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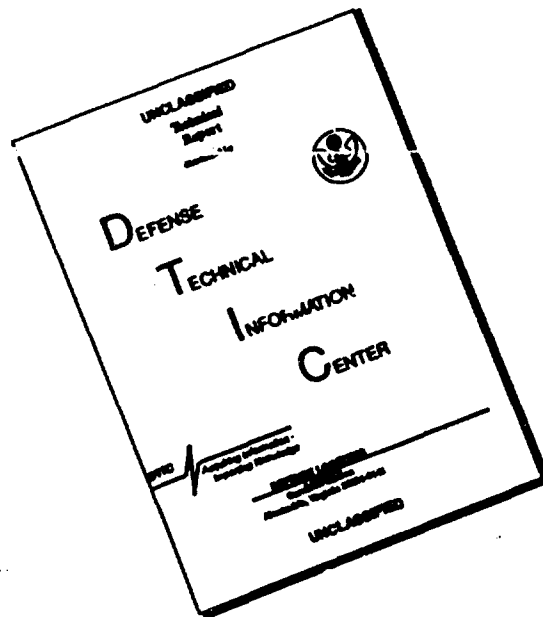
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**DYNAMICS OF THE IMMUNOALLERGIC AND MORPHOLOGICAL REACTIONS
IN EXPERIMENTAL VACCINATION AGAINST ANTHRAX**

Following is the translation of an article by E. M. Shlyakhov, I. G. Shroyt, and T. A. Burdenko, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 3, 1965, pages 106--111. It was submitted on 1 Aug 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.

The administration of the STI anthrax vaccine to sensitive animals (guinea pigs, rabbits, sheep) causes an immunological reorganization, in particular a sensitization of the tuberculin type, made apparent in 1957 by means of setting up cutaneous-allergic tests with the adequate allergen "anthraxin" (Shlyakhov, 1958, 1960, 1961, 1964; Shlyakhov and Shvarts, 1962, 1964; Shlyakhov and Shroyt, 1964). Another aspect of the postvaccinal reorganization in anthrax -- immunomorphological -- has been dealt with in a comparatively small number of works (Chalisov and Tamarin, 1946; Gefen and Gordon, 1961; Gusman and Migulina, 1963). These works make it possible to form concepts concerning the dynamics and nature of cellular processes in experimental anthrax vaccination, depending on the species of animal, vaccines used, and the methods of their application.

As it follows from what has been stated above, the immunoallergic and morphological reorganization of the organism of vaccinated animals has been studied very isolatedly. Nevertheless these phenomena are no more than two sides of a single process. Therefore, we considered it necessary to carry out a combined study of anthraxin tests and the cellular reactions in vaccinated animals, that is, exposing the morphological resonance of the sensitization process.

Besides the generally accepted methods of morphological investigation, we took into consideration the dividing and dystrophically changed cells of the spleen, and also carried out a quantitative determination of the plasmatic reaction in the various structures of this organ.

It is known that the mitotic activity of the cells of the spleen reflects the immunological state of the organism (Zalkind, 1958, 1964, and others), and the profoundness of the process of cellular alteration depends on the biological properties of the antigen. It has been shown that both of these processes are interrelated (Shroyt, 1963; Shroyt and Koslyuk, 1964).

For the investigation we used guinea pigs weighing 300--500 grams. Two groups of animals (270 and 184 pigs) were vaccinated under the skin of the right groin correspondingly with 40 million and one million spores of the STI-1 anthrax strain.

The tests with anthraxin (chemical) were set up in separate groups of animals in 2, 5, 7, 15, 30, 60, and 115 days following vaccination. The reaction was considered after 24 hours and evaluated according to the scale which developed (Shlyakhov, 1964), after which the animals were destroyed.

For the purpose of morphological investigation, fragments of the spleen and the regional (inguinal) and distant (bifurcate, axillary, mesenteric) lymph nodes were fixed in 10% neutral formalin and Carnoy's fluid, and poured in paraffin. The sections were stained with hematoxylin-eosin, according to Brash and according to Feulgen.

For calculating mitoses we used a 10X eyepiece, equipped with a rectangular diaphragm with an area of 15 mm², and a 90X immersion objective. The calculation of the mitotic activities of the cells were carried out: a) in 200 fields of vision within the limits of the light centers of the follicles, b) in 200 fields of vision throughout the entire preparation (in the red and white pulp). All the dividing cells were attributed to the four phases of mitosis. At the same time we considered the number of dystrophically changed cells (pyknosis, corrugation, vacuolization, etc.). When investigating the preparations stained according to Brash, we conducted the calculation of pyroninophilic cells in 300 fields of vision separately in the light centers of the follicles, in the perifollicular zone and the red pulp according to the method proposed by us (Shroyt). The indices of mitotic activity and pyroninophilia were also studied in a control group on 8 healthy guinea pigs of the same age and weight as the animals used in the test series.

In order to guarantee the greatest objectivity of the evaluation, the results of the investigations were processed mathematically and expressed in numerical indices.

In the course of the first 8 days following the administration of 40 million spores of the STI-1 strain, an inflammatory reaction with an expressed edema developed at the site of inoculation, then the process regressed. Not once was the formation of abscesses or foci of necrosis noted.

In the inguinal lymph node regional to the site of administration of the vaccine an expressed hyperplasia of the follicles and an augmentation of the germ centers were observed. The sinuses were expanded and filled with desquamated cells of endothelium, reticular cells and macrophages. A large number of mitotically active cells appeared both in the follicles and in the brain cords. These processes increase by the 6--8th day following

vaccination. In the course of this same period there appear in the follicles and the brain cords of the lymph nodes a significant number of large cells, rich in RNA, which are regarded as immature elements of a plasmatic order, and also small accumulations of plasmatic cells. Many large cells were noted in the red pulp. The nuclei of these cells were rich in DNA. On the 16th and 31st day following the vaccination the intensity of the hyperplasia process had changed little, the number of cells of a plasmatic order remained significant; along the sinus passages the number of cells with nuclei rich in DNA gradually decreased.

Subsequently (61st and 116th day after immunization) the immunomorphological reaction regressed, the follicles acquired a solid structure, and mitoses became less. At the same time the number of mature plasmatic cells increased.

In the remote (bifurcate, axillary, mesenteric) lymph nodes the symptoms of hyperplasia were expressed to a lesser degree and were somewhat lagging in comparison with the regional node. Thus, the intensity of changes in them on the 6th day following immunization corresponded to the picture observed in the regional node on the 3rd day of the test. The morphological changes in the spleen were similar to the changes in the regional lymph node.

In the lungs, besides the symptoms of hyperplasia of the lymphoid follicles and cellular transformation, in places there was noted a thickening of the interalveolar septa; such sectors often contained cells of a plasmatic order. The immunomorphological reaction of the pulmonary tissue was more expressed by the end of the first month following vaccination.

The changes in the guinea pigs which had received one million spores of the STI-1 vaccine differed from those described above mainly in intensity. On the 6--8th day the symptoms of hyperplasia were expressed very moderately and abated by the 31st day following immunization.

Thus, the immunomorphological reaction in guinea pigs, inoculated with an optimal dose of anthrax vaccine, lasted for a considerable period -- four months. The nature of this reaction was the sum total of the effects of hyperplasia of the reticulo-endothelial system and cellular transformation, and was devoid of any traits which are specific for the anthrax antigen.

For the purpose of a more detailed characterization of morphological processes following the development of postvaccinal immunity, we conducted quantitative-qualitative determinations of the effects of cellular transformation.

As the calculations showed, in the control animals the mitotic activity of the cells of the spleen in the light centers of the follicles was equal on an average to 9.6 for 1,000 cells (from 8.9 to 12.20/00, the number of dystrophically changed cells (with the symptoms of pyknosis, corrugation,

vacuolization, etc.) equaled on the average 8.60/oo, and the number of cells with the symptoms of intensive pyroninophilia (with an evaluation of the intensity of staining at +++ and ++++) in the light centers of the follicles, the perifollicular zone and the red pulp -- 94 per 1,000.

Based on the data presented in tables 1 and 2 it is possible to conjecture on the changes in the quantitative parameters of the three cited forms of cellular changes. From the tables it follows that in the guinea pigs which received 40 million spores of the STI-1 strain the mitotic activity increased from the very beginning of the test and reached its maximum by the end of the month. Simultaneously the number of degeneratively changed cells increased. Both processes were normalized by the end of the second month following immunization. The index of pyroninophilia had become larger on the 3rd day of the test already, increased up to the 6--8th day, and then decreased smoothly up until the end of observation.

In the guinea pigs which received one million spores, the activation of mitotic activity and the index of degeneratively changed cells differed little from the corresponding indices of control animals (the fluctuations were statistically insignificant). The number of pyroninophilic cells increased, but less intensively. At the height of the process of plasmatization the index was identical with that noted on the 3rd day in animals inoculated with 40 million spores.

In order to ascertain the presence of a correlation between the immunoallergic and morphological phenomena, we compared the dynamic levels of the indices of cellular changes with the data of anthraxin reactions. The results of this comparison, which are presented in table 3, testify to a chronologically different evolution of immunological (in the wide sense) reactions of an organism following the administration of antigen. In the spleen of a vaccinated guinea pig the plasmatization of cells developed earliest of all; pyroninophilia reached its maximum already on the 6th day. The lowering of the indices of pyroninophilia which was observed later corresponded in time with the greatest intensity of the processes of cell disruption which set in by the end of a month following immunization. The reaction to anthraxin, both in frequency and intensity, increased dynamically from the earliest periods following immunization and reached its peak on the 16th day. The maximum level of pyroninophilia preceded and, apparently, influenced the subsequent growth in the indices of the tests with anthraxin.

It is noteworthy that the evolution of the level of mitotic activity of the cells of the spleen is considered an intimate and accurate index of immunogenesis. Having increased systematically during the first 8 days following immunization, the indices of mitotic activity decrease somewhat by the 16th day following immunization in order to then reach the peak by the end of a month and to subsequently adhere to the level of the indices detected in the control animals. The decrease in the level of mitotic

activity is apparently connected with the intensification of cell disruption, which is noted by this period. The periods for the appearance of increased cellular destruction correspond with the periods of the mass death of the microbial cells of the vaccine, that is, with the moment of the immunoe-
limination of the antigen by competent cells.

A certain temporary lowering of the index of the anthrax cutaneous-allergic tests, which sets in by a month following vaccination, corresponded, thus, with the greatest expressiveness of the process of mitotic activity and the mass death of cells which are stimulated to the increased vital activity.

Subsequently during the multiplication of part of the immunologically competent cells, the anthrax index levels out somewhat so that after 3--4 months it is decreasing regularly. This drop correlated with the normalization of the indices of both the mitotic activity and the degenerative changes (see drawing).

The increase of the indices of positive tests with anthrax took place parallel with the increase of mitotic activity and behind the increase of pyroninophilia of the cells. This indicates the participation of the process of cell plasmation and their intensified multiplication in the development of a slowed-down, increased sensitivity to the administration of the antigen. Together with this, the synchronism of the described phenomena speaks in favor of the opinion which appraises the slowed-down hypersensitivity as one of the variants of the immunological response of the organism (Demeshek, 1963; Dixon, 1963).

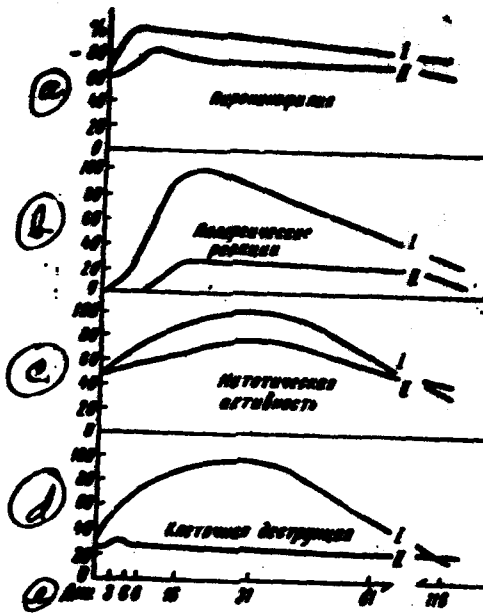
Conclusions

1. Between the evolution of the anthrax reactions and the immunomorphological cellular changes there are specific chronologically outlined bonds.
2. The manifestations of the enumerated reactions represent an expression of the successive implication of different links of the adaptation mechanisms in the single process of the interaction of the organism with the factors of the external medium.

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Periodicity of allergic and cellular reactions (in %) in guinea pigs, immunized subcutaneously with 40 million (I) and 1 million (II) spores of STI-1.

- a. Pironinophilia
- b. Allergic reactions
- c. Mitotic activity
- d. Cellular destruction
- e. Days

Table 1

Indices of cellular changes in the spleen of guinea pigs, inoculated subcutaneously with 40 million spores of the STI-1 strain

Index	Changes (in o/oo) in various days following vaccination						Control
	3rd	6th	8th	16th	31st	61st	
Dividing cells	12.2	14.9	15.4	10.3	19.1	9.4	10.6
Perishing cells	16.4	22.0	27.6	28.7	30.8	11.2	8.5
Cells with intensive pyroninophilia	120.6	154.4	140.3	140.7	127.5	116.6	117.8
							94.0

Table 2

Indices of cellular changes in the spleen of guinea pigs, inoculated subcutaneously with a dose of 1 million spores of the STI-1 strain

Index	Changes (in c/oo) in various days following vaccination							Con- trol
	3rd	6th	8th	16th	31st	61st	116th	
Dividing cells	9.3	11.5	10.6	12.1	14.9	7.0	8.3	9.6
Perishing cells	10.7	7.0	7.0	9.1	8.8	7.5	8.9	8.6
Cells with intensive pyroninophilia	93.4	102.5	122.3	106.2	106.4	108.5	102.9	94.2

Table 3

Indices of the anthraxin tests and cellular changes in guinea pigs, inoculated subcutaneously with 40 million spores of the STI-1 strain (optimal index 100)

Index	Changes in various days following vaccination								Control
	3rd	6th	8th	16th	31st	61st	116th		
Positive tests with anthraxin	3.0	30.0	55.0	100.0	79	91.0	40.0	0	
Intensity of anthrax reaction	4.6	28.5	53.0	100.0	79	60.0	39.0	1.2	
Dividing cells	64.0	78.0	80.0	54.0	100	49.0	55.5	51.3	
Perishing cells	53.2	71.5	89.5	93.2	100	36.4	30.0	28.0	
Pyriminophilic cells	78.0	100.0	91.0	91.0	82	75.0	76.0	60.9	