CHARACTERISTICS OF ASEPTIC INFLAMMATION IN GERMFREE GUINEA PIGS

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Aseptic inflammation develops more slowly and less intensively in germfree animals than in ordinary guinea pigs (slow formation of poorly developed leukocyte barrier and capsule). The barrier capacity of an inflammatory focus in germfree animals relative to staphylococci is also reduced.

Few investigations of the inflammatory reaction in germfree animals have so far been made, and none by Soviet workers [5, 6, 8, 9]. However, germfree animals are characterized by a number of interesting features, and in particular, their lowered level of general resistance [7] suggests differences between their inflammatory reaction and that of ordinary animals.

The object of the present investigation was to study aseptic inflammation in germfree animals.

EXPERIMENTAL METHOD

Sixteen germfree guinea pigs aged one month, obtained by hysterotomy and reared in isolators of the writer's own design, were used in the experiments. The apparatus and the methods used to rear germfree animals were described previously by the writer [2, 3]. Control experiments were carried out on ordinary animals of the same age (17 guinea pigs), receiving a sterile diet similar to that given to the germfree animals, and also on animals receiving an unsterile diet (130 animals). Selye's [10] model of aseptic inflammation, the "pocket granuloma," as modified by V. S. Zueva, was used. An injection of 25 cm^3 air was given into the subcutaneous cellular tissue in the interscapular region, and 1 ml of a 10% suspension of mustard in mineral oil was then given through the same needle. The mustard suspension was sterilized in an autoclave. The inflammatory reaction was studied on the first, third, and seventh days of development. At postmortem examination on the animals at these times the capsule of the inflammatory focus was separated from the underlying healthy tissues, and the volume of exudate and the size and weight of the capsule were measured. The number of leukocytes per ml exudate was counted. A differential cell count was carried out on films of exudate stained by Giemsa's method. Histological sections were stained with hematoxylin-eosin and by Van Gieson's method. The cell composition of the inflammatory focus was studied under the microscope. For quantitative analysis of the development of each component of the focus separately, the thickness of the leukocyte barrier and of the connective-tissue capsule was measured by means of a type MOV-1-15 ocular micrometer at four different places in each of three sections by the method of Taylor et al. [11]. At each period the mean values for the corresponding experimental group were determined and the significance of the differences assessed. The barrier capacity of the inflammatory focus also was determined. For this purpose, 500 million bacterial cells of an 18-h culture of staphylococci of the "Zhaev" strain in 0.25 ml physiological saline were injected into the focus 15 min before the animal was due to be sacrificed, and blood cultures on Petri dishes with agar were subsequently prepared. This dose was determined beforehand as the minimal dose which, when injected into a focus of aseptic in-

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TABLE 1. Thickness of Leukocyte Barrier and Connective-Tissue Capsule in Focus of Aseptic Inflammation (in mm) in Germfree and Control Animals ($M \pm m$)

Inflammatory structures	Time of investigation (days)	Germfree animals	Control animals	P
Leukocyte barrier	1-st	0,056±0,0058	0,278±0,001	<0,001
	3-rd	0,101±0,012	0,376±0,002	<0,001
	7-th	0,093±0,028	0,541±0,04	<0,001
Capsule	1-st	absent	absent	—
	3-rd	»	0,460±0,019	—
	7-th	0,251±0,012	0,987±0,086	<0,001

flammation in an ordinary animal would cause the appearance of single bacterial cells in the blood (the "minimal aggressive dose") [1].

EXPERIMENTAL RESULTS

Inflammatory changes in the germfree animals were slower to develop and less marked in degree than in ordinary animals. External examination showed that the inflammatory focus in ordinary animals was of considerable size. This difference was seen particularly clearly during the first 24 h. When the inflammatory focus in germfree animals was examined at autopsy, the degree of hyperemia was slight, and macroscopic evidence of the formation of a focus appeared only on the third day. In the germfree animals during the first day of inflammation fixation of mustard particles on the inner surface of the "pocket" was not observed, as it was in ordinary animals, and there was no exudate. A trace of exudate (not more than 0.2 ml) appeared on the seventh day of development of inflammation. Formation of a focus in the control animals was observed during the first day. At autopsy exudate (about 1 ml) was discovered, and its amount increased at subsequent periods up to 3-5 ml. In the ordinary animals the exudate consisted mainly of neutrophils (on the first day), but gradually its content of mononuclear cells increased. After excision of the capsule, the very slight development of granulation tissue in the germfree animals was noticeable, whereas in the ordinary animals numerous bands of connective tissue with newly formed blood vessels were visible. Externally, the hyperemia around the focus of inflammation was slight in the germfree animals by contrast with the ordinary guinea pigs. Examination of the wall of the inflammatory focus from germfree animals under the microscope revealed infiltration by far fewer cells than in the control animals. This was particularly conspicuous during the first day. At this time, minimal infiltration was observed in the germfree animals and no leukocyte barrier had yet been formed. Next to the zone of necrosis, containing the products of cell destruction, there was a zone of healthy tissue, in which infiltration was slight in degree, and which contained a very few cells at the boundary of the focus. In the control animals at this time, marked infiltration and a clearly defined leukocyte barrier, consisting mainly of neutrophils, were visible. Examination of the cell composition of films of exudate at this period showed the presence of 20.4% neutrophils, 4.6% lymphocytes, and 72.4% polyblasts in the germfree animals, compared with 84% neutrophils, 5% lymphocytes, and 11% polyblasts in the ordinary animals. Predominance of polyblasts in the exudate from the germfree animals was relative, having regard to the extremely small total number of cells. For instance, in 100 fields of vision in impression films taken from the inflammatory focus in germfree animals, on the average 200 cells were counted, but the number in ordinary animals was 7.5 times larger (mean 1500 cells). Later, the increase in size of the leukocyte barrier and the growth of the connective-tissue capsule were considerably more marked in the ordinary animals than in the germfree guinea pigs. The inflammatory response of the control animals receiving a sterile diet was not significantly different from that in ordinary animals. The results of measurement of the thickness of the leukocyte barrier and connective-tissue capsule are given in Table 1.

Determination of the barrier capacity of the inflammatory focus on the first day showed that it was much lower in the germfree animals. The results of blood culture tests after injection of staphylococci into the focus, for instance, revealed abundant growth of microorganisms in the germfree animals (up to 2500 bacterial cells/ml blood), whereas only solitary bacteria were seeded from ordinary animals. In the subsequent periods of inflammation, permeability of the focus for bacteria fell appreciably in the germfree animals (only solitary bacteria were seeded on the seventh day). At this period, no bacteria were found in the blood of the ordinary animals.

Data in the literature on inflammation in germfree animals are very few in number and contradictory in nature [6, 8]. Moreover, they tend to be purely descriptive in nature. An attempt was therefore made to

estimate the development of the inflammatory reaction in germfree animals quantitatively, using Selye's method [10]. The results showed that development of the inflammatory reaction in germfree animals is considerably retarded both in time and in the degree of intensity of its various manifestations, compared with that in ordinary animals. This was particularly clearly reflected in migration of neutrophils into the focus of inflammation. The results of a study of the mechanisms of formation of the inflammatory focus [4] show that a neutrophil reaction is the earliest cellular reaction in inflammation. The results indicate a retarded leukocyte reaction in germfree animals in response to an inflammatory stimulus. This is in agreement with results obtained by other workers who observed less marked development of inflammatory changes in germfree animals [5, 8, 9]. In this study of the causes of inadequate development of the inflammatory reaction in germfree animals, an attempt was made to eliminate as far as possible the influence of dietary deficiencies. The control experiments revealed no effect of sterilization of the diets, because, even allowing for the destructive action of sterilization on the constituents of the diet, it still remained adequate for the germfree animals.

The experimental results demonstrate the considerable retardation of development of aseptic inflammation in germfree guinea pigs compared with ordinary animals, both in the time and in the degree of intensity of its individual manifestations.

LITERATURE CITED

1. V. M. Berman and E. M. Slavskaya, in: Problems in Age Reactivity in Infectious and Immunologic Processes [in Russian], Leningrad (1955), p. 48.
2. G. I. Podoprigora, in: Abstracts of Proceedings of the 13th Conference of Junior Research Workers at the Institute of Normal and Pathological Physiology [in Russian], Moscow (1967), p. 42.
3. G. I. Podoprigora and A. L. Karpovskii, in: Abstracts of Proceedings of the 14th Conference of Junior Research Workers at the Institute of Normal and Pathological Physiology [in Russian], Moscow (1968), p. 52.
4. A. M. Chernukh, The Infectious Focus of Inflammation [in Russian], Moscow (1965).
5. G. D. Abrams and J. E. Bishop, Arch. Path., 79, 213 (1965).
6. G. I. Brody and J. E. Bishop, Arch. Path., 76, 126 (1963).
7. T. D. Luckey, Germfree Life and Gnotobiology, New York (1963).
8. M. Miyakawa, N. Isomura, H. Shirasawa, et al., Acta Path. Jap., 8, 79 (1958).
9. R. Rovin, in: M. Miyakawa and T. D. Luckey (editors), Advances in Germfree Research and Gnotobiology, Cleveland (1968), p. 180.
10. H. Selye, in: Mechanisms of Inflammation. Symposium, Montreal (1953).
11. P. Taylor, C. Tejada, and M. Sanches, J. Exp. Med., 126, 4 (1967).