SOME CLINICAL, HEMATOLOGICAL AND IMMUNOLOGICAL MANIFESTATIONS OF THE EFFECT PRODUCED BY FREUND-TYPE ADJUVANT ON ALBINO RATS

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V. V. Sura, V. A. Kolaev, and I. V. Konstantinova

Institute of Experimental Biology (Director—Assistant Professor Yu. I. Borodin, Scientific Leader—Professor E. N. Meshalkin) of the Ministry of Public Heath of RSFSR, Department of Hospital Therapy (Head—Professor A. A. Demin) of Nobosibirsk and the Department of Experimental Biology (Head—Professor B. B. Fuks) of the Institute of Cytology and Genetics of the Siberian Division of AN USSR, Novosibirsk

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Adjuvants, in the formula proposed by Freund as well as in their various modifications, are widely used at present for production of experimental diseases, in the pathogenesis of which the autoimmune processes are of significance. The nature of the disease produced depends on the nature of the cellular antigen mixed with the adjuvant [2, 3, 6, 7, 8, 19].

At the same time, introduction of adjuvants and even their individual fractions (without cellular antigens) into animals (white rats, hamsters, white mice, etc.) results in formation of hyperplasia of reticulo-endothelial cellular elements in many organs and occasionally in development of interstitial nephritis. Of particular note is the proliferation of plasma cells [9, 17]. Similar changes are also described in the course of experimental vaccination with BCG [4].

Along with these immuno-morphological changes administration of the adjuvant under certain experimental conditions may lead to peculiar, clinically described pathology. Thus, the action of incomplete adjuvant with foreign protein in animals subjected to increased salt content and unilateral nephrectomy, led to a peculiar syndrome (proliferation of endocardial endothelium, intracapillary nephritis, myeloid metaplasia in organs) named endotheliomyeloma [1, 15].

Intracutaneous or subcutaneous injection of adjuvant containing lipid D fraction of the tubercle bacillus leads regularly (in 88% of cases) to arthritis, as well as iridocyilitis, dermatitis and uretritis. In general, the clinical manifestations resembled Reuter's syndrome in humans [11-13, 18]. Development of PPLO infections in emerging arthritis in rats as a result of administration of adjuvant, was not considered to be significant.

The purpose of the present study was to determine the clinical, hematological, and immunological characteristics of the effect of adjuvant under different experimental conditions.

EXPERIMENTAL METHODS

The study was carried out on 104 white rats (65 females and 39 males) 100 to 200 g in weight. A saline oil emulsion, consisting of 1.5 ml lanolin, 8.5 ml paraffin oil, 20 mg BCG (live or heat-killed) and 2 ml of physiological saline or an equal amount of the supernatant fluid, obtained the centrifugation of homogenate of rat kidney cortex in 50% (by weight) physiological saline, was used as an adjuvant. Before preparation of the homogenate the kidneys were carefully washed with physiological saline through the peritoneal part of the aorta in a live, anaesthetized animal. The adjuvant was prepared independently using aseptic technique. These ingredients were carefully mixed at 56° for 15-20 min and 0.5 ml amounts were injected into rats at 9 to 10 day intervals; 95 animals were injected intraperitoneally and 9 subcutaneously. There were 7 subcutaneous injections; most animals received 4 intraperitoneal injections (a small number of rats received 3-6 injections). In a few cases, the last injection was

Fig. 1. Dermatitis, with appearance of skin disease, in a rat injected with an adjuvant.

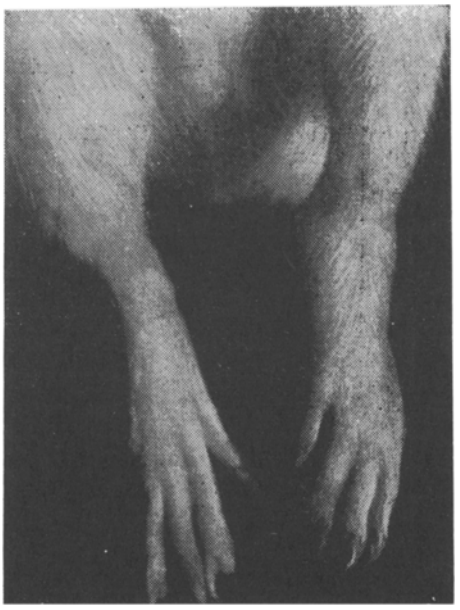

Fig. 2. Arthritis in a rat after injection with an adjuvant.

administered after a long interval of 20-30 days; as a rule, increasing the interval led to a bad general condition of the animals. The length of observation varied from 30 days to 8 months. In addition, 10 rats were sacrified during the first 13 days, 1-2 days after a single intraperitoneal injection of the adjuvant. All animals were divided into 4 basic groups according to the composition of the adjuvant: 46 rats received the adjuvant with live BCG without kidney tissue, 16 received the adjuvant with killed BCG but without kidney tissue, 37 adjuvant with live BCG and kidney tissue homogenate and 5 with killed BCG and kidney tissue homogenate. Nine animals were partially or completely splenectomized, 10 rats received prednizol (5 mg per 100 g of animal).

Laboratory analysis of blood was carried out on all animals (hemoglobin level and differential blood counts; in some, total WBC count, total RBC count, ROE determination and urine were also determined). Total serum protein was determined by a refractometric method and paper electrophoresis was used to establish serum protein fractions; the level of residual blood nitrogen was also determined. Analysis of bone marrow and spleen smears were carried out on sacrificed animals. The serum was tested for antibodies to DNA by the passive hemagglutination method of Boiden and Stavitskii, using the reaction of specific inhibition. Sensitization of tannic acid treated sheep erythrocytes by solution containing DNA (0.25 mg/ml) was carried out in a phosphate buffer at pH 6.4 in cold (4°). Denatured DNA, isolated by P. I. Salganik, using Kirby's method (phenol extraction) from calf thymus and from Escherichia coli in the Laboratory of Nucleic Acids of the Institute of Cytology, has been used in this reaction.

RESULTS

Certain general conclusions were formulated, regardless of the many different clinical symptoms by the animals.

I. Accumulation of fluid in the peritoneum was observed in all animals injected intraperitoneally with an adjuvant (except in rats receiving prednizol), it was more evident 3-4 days after injection. The fluid had the appearance of an exudate; its protein content varied from 3-9%, lymphocytes were the predominating cellular elements. Distinct cellular lupus phenomenon was observed on incubation of the exudate with the blood of healthy rats.

II. Skin changes were apparent in 56 rats; they were characterized by moderate or distinct epilation, dermatitis, at times with skin ulceration (Fig. 1). Hemorrhages were observed in some rats. In addition to the skin changes in 4 rats subjected to intraperitoneal injection of adjuvant without kidney tissue, there developed destruction of joints of the appendages, characterized in 3 animals by a slight swelling, reddening and destruction of function. In one animal only arthritis was characterized by chronic duration, onset of a significant deformity (Fig. 2) and by osteoporosis. This animal also showed iridiocyilitis with deposits.

III. All animals had proteinuria. The protein content in the urine, as a rule, was not excessive, varying within the limits of 0.1-0.9%. These quantities of protein were observed usually 15-22 days after the first injection of an

Titers of Antibodies to DNA in Serum of Rats Injected Intraperitoneally with the Adjuvant with BCG Vaccine

Rat No.	Antibody titer: In reaction with bacterial DNA	Antibody titer: In reaction with thymus DNA	Number of injections of the adjuvant	Time between the last injection of the adjuvant and sacrifice of animals (in days)
	Injection of the adjuvant with live BCG vaccine			
1	1:160	1:40	5	14
3	1:20	–	5	14
6	1:20	1:10	4	42
7	1:20	–	4	42
B/n	1:20	1:20	4	45
15	1:20	1:20	5	14
16	1:40	1:10	5	14
30	1:40	–	4	14
	Injection of the adjuvant with killed BCG vaccine			
2	1:80	1:80	6	16
4	1:40	–	5	40
5	1:20	1:20	6	14
8	1:20	–	6	16
11	1:10	1:20	5	45
12	1:10	1:40	5	40
113	1:160	1:10	6	14
14	1:160	1:80	5	40

adjuvant. Subsequently, not significant increase in proteinuria was observed in some rats (to 1.2-1.5%). The level of proteinuria in rats, injected with the adjuvant containing kidney tissue, did not differ significantly from the level of proteinuria in animals injected with adjuvant alone. Only in one rat, subjected to partial splenectomy and injected intraperitoneally with the adjuvant with kidney homogenate, proteinuria gradually increased and reached 16.5% at the beginning of the fifth month of observation.

IV. Changes in red blood cells were characterized in 41 rats by development of definite normochromic anemia. The tendency for development of anemia was shown usually after 2-3 intraperitoneal injections of an adjuvant and was observed in all groups, particularly in the male rats, receiving intraperitoneal injections of the adjuvant with live BCG. In some animals the hemoglobin level prior to their death decreased to 2 g% and even to 1 g%, and the RBC count to 960,000 per ml. The phenomenon of RBC agglutination was observed in some animals. Four rats in different groups developed erythrocytosis (up to 15,340,000 cells per ml) with formation of erythroblasts in peripheral blood and proerythroblasts in the spinal fluid. No significant changes in RBC have been observed in 59 rats. Significant qualitative and quantitative changes have observed in WBC. After 1-2 injections of an adjuvant there was a neutrophilic leucocytosis, followed by leucopenia with degeneration in the neutrophils (hypersegmentation and fragmentation of the nuclei, vacuolization of the protoplasm); the number of monocytes and plasma cells increased. The phenomenon of thrombo- and leucoagglutination became apparent. The lupus-cell phenomenon appeared in the peripheral blood and the bone marrow. Lupus cells as well as free lupus bodies were encountered (Fig. 3).

Changes in the bone marrow were characterized by a tendency to lymphoidal-plasmocytic, promyelocytic, proerythroblastic reactions, basophilia of cellular elements and activation of phagocytosis by reticuloendothelial cells. Definite leukemic reaction with the shift to hemocytoblasts was observed in some animals. In megakaryocytic development there was a significant decrease in mature megakaryocytes; naked "nuclei" were observed with pycnotic changes, as well as immature megakaryoblasts without fragmenting and degranulated cytoplasm. The cytological changes in the spleen were characterized in general by lymphoblastic and plasmocytic proliferation of cellular elements, increase in mitotic figures, general hyperplasia of cellular elements, and phagocytosis, specifically phagocytosis of erythrocytes.

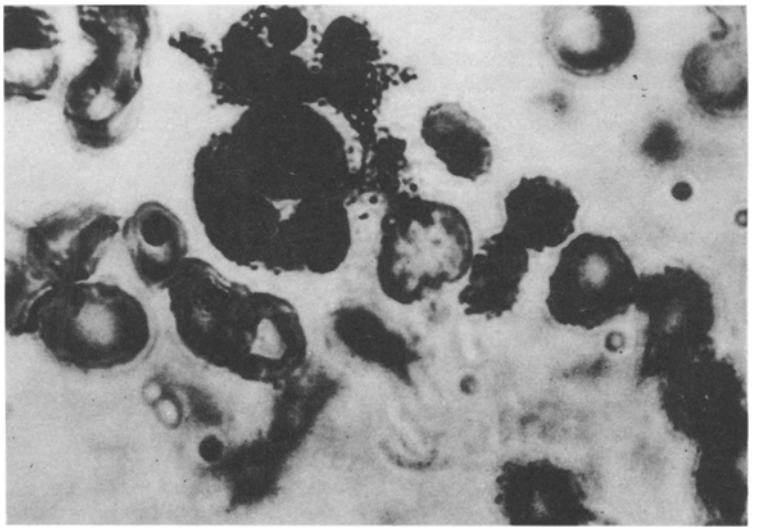

Fig. 3. The rosette phenomenon. Reit-Romanovsky stain. Objective 90 × ocular 10 ×.

The serum protein level was studied in 62 animals. A significant decrease in the protein level (to 5.3 g %) was noted in rats with anemia. In the absence of anemia the serum protein level remained normal or had a tendency to increase, reaching 9-9.2 g% in some rats. In electrophoretic study of serum proteins, carried out on all animals, there was an increase in the level of γ-globulin (up to 20-24%), with a peak in the region of α_1-α_2 globulins (to 38-39.36%), hypoalbumenemia. Changes in the protein fraction were noted 9-10 days after the first injection of the adjuvant (and in some animals on the 2nd-3rd day). They became more pronounced with the progress of the disease. The level of the remaining nitrogen was determined selectively in 26 rats, in which other clinical symptoms—anemia and proteinuria above the normal level—would have been secondary symptoms of kidney insufficiency. The level of the remaining nitrogen did not increase above normal in 20 rats, and it was somewhat above normal in 6 rats, reaching 60-65 mg%.

VI. Serum of 46 animals was analysed for antibodies to DNA; of these 33 showed antibodies to DNA with titers up to 1:160. The use of bacterial DNA led to more consistent and higher titers of antibodies. No significant differences in antibody titers were observed in animals injected with an adjuvant to which has been added either live or killed BCG (see table). Initial appearance of antibodies to DNA (in titers up to 1:10) was recorded only 5 days after the first injection of an adjuvant. Antibodies to DNA in titers up to 1:80 were regularly observed in animals after 200 days or longer following the last injection of an adjuvant. In completely splenectomized rats, antibodies to DNA showed titers of 1:40. It was not possible to establish a clear correlation between the extent of clinical symptoms, the expression of lupus-cellular phenomenon, and the presence of antibodies to DNA and their titers.

VII. The rats injected intraperitoneally with live BCG exhibited a more severe illness. Thus, if in a group of animals in the chronic experiment, injected intraperitoneally with the adjuvant containing live BCG, 18 of the 64 rats succumbed, then only 2 out of 21 rats injected with an adjuvant with killed BCG died. The primary cause of death of most animals was progressive anemia: 3 rats under conditions of progressive starvation, 3 with observable erythrocytosis. When injected with an adjuvant in the course of prednizol therapy there was no observable accumulation of fluid in the peritoneal cavity, no skin disorders, proteinuria was within normal physiological values and blood dyscrasias, including the lupus-cellular phenomenon, were absent or were present to a slight degree. However, all pathological symptoms returned after withdrawal of prednizol.

In this manner, the adjuvant with BCG vaccine leads to development in rats of distinctive symptom complex, characterized by skin damage, proteinuria, hematological changes, significant shifts in serum protein and the appearance of antibodies to DNA. Some of these manifestations—lympho-plasmocytic reaction in the bone marrow and in the spleen, erythro and leucoagglutination, thromboagglutination, the expressed protein shifts—point to significant changes in immunogenic processes, in general, leading to their activation. The presence of erythrophagocytosis, noted in particular in the spleen, can be considered as demonstration of auto-aggressive processes [5]. Of

considerable interest is the appearance of real lupus-cellular phenomenon, established during continuous observation in all animals. This confirms the appearance in the blood or other fluids of the body of the lupus-cellular factor (γ-globulin with 7S sedimentation constant), which is the antibody to the nucleoprotein [10]. Along with this, antibodies to DNA appeared in a significant number of animals used in chronic experiments; these antibodies are encountered with greatest regularity in systemic human lupus and are looked upon as being the most important antinuclear factor [10, 14].

The obtained results allow us definitely to speak about the appearance of antinuclear antibodies in rats. Their formation is probably determined by osmosis of the products of cellular breakdown from the peritoneal cavity, with the background of activation of immunogenic processes. In many of its characteristics (dermatitis with ulcerative destruction of the skin, arthritis in some animals, lupus-cellular phenomenon, antibodies to DNA) the resulting syndrome closely resembles systemic human lupus. The absence of expressed kidney pathology in most rats receiving the protein along with the adjuvant (nearly 5 mg per injection) is probably correlated with the fact that the above dose is considerably higher than that which usually leads to expressed pathology [8]. For example, the inhibitory action of homologous brain extract on the development of experimental encephalomyelitis is well known [16]. One may assume that subsequent studies of "adjuvant diseases" [11] will lead to clarification of the pathogenesis of autoimmune diseases and along with them also of systemic lupus.

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