CHANGES IN THE AUTOIMMUNOLOGIC REACTIVITY OF WISTAR RATS RECEIVING FREUND'S COMPLETE ADJUVANT

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Injection of Freund's complete adjuvant into Wistar rats stimulates autoimmunologic reactivity. An etiologic link is postulated between the increased autoimmunologic reactivity and the reduced resistance of these animals demonstrated previously to the action of a carcinogenic factor.

Experiments have shown that injection of Freund's complete adjuvant (FCA) into Wistar rats 10-12 days before administration of a carcinogen lowers the resistance of the experimental animals to tumor development [1, 2]. A possible connection between this phenomenon and the stimulation of autoimmune processes has been postulated.

It was therefore decided to study changes in the autoimmunologic reactivity of Wistar under the influence of FCA alone and together with a carcinogen.

EXPERIMENTAL METHOD

Experiments were carried out on 78 Wistar rats aged 6-7 months. The carcinogen used was 20-methylcholanthrene (3 mg in 0.3 ml apricot oil per rat). Twenty animals received FCA only, in a dose of 0.04 ml injected into the plantar pad of a hind limb 10-13 days before sacrifice. Fourteen animals received FCA in the same way but, 3 days before sacrifice, the carcinogen was injected intramuscularly (0.2 ml) and into the subcutaneous adipose tissue of the opposite thigh (0.1 ml). Another 10 animals received the carcinogen only, 3 days before sacrifice. Nine rats received FCA only, 64 days before sacrifice and 25 animals acted as the control.

The animals' sera were studied in the gel-diffusion test [4] with saline extracts from various tissues and organs. Extracts of the liver, kidneys, spleen, thymus, retroperitoneal lymph glands, thyroid gland, synovial membranes of the intact limbs and of limbs affected by polyarthritis, the femoral muscles (intact and from the region of injection of the carcinogen), and subcutaneous adipose tissue (intact and from the region of injection of the carcinogen) and also tissue extracts from the region of injection of FCA were studied. The extracts were prepared under standard conditions: 2 parts by weight of 0.9% NaCl solution were added to 1 part of the tissue or organ. The sera were tested separately. The serum was placed in the central well and extracts from the organs and tissues in the peripheral wells. In the direct tests sera and tissues within one experimental or control group were used; in the crossed tests sera of the experimental animals and tissues of the control animals were used. Antigens against Mycobacterium tuberculosis also were used in various dilutions in the tests in the form of a nonsensitizing (but active in allergic skin tests) polypeptide fraction of M. tuberculosis cells (initial concentration 50,000 tuberculin units/ml) and an antigen prepared by boiling BGC vaccine for 30 min (initial concentration 5.0 mg bacterial cells/ml). Sera of the experimental and control animals were tested in parallel.

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TABLE 1. Results of Precipitation Tests of Sera from Control Animals and Animals Receiving FCA 10-13 Days before Sacrifice with Antigens from Liver, Kidney, and Muscle Tissue

Group of animals	Liver			Kidney			Muscle		
	1	2	3	1	2	3	1	2	3
Control	25	22 (0,88)	1:1,47	20	19 (0,95)	1:1,82	14	0 (0,00)	—
Receiving FCA	20	20 (1,00)	1:2,22	10	8 (0,80)	1:1,82	10	5 (0,50)	1:2,22
Receiving carcinogen (3 days previously)	10	5 (0,50)	1:2,00	10	10 (1,00)	1:1,47	10	0 (0,00)	—
Receiving carcinogen (3 days) and FCA (13 days previously)	14	9 (0,64)	1:2,56	9	9 (1,00)	1:2,77	9	9 (1,00)	1:2,13

Legend: 1) total number of sera; 2) number of sera giving positive reaction in absolute and relative (in parentheses) values; 3) mean titer of reaction

EXPERIMENTAL RESULTS

The sera of the control and of all the groups of experimental animals mentioned give negative reactions (absence of precipitation lines) with antigens from tissues of the thyroid gland, thymus, retroperitoneal lymph glands, synovial membranes of the joints, whether normal or affected by inflammatory disease, the subcutaneous adipose tissue whether intact or from the region of injection of the carcinogen, and with tissue antigens from the region of injection of the FCA. Precipitation lines were given with antigens from long tissue and spleen by one and six respectively of the 25 control sera; the results of tests performed on the sera of animals receiving FCA (after 10-13 days) and the carcinogen, separately or together, with these antigens were negative.

The sera of the control animals, like the animals treated with FCA (after 10-13 days) did not give precipitation lines with the M. tuberculosis antigens used in the initial concentration or in dilutions of up to 1:64. The results of tests of the sera of animals killed 10-13 days after receiving FCA and the sera of the control animals with antigens from liver, kidney, and muscle tissues are shown in Table 1.

The results in Table 1 show that the sera of the rats treated with FCA gave positive reactions in more cases than the control sera. This rule is also characteristic of the sera of rats receiving the carcinogen after FCA compared with the sera of animals receiving the carcinogen only. Differences in the frequency of positive tests with antigen from intact muscle between the two groups compared are statistically significant: $P < 0.01$ and $P < 0.001$ respectively. It is also clear from the Table that the sera of the animals receiving FCA alone and FCA followed by the carcinogen reacted, and did so in higher titers, than the control sera and the sera of the animals receiving the carcinogen alone. In the tests with muscle tissue antigens these sera gave a double precipitation line (Fig. 1).

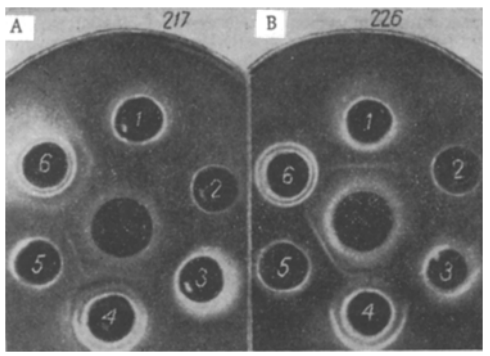

Fig. 1. Results of gel-diffusion test between sera of animals receiving carcinogen alone (A) and carcinogen after FCA (B) and various tissue and organ antigens: 1) antigens from liver tissue, 2) lymph glands, 3) spleen, 4) muscle from intact limb, 5) kidneys, 6) muscles from region of injection of carcinogen. Remainder of explanation in text.

The differences between the sera of the experimental and control animals in the results of the tests with muscle antigens from the region of injection of the carcinogen were the same as in the reactions with antigens from intact muscle. In the latter, however, the precipitation lines were usually less clear.

The results of the tests with sera and antigens from the organs and tissues of animals killed 64 days after receiving SCA were as follows. Five of the nine sera gave precipitation lines with antigens from lung tissue and all nine gave precipitation lines with antigens from spleen tissue. The differences in the number of experimental and control sera re-

acting positively with antigens from lung and spleen tissues were statistically significant: $P < 0.01$ and $P < 0.001$ respectively.

The sera of the animals receiving FCA 64 days before sacrifice reacted with antigens from the liver and kidney tissues just like the control sera. However, the titer of the reactions in the experimental series did not exceed that in the control. For instance, the sera of the experimental animals gave a positive reaction with the liver tissue antigens in a titer of 1:2, compared with titers of 1:2 and 1:4 for the control sera. Neither the experimental nor the control sera reacted with muscle antigens.

The sera of the rats killed 10-13 days and 64 days after receiving FCA reacted positively with extracts of the organs and tissues in the direct tests and gave precipitation lines in the crossed tests also, usually in the same titer. If antigens obtained from the experimental and control animals were placed in the peripheral wells of a group and the reacting serum was placed in the center, the resulting precipitation lines merged into one continuous line. This merging also took place if antigens from different organs and tissues were placed in neighboring wells. This points to the absence of individual or organ specificity in the antibodies formed.

The results of the crossed tests between antigens of the control rats and the sera of animals receiving FCA and the absence of precipitation lines with antigens of M. tuberculosis and also with tissue extracts from the region of injection of FCA are evidence that bacterial antigens played no part in the formation of the precipitates in these tests. These results also show that in the early stages of the response to FCA (10-13 days after its injection) the autoimmunologic reactivity of the experimental animals was stimulated. Injection of the carcinogen did not significantly alter this state, and on the first days it acted when the titer of autoantibodies was increased. These results confirm the view [1, 2] that an increased titer of autoantibodies is one possible factor reducing the antitumor resistance of Wistar rats injected with a carcinogen in the early stages of the response to FCA.

Autoantibodies, by altering the functional state of the tissues, may also influence their antitumor resistance. Cellular immunity, connected in the general view with resistance to tumors, may also be lowered when autoimmunologic reactivity is increased [9, 11, 12].

With the passage of time after the administration of FCA, the autoantibody titer falls. Corresponding to the dynamics of the autoantibody titer, the antitumor resistance of the animal changes after receiving FCA and BCG [3]. The active immunologic principle of FCA is known to be the killed M. tuberculosis cells. By the use of avirulent M. tuberculosis cells (BGC), results indicating that initially irritation and damage to the organs and tissues of the recipient take place, followed by a gradual return to normal, have been obtained [5-8, 10]. The present writers consider that this dynamics of the functional state of the tissues may be partly responsible for the changes in the autoantibody titer and may be one of the factors determining the state of antitumor resistance of the recipient in the various stages of its immunologic response.

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