ROLE OF 7S and 19S ANTIBODIES IN THE MECHANISM OF STIMULATION AND REGRESSION OF LEUKEMOGENESIS INDUCED IN MICE BY RAUSCHER VIRUS

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Irreversible progression of Rauscher virus leukemia in C57BL/6 mice was found to be associated with persistence of production of 7S antibodies against group-specific mouse leukemic antigen (GSA), capable of blocking in vitro the cytotoxic effect of 19S antibodies of the same specificity and strongly stimulating leukemogenesis in vivo. In the regressive form of leukemia a larger production of antibodies against GSA is observed, and antibodies subsequently appear against the type-specific antigen. Cytotoxic 19S antibodies blocked by the 7S fraction also were discovered in the serum of BALB/C mice, which are sensitive to Rauscher virus.

Previous work by the writers has shown that the genetically determined resistance of C57BL/6 mice to the leukemogenic action of Rauscher virus (RV) can be suppressed by means of Freund's complete adjuvant (FCA). Mice infected with the virus only do not develop leukemia, whereas the injection of FCA before or together with RV gives rise either to a reversible (spontaneously regressive) or to an irreversibly progressive leukemia. Investigations of the dynamics of the humoral immune response have linked the mechanism of this phenomenon of stimulation of leukemogenesis by FCA with the appearance of an early peak of antibodies against the group-specific antigen (GSA) of mouse leukemias. It has also been shown that regression of Rauscher's disease in some mice is associated with the development of a second peak of antibodies against type-specific antigen (TSA), whereas no second peak of the immune reaction appears in animals with irreversible progressive leukemia. In the course of these experiments it was found that the cytotoxic activity of the sera of the first peak (anti-GSA) is inversely proportional to their ability to stimulate leukemogenesis in vivo, and on this basis it could be postulated that these sera contain at least two components, bearing an antagonistic relationship to one another.

The object of this investigation was to study the separate properties of 7S and 19S fractions of the sera of mice infected with Rauscher virus together with Freund's adjuvant, because the 19S antibodies are known

TABLE 1. Effect of 7S Fractions of RV–FCA Sera on Leukemogenesis in BALB/c Mice (mean number of plaques in spleen of recipient mice)

Expt. No.	Serum fraction		
	7S of uninfected C57BL/6 mice (control)	7S of RV–FCA mice of first peak	7S of RV–FCA mice of second peak
1	14.8 ± 2.0	53.5 ± 5.1	3.1 ± 0.5
2	7.6 ± 1.1	36.0 ± 5.2	2.3 ± 0.2

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TABLE 2. Cytotoxic Reaction of BALB/c Mice with Isologous Cells of Rauscher's Leukemia (percentage of dead cells)

Expt. Number	Control of serum of uninfected BALB/c mice			Sera on third day after injection of RV			
	whole	19S fraction	7S fraction	whole	19S fraction	7S fraction	blocking effect of 7S fraction
1	18	15	12	19	46 (0,32)	22	80
2	12	13	10	12	35 (0,26)	11	100
3	10	11	10	18	45 (0,38)	16	88

Note. Index of cytotoxicity given in parentheses.

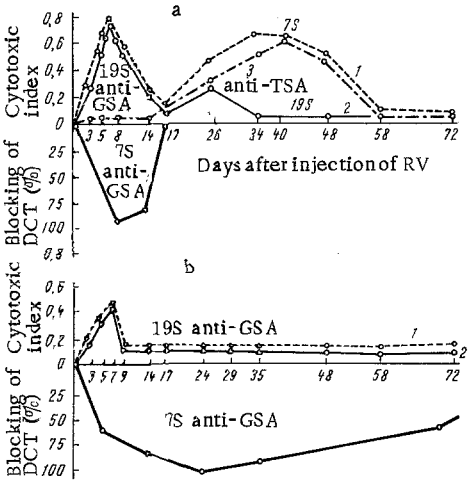

Fig. 1. Dynamics of humoral immune response of C57BL/6 mice inoculated with FCA–RV: a) antibodies of mice with regressive leukemia; b) antibodies of mice with irreversibly progressive leukemia. Ordinate: above abscissa, cytotoxic index of whole serum (1), of 19S fraction (2), of 7S fraction (3); below abscissa, percentage neutralization of DCT of sera of first peak by 7S antibodies (block effect in %); abscissa: days after injection of RV.

to be more effective than the 7S antibodies in cytotoxic reactions in vitro, while the 7S antibodies can induce the phenomenon of stimulation of malignant growth [2, 4, 6].

EXPERIMENTAL METHOD

C57BL/6 and BALB/c mice aged 1-1.5 months were used. The C57BL/6 mice were divided into two main groups: those of group 1 were infected intravenously with whole plasma from leukemic BALB/c mice in a dose of 0.4 ml per mouse; the mice of group 2 received 0.2 ml of the same plasma in a dilution of 1:24. The titer of the RV samples was 10^3-10^4 p.f.u./0.2 ml. Half of the mice of each group were inoculated with FCA (Difco, U.S.A.) simultaneously with the injection of RV, in a dose of 0.1 ml intraperitoneally; each of the four subgroups of mice contained 25-30 animals. The control mice received FCA alone or remained intact. Altogether more than 350 C57BL/6 mice were used in the repeated series of experiments. Blood for the serologic tests was taken from the retro-orbital sinus at various times after infection, and sera from mice of the same group were pooled. The sera was studied by the following methods.

1. The direct cytotoxicity test (DCT) in vitro [1], using whole sera and 19S and 7S fractions obtained by gel filtration on Sephadex G-200, equilibrated with 0.15 M NaCl in tris-HCl buffer, 0.05 M (pH 7.3); in the course of elution the concentration of immunoglobulins was lowered by 10-15 times. Sera treated with 2-mercaptoethanol (2ME), which selectively destroys IgM but does not inactivate 7S antibodies [7], also were used.

2. The presence of "blocking" antibodies in the 7S fractions of the sera was tested in vitro by a method based on suppression of the DCT of the antiserum of 19S fraction by preliminary treatment of the leukemic cells with 7S antibodies (the block effect). The degree of the block was calculated by the formula: $X = (a-b)/(a-c) \cdot 100$, where a is the percentage of dead cells in the reaction with antiserum after incubation with the 7S fraction of normal serum; b the same with antiserum after incubation with the test 7S fraction; and c the same with normal serum after incubation with normal 7S. The fractions of sera used in the tests were first tested for toxicity (with normal syngenic cells and with leukemic cells without complement) and for absence of anticomplementarity.

3. Neutralization of the DCT and the blocking effect by preliminary absorption of the fractions with cells of Rauscher's leukemia, of spontaneous leukemia in AKR mice ($5 \cdot 10^7$ per 0.06 ml serum), and lymphocytes of intact C57BL/6 and BALB/c mice.

EXPERIMENTAL RESULTS

It was noticed earlier that if C57BL/6 mice are inoculated with the same dose of RV and FCA, the outcome of the disease varies among individual animals. If the mice were infected with two different doses of virus, differing by more than one order of magnitude, these differences were more significant: the disease progressed in most animals inoculated with the "high" dose of RV and they died from leukemia by the end of the 3rd month, whereas involution of the leukemic changes was observed in most mice infected with the "low" dose of RV.

Dynamics of Properties of 19S and 7S Fractions of Sera of Mice with Spontaneously Regressive Rauscher's Leukemia. The DCT results (Fig. 1a) show that the 19S antibodies were completely responsible for the first peak of immunologic activity of the sera against GSA. At the beginning of the second peak of immunologic activity (anti-TSA) both the 19S and 7S fractions were cytotoxic, but later the DCT was positive only on account of activity of the 7S antibodies. The blocking-effect method revealed noncytotoxic antibodies in the 7S fractions of the sera of the first peak, capable of significantly blocking the cytotoxic activity of the 19S fractions or of whole sera of the first, but not of the second, peak. Complete exhaustion of the blocking ability of the 7S fractions as a result of absorption by cells of Rauscher's leukemia and of spontaneous leukemia of AKR mice, containing considerably amounts of GSA, confirmed the immunospecificity of the blocking effect of the 7S antibodies. Activity of the whole serum is evidently dependent on the balance between 19S and 7S antibodies: the cytotoxicity of the highly active sera (cytotoxic index over 0.7) could not be blocked by the 7S fraction, but dilution of the same sera made the blocking effect highly significant; the 7S fractions of uninfected mice, as well as those of mice inoculated with RV alone or with FCA alone, did not possess blocking properties. On the other hand, fractions of sera of the second peak from mice inoculated with RV and FCA did not block the DCT of sera of either the first or the second peak.

Properties of 19S and 7S Fractions of Sera of Mice with Irreversibly Progressive Leukemia. The DCT results (Fig. 1b) show that with the sera of the "progressive" mice there was only one very small "early" peak, of short duration, due to 19S antibodies; later the cytotoxic effect was completely absent. At the same time the blocking-effect method revealed the presence of very long-persisting antibodies in the 7S fractions of the serum, blocking the cytotoxic activity of the 19S antibodies against GSA, but not of the sera of the second peak from "regressive" mice. The blocking effect was completely abolished by exhaustion of the 7S fractions by AKR leukemic cells.

Modification of Leukemogenesis in Vivo by 7S Fraction of RV–FCA Sera. Twenty-four hours after the RV injection, the BALB/c mice received an intravenous injection of 0.3 ml of the test fraction of sera of C57BL/6 mice; the effect was judged by the number of colonies in the spleens of recipient mice killed on the seventh day. The 7S antibodies of the "progressive" mice and of the 7S fractions of sera of the first peak of the "regressive" mice (anti-GSA) were found to stimulate leukemogenesis to a highly significant degree, whereas the 7S fractions of the second peak inhibited production of leukemic colonies in the spleen of the BALB/c mice (Table 1). The following general conclusions can be drawn from these results.

The essence of the immunologic mechanism of irreversible depression of resistance of the C57BL/6 mice to RV by Freund's adjuvant is evidently stimulation of the production of 7S antibodies against GSA. These antibodies block the cytotoxic effect of the same specificity and they can strongly stimulate leukemogenesis in vivo. The progression of leukemia is accompanied by cessation of the production of "protective" 19S antibodies and by persistence of the production of "blocking" 7S antibodies, which, by competition, possibly inhibits the development of other immune reactions (antiviral, cytotoxic anti-TSA antibodies, reaction of delayed hypersensitivity).

In the reversible (regressive) form there is a much greater production of 19S anti-GSA antibodies, soon after which production ceases of both 19S and 7S components of the anti-GSA reaction, ordinary (in animals infected with RV only) anti-TSA antibodies appear, and the animals recover. The phenomenon can be regarded as linked with that of "antigenic modulation" (disappearance of GSA from the cell surface) in the presence of cytotoxic antibodies [3, 5]. On the basis of these results it was postulated that the absence of any marked humoral immune response in leukemia, at least in the earliest stages of the disease, may be due, not to the phenomenon of immunologic tolerance or virus-induced immunodepression, but to inhibition (autoblocking) of the cytotoxic 19S antibodies by 7S antibodies. The results of experiments on BALB/c mice, which are highly sensitive to RV, confirm that cytotoxic 19S antibodies, blocked in vitro by the 7S fraction, appear in the serum of these mice taken on the third day after infection, whereas the whole serum is completely inactive in the DCT (Table 2).

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