LOCAL SCHWARTZMANN'S PHENOMENON IN GERMFREE GUINEA PIGS

O. V. Chakhava, B. B. Pershin, and N. M. Shustrova

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To reproduce the local Schwartzmann phenomenon, heat-killed cells of Citrobacter or Escherichia coli were injected intradermally and intravenously into 11 germfree guinea pigs, two guinea pigs monoassociated with Citrobacter, and 25 control guinea pigs. All the germfree animals were refractory to the phenomenon, whereas 20 of the 25 control animals gave a positive reaction. A zone of infiltration at the site of intradermal injection was present in all the control guinea pigs but in none of the germfree animals. The possible primary and secondary mechanisms of suppression of the Schwartzmann phenomenon in the absence of the normal microflora are discussed.

KEY WORDS: Schwartzmann phenomenon; germfree animals.

Investigation of the Schwartzmann phenomenon is necessary primarily because of its similarity with hemorrhagic-necrotic tissue lesions in various infectious diseases [4, 12].

"The discovery of this phenomenon led immunologists into a blind alley; although very similar to reactions of increased sensitivity, it clearly was not immunological in nature" [5]. However, experimental evidence of the immunological nature of the trigger mechanisms of the phenomenon was obtained in some laboratories.* It was shown that the Schwartzmann phenomenon is completely absent in rabbits if a mixture of purified endotoxin and monospecific serum is used for the reacting injection [14]. A similar effect was obtained with guinea pigs injected with anti-gamma-globulin serum before the reacting injection of Escherichia coli lipopolysaccharide [11]. The phenomenon could also be prevented by passive immunization with O-antiserum (19S-immunoglobulin), clearing the blood rapidly from circulating endotoxin. The "nonspecific" nature of the Schwartzmann phenomenon, as was previously considered, was shown to be apparent and due to the presence of common antigens between unrelated species of bacteria [6].

After inducing the Schwartzmann phenomenon by chemical haptens (neoarsphenamine, etc.) after preliminary sensitization of animals, Frey et al. [7] concluded that the mechanisms of delayed hypersensitivity and of the Schwartzmann phenomenon are very similar.

The hypothesis was put forward that sensitivity to the Schwartzmann phenomenon is the result of sensitization of the organism by the symbiotic microflora [13]. In this connection it is interesting that germfree mice proved more resistant to the lethal action of E. coli endotoxin of murine origin than ordinary animals of the same genetic line [8].

The information given above, on the one hand shows the immunologic nature of the Schwartzmann phenomenon, and on the other hand it confirms the view that increased sensitivity to endotoxins of gram-negative bacteria is the result of prolonged sensitization of the organism by antigens of bacteria of the normal

* The external manifestation of the reaction and the pathophysiological changes of the phenomenon are surveyed in [1].

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.)
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TABLE 1. Local Skin Reaction and Schwartzmann Phenomenon in Germfree, Monoassociated, and Ordinary Guinea Pigs

Group of animals	Age (in days)	Bacteria administered	Skin reaction*			Result†	Total
			I	H	N		
Germfree	34—42	0-77	—	—	—	0/4	
Germfree	26—38	Vi-strain	—	—	—	0/7	
Monoassociated	26—32	Vi-strain	—	—	—	0/2	0/13
Ordinary, or control, same age as experimental animals	34	0-77	+	—	—	0/1	
	34	0-77	+	+	—	1/1	
	33	0-77	+	—	+	1/1	
	21—28	Vi-strain	+	—	—	0/3	14/18
	31—41	Vi-strain	+	+	—	3/3	
	31—41	Vi-strain	+	+	+	9/9	
Ordinary, not corresponding in age to other two groups of animals	102	Vi-strain	+	—	+	2/2	
	>180	Vi-strain	+	+	+	3/3	
	>180	Vi-strain	+	+	—	1/1	6/7
	>180	Vi-strain	+	—	—	0/1	

*I — firm infiltration easily detected by palpating skin; H — hemorrhages in skin; N — necrosis of skin.
† Numerator gives number of guinea pigs with Schwartzmann phenomenon; denominator gives total number of guinea pigs.

microflora [8, 13].

The object of this investigation was to study the local Schwartzmann phenomenon in experiments on germfree and ordinary animals.

EXPERIMENTAL METHOD

Germfree guinea pigs were obtained and reared, and their germfree status was established as described previously [2]. Monoassociated guinea pigs were obtained by associating germfree newborn animals with Citrobacter, strain No. 5396/38, containing Vi-antigen ($10 \cdot 10^7$ bacterial cells per guinea pig, one dose given with the food). To reproduce the Schwartzmann phenomenon, heat-killed (70° C, 1 h) suspensions of the above-mentioned bacteria or of E. coli serotype 0-77, isolated by the writers from feces of ordinary guinea pigs, were used. For the preparatory injection 0.05 ml ($4 \cdot 10^9$) of a suspension of bacterial cells was injected intradermally, and 24 h later the reacting injection was given — 0.5 ml ($4 \cdot 10^9$) of a suspension of bacterial cells intravenously. The reaction was read 24 h after the second injection and was regarded as positive if hemorrhages and (or) necrosis of the skin appeared at the site of the intradermal injection after intravenous injection of the same bacteria [6]. The presence of erythema and of a palpable zone of infiltration with edema alone was regarded as absence of the phenomenon, for changes of such a nature can take place even before the intravenous reacting injection.

EXPERIMENTAL RESULTS

The results of experiments in which attempts were made to reproduce the Schwartzmann phenomenon by the use of a suspension of killed bacteria as the antigens are summarized in Table 1. Altogether 13 gnotobiotic animals (11 germfree and two monoassociated) were used. The number of Citrobacter cells per gram of feces from the latter reached 10^9-10^{11}. As Table 1 shows, none of the 13 experimental guinea pigs gave a cutaneous reaction: not only the basic features of the phenomenon, namely hemorrhages and necrosis, were absent, but no infiltration was found at the site of the intradermal injection. The absence of the phenomenon in the monoassociated animals can be attributed to the fact that the monoflora usually does not exert a strong enough sensitizing action on gnotobiotic animals [8].

Of the 25 control guinea pigs, the phenomenon was reproduced in 20 and infiltration and edema at the site of the intradermal injection occurred in all control animals even if the phenomenon was negative. In this connection it is important to note that a local reaction after the preparatory injection is regarded as an immunologically specific component of the Schwartzmann phenomenon [13].

It is interesting to note that all the gnotobiotic animals reacted to the procedure identically (negatively), whereas among ordinary animals individual variations in the manifestation of the local skin reaction and the phenomenon as a whole were observed.

When these facts are discussed it must be remembered that germfree animals differ from ordinary not only in the absence of a normal microflora: microbiological sterility evokes a number of primary and secondary interconnected changes in various organs and systems of the gnotobiotic animal [3]. Some of these changes, for example, hypercorticism and hyperplasia of lymphatic tissue, may perhaps have played a role of considerable significance in the cutaneous areactivity of the experimental guinea pigs. The blood-clotting system, to which some workers have attached decisive importance in the realization of the Schwartzmann phenomenon [1], functions perfectly normally in germfree animals kept on a balanced diet [10].

Whatever the direct mechanism of the phenomenon now revealed, it is clear that the primary cause of the refractoriness of the germfree guinea pigs to the Schwartzmann phenomenon is absence of interaction between the organism as a whole and the normal microflora. For the same reason, evidently, the reaction of delayed hypersensitivity is depressed in germfree animals [9].

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