactory in practice for maintaining germfree guinea pigs and rats [9, 10]. The diets were sterilized in a vacuum autoclave at 121°C for 25 min. Throughout the period of the gnotobiological experiments, the sterility of the germfree animals was kept under strict control. The microbiological control followed the general pattern [11] with slight modifications [4].

Activity of the barrier-fixing function was assessed from the level of the bacteriemia and quantitative bacteriological investigation of the regional lymph nodes and spleen. The bacteriemia was determined by seeding tenfold dilutions of blood samples (0.5 ml) on Endo's agar in petri dishes followed by counting the number of colonies growing per milliliter of blood. During bacteriological investigation of the lymph nodes and spleen a weighed sample of tissue was homogenized in 1 ml of sterile physiological saline. Samples of 0.5 ml of the corresponding tenfold dilutions of homogenate were seeded on Endo's agar in petri dishes. The number of growing colonies was calculated per 100 mg of tissue.

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 6, pp. 706-709, June, 1977. Original article submitted September 10, 1976.

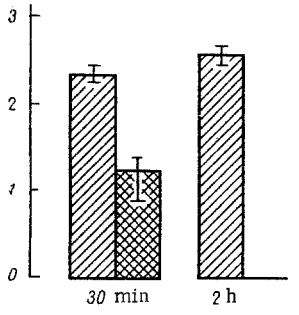

Fig. 1

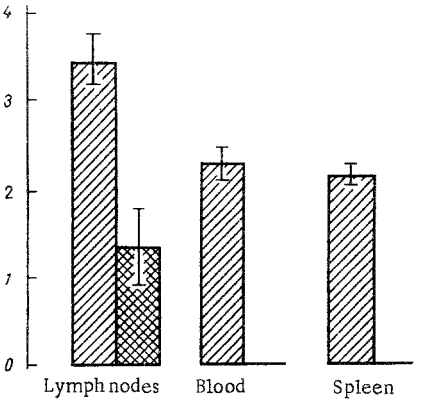

Fig. 2

Fig. 1. Bacteriemia in germfree and ordinary guinea pigs 30 min and 2 h after parenteral injection of E. coli 055. Cross-hatched columns represent ordinary guinea pigs; obliquely shaded columns represent germfree guinea pigs. Ordinate, log. of number of microbes in 1 ml of blood.

Fig. 2. Bacteriemia and distribution of microorganisms in lymph nodes and spleen of germfree and ordinary rats 24 h after parenteral injection of E. coli 055. Cross-hatched columns represent ordinary rats; obliquely shaded columns represent germfree rats. Ordinate, logarithm of number of microbes in 100 mg of tissue or 1 ml of blood.

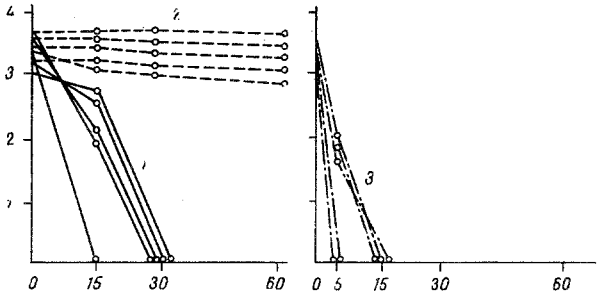

Fig. 3. Clearance of blood following injection of E. coli 055 into germfree and ordinary guinea pigs: 1) ordinary guinea pigs; 2) germfree guinea pigs; 3) germfree guinea pigs after opsonization of bacteria. Abscissa, time (in min); ordinate, logarithm of number of microbes in 1 ml of blood.

The test microorganism was a pathogenic strain of Escherichia coli 055. The barrier-fixing function was determined by subcutaneous and oral methods of infection. As a first step, in experiments on ordinary animals the minimal agressive dose was determined, i.e., the smallest number of microorganisms which, when introduced by one of the above methods into the bloodstream, led to demonstrable bacteriemia after 30 min. In the subcutaneous method of estimating the barrier-fixing function, $5 \cdot 10^8$ bacterial cells in 0.1 ml physiological saline were injected subcutaneously into the animal's hind limb, whereas by the oral method of infection $1 \cdot 10^{10}$ bacterial cells were given in 1 ml of physiological saline.

The fixing capacity of the deep barrier systems was determined from the time taken for E. coli 055 cells, injected into the heart in a dose of 125,000 cells in a volume of 0.5 ml, to disappear from the bloodstream. Each group of experimental (germfree) and control (ordinary) animals infected by one of the above methods contained eight to ten animals.

EXPERIMENTAL RESULTS

The results showed that 30 min after subcutaneous injection of E. coli 055 the indices of bacteriemia in the germfree guinea pigs were much higher than those in ordinary animals. The bacteriemia in the ordinary animals, moreover, unlike that of the germfree animals was transient in character (Fig. 1). Quantitative bacteriological investigations of the regional (inguinal) lymph nodes 2 h after infection showed higher fixing capacity in the ordinary animals. Whereas there were $2 \cdot 10^5$ bacterial cells for every 100 mg of lymphoid tissue in the germfree animals, in the ordinary animals the number of microorganisms was $4 \cdot 10^6$.

In the germfree Wistar rats 24 h after parenteral infection bacteria were found in the regional lymph nodes, blood, and spleen, whereas in ordinary animals microorganisms were detected only in the regional lymph nodes, and in much smaller numbers. These findings indicate higher activity of the deep barrier-fixing systems in ordinary animals (Fig. 2).

A depressed barrier function of the lymphoid system of the intestinal tract also was found in the germfree animals. Whereas in ordinary guinea pigs after oral contamination only transient bacteriemia was found, disappearing after 24 h of observation, the bacteriemia in the germfree animals increased progressively, causing death of the animals in the course of 2-3 days.

Depressed barrier-fixing function also was observed in the deep barrier systems of the germfree animals. After injection of E. coli 055 into the heart of the germfree guinea pigs persistent bacteriemia was observed. Meanwhile, in ordinary animals the bacteria disappeared rapidly from the blood stream (15-30 min after infection). Further investigations showed that the depressed phagocytic activity of the reticuloendothelium was largely due to a deficiency of antibodies against E. coli 055 in the germfree guinea pigs [8]. After preliminary opsonization of the bacteria with specific antiserum in dilutions of 1 : 10 and 1 : 100 (agglutinating titers 1 : 1024) the blood clearance indices of the germfree animals were restored to the normal values observed in ordinary animals (Fig. 3).

As a result of these experiments on germfree animals depression of the fixing properties of both the superficial and deep barrier systems was established. On the one hand, this points to the important role of the microbial factor in the formation of the barrier-fixing mechanisms of resistance, and on the other hand it emphasizes the systemic character and the close interconnection between the barrier-fixing function and other manifestations of the immunobiological reactivity of the body.

Considering that the mechanism of the barrier-fixing function and of the processes of phagocytosis have, in principle, much in common, it can be suggested that the morphological substrate of the barrier-fixing function with respect to the whole organism is made up of elements of the reticuloendothelial system or, in the new nomenclature, the mononuclear phagocytic system [7].

The immediate causes of depression of the barrier-fixing function of the mononuclear-phagocytic system in the germfree animals may have been a deficiency of humoral (opsonizing) factors such as antibodies and complement, and also the deficient developement of the mechanisms of cellular reactivity to the pathogenic agent introduced. Enhancement of the barrier-fixing function by vaccination and endotoxin has been observed previously in ordinary animals [1, 2]. Meanwhile the presence of a definite barrier-fixing function even in germfree animals, intact from the immunological standpoint, suggests that the acquired humoral factors merely facilitate this process by forming immune complexes with foreign antigens that provide an adequate biological signal for activation of the cells of the mononuclear-phagocytic system. The further study of the precise mechanisms and principles of the barrier-fixing function and its dependence on the microbial factor is an essential step in the study of controlled regulation of the resistance of the organism.

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INACTIVATION OF STEM CELLS BY ALLOGENEIC LYMPHOCYTES: COMPETITION BETWEEN T_1 AND T_2 SUBPOPULATIONS

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UDC 612.6.02.017.1-06:612.112.94

Transplantation of bone marrow cells mixed with allogeneic T lymphocytes into irradiated recipients is accompanied by inactivation of stem cells of the graft. Lymphocytes from lymph nodes of T mice possess greater inactivating power than T lymphocytes from the spleen. In the case of the combined action of T lymphocytes from the spleen and T lymphocytes from the lymph nodes, inactivation of the stem cells was slight in degree or absent altogether.
KEY WORDS: T lymphocytes; stem cells.

Combined transplantation of genetically foreign cells of hematopoietic tissues into irradiated recipients is known to be followed by inactivation of the stem colony-forming units (CFU) of the grafts by the allogeneic lymphocytes of the mixture [2]. It has been shown that the chief, it not the exclusive inactivating role in these processes is played by T lymphocytes [1].

The object of this investigation was to continue the study of the inactivating power of T lymphocytes and, in particular, of their subpopulations with affinity for the spleen (T_1) and for the lymph nodes (T_2) of the recipients and also the interaction between these cells in the processes of CFU inactivation.

EXPERIMENTAL METHOD

Experiments were carried out on CBA, C57BL/6J (C57BL), and (CBA × C57BL/6)F_1 mice. The donors of thymus cells were CBA mice aged 4-7 weeks and the donors of bone marrow cells were C57BL mice aged 2-5 months. The recipients were CBA and (CBA × C57BL)F_1 mice aged 2-5 months irradiated in a dose of 850-900 rad 24 h before transplantation of cell suspensions. The dose rate was 170-149 rad/min. To obtain T mice, $3 \cdot 10^7$-$5 \cdot 10^7$ syngeneic thymus cells were injected into lethally irradiated CBA mice and, 7 days later, the spleen (Spl) and inguinal, popliteal, mesenteric, and submandibular lymph nodes (LN) were removed for the preparation of cell suspensions. The cells obtained were mixed in various proportions with bone marrow cells of C57BL mice and the cell mixtures were transplanted into lethally irradiated (CBA × C57BL)F_1 mice. Animals receiving bone marrow cells of C57BL mice only served as the control. In all the experiments cell suspensions were injected intravenously into the irradiated mice. The methods used to prepare the cells were described previously [3]. The recipients were killed 7-8 days after transplantation, their spleens were removed and fixed in Bouin's solution, after which the number of macroscopically visible colonies was counted. The decrease in the number of colonies in the experimental group of animals compared with the controls was expressed as the index of CFU inactivation [3].

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. Institute of Biophysics, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 6, pp. 709-711, June, 1977. Original article submitted November 30, 1976.