IN VITRO BLOCKING ACTIVITY AND BIOLOGICAL EFFECT OF IgG (7S-ANTIBODIES) ISOLATED FROM SPLEEN CELLS AND SERUM OF MICE DEVELOPING RAUSCHER'S LEUKEMIA

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Immunoglobulins covering the surface of leukemic cells in the initial stage of Rauscher's leukemia in vivo and detectable in eluates from spleen cells of C57BL/6 and BALB/c mice, and also eluates of serum 7S-antibodies, when tested in vitro, block the cytotoxic effect of isologous and homologous 19S-antibodies against the group-specific antigen of mouse leukemic cells. The blocking antibodies (7S-IgG) isolated from both sources possess similar biological activity by stimulating the development of leukemia in experiments in vivo.

KEY WORDS: leukemia in mice; antitumor antibodies; carcinogenesis and antibodies; antibodies on leukemic cells.

In recent years the study of immunologic factors leading to progression of malignant tumors has been a favorite research topic. The effect of blocking the cytotoxic action of immune lymphocytes with humoral antibodies has been demonstrated in various experimental systems [1, 3, 5, 8, 9] and in certain human tumors [4, 6, 7]. Using Rauscher's leukemia as the model, the phenomenon of blocking the cytotoxic action of humoral antibodies of the 19S-type in vitro with serum 7S-antibodies has been shown [2].

In a previous paper the authors described how it was possible to isolate IgG possessing the properties of antibodies to the surface antigens of leukemic cells from spleen cells and the serum of mice inoculated with Rauscher's virus (RV) mixed with Freund's complete adjuvant (FCA) in the early stage of development of leukemia.

This paper describes the results of a study of the blocking activity in vitro of eluates from spleen cells (ESC) obtained at low pH values and also of eluates of serum antibodies (ESA) isolated by the use of leukemic cells as the immunosorbent and also the effect of their injection in vivo.

EXPERIMENTAL

Heparinized plasma from BALB/c mice with leukemia was used as the Rauscher's virus; the titer of the RV preparations was 10^4-10^5 plaque-forming units in 0.2 ml. An intraperitoneal injection of 0.1 ml FCA (Difco, USA) was given to C57BL/6 mice aged 1.5-2.5 months, and 6 days later the animals were given RV by intravenous injection in a dilution of 1 : 5-1 : 10 and in a volume of 0.2 ml. BALB/c mice were infected with RV (10^{-2}) in the same combination with FCA, or with RV alone. Blood was taken from the retro-orbital sinus starting on the third day after inoculation and continuing until the end of the second week, and the sera of the mice of each group were pooled. To prepare the ESC spleens, from 10 to 30 mice of a particular group at each time were used. The methods of obtaining ESC at low pH values (2.8-2.6) and of preparing ESA by the use of leukemic cells previously fixed with 1% glutaraldehyde as the immunosorbent, as well as the conditions for fractionating the sera on Sephadex G-200, were described in the previous communication.

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TABLE 1. Blocking Activity of ESC from Mice Developing Rauscher Leukemia (M ± m)

Materials investigated by block test	Percentage blocking of cytotoxicity of 19S-fraction of serum	
	from BALB/c mice (4 days after injection of RV+FCA)	from C57BL/6 mice (7 days after injection of RV+FCA)
Control washings (pH 7.2) from spleen cells of mice inoculated with RV+FCA	–	–
ESC of BALB/c mice:		
intact mice	27 ± 4.4	23 ± 6.7
mice with leukemia:		
more than 20 days after injection of RV	20 ± 4.7	15 ± 6.3
6 days after injection of RV	62 ± 6.5	
6-8 days after injection of RV+FCA	78 ± 7.2	
ESC of BALB/c mice (6-8 days after injection of RV+FCA):		65 ± 7.9
absorbed by spleen cells of intact BALB/c mice	72 ± 3.6	
absorbed by leukemic BALB/c cells	6.3 ± 1.7	
ESC of C57BL/6 mice:		
intact mice		12 ± 5.7
10-12 days after injection of RV+FCA	92 ± 6.4	85 ± 10
ESC of C57BL/6 mice (10-12 days after injection of RV+FCA):		
absorbed by spleen cells of intact BALB/c mice	85 ± 7.0	74 ± 9.6
absorbed by leukemic BALB/c cells	12 ± 5.4	9 ± 3.3
absorbed by cells of spontaneous leukemia of AKR mice	17 ± 7.4	14 ± 7.3

TABLE 2. Blocking Activity of Antibodies Eluted after Adsorption of Sera of Mice Developing Leukemia on Leukemic BALB/c Cells (M ± m)

Materials investigated by block test	Percentage blocking of cytotoxicity of 19S-fraction of serum	
	from BALB/c mice (4 days after injection of RV+FCA)	from C57BL/6 mice (7 days after injection of RV+FCA)
Control washings (pH 7.2) from BALB/c leukemic cells previously incubated with sera of mice inoculated with RV+FCA	–	–
Eluates from cells of intact BALB/c mice previously incubated with sera:		
BALB/c (4 days after injection of RV+FCA)	28 ± 3.9	
C57BL/6 (8 days after injection of RV+FCA)		32 ± 6.3
Eluates from leukemic BALB/c mice previously incubated with sera:		
of intact BALB/c	24 ± 4.3	
" C57BL/6		22 ± 3.6
BALB/c (5 days after injection of RV)	64 ± 2.9	
BALB/c (4 days after injection of RV+FCA)	90 ± 7.6	
C57BL/6 (8 days after injection of RV+FCA)		95 ± 5.0
ESA of mice developing leukemia, exhausted with:		
cells of intact BALB/c mice	86 ± 7.4	80 ± 7.5
leukemic BALB/c cells	8 ± 3.3	12 ± 3.3

The blocking activity of the ESC, ESA, and 7S-fractions of the sera was estimated by means of a block test of the inhibition of cytotoxicity of the serum or 19S-fraction of the first peak from mice inoculated with RV–FCA by the method described previously [2]. The degree of the block was calculated by the equation

$$X = \frac{a - b}{a - c},$$

where a is the percentage of dead cells in the reaction with the immune serum after incubation with the 7S-fraction of serum from intact mice or with control "washings" from spleen cells at pH 7.2; b – immune serum after incubation with the 7S-fractions, ESC, or ESA for testing; c – with normal serum after incubation with normal serum or with the control "washings" (pH 7.2) from spleen cells of intact mice. The result was regarded as significantly positive if the cytotoxic reaction was blocked by more than 60%.

The biological activity of the blocking antibodies was investigated by injecting ESC or ESA (heated to 56°C for 30 min) intravenously in a dose of 0.2 ml into BALB/c mice 24 h after the animals received an injection of RV (10-12 mice in a group). The effect was judged by counting the foci of proliferation of the leukemic cells (plaques) on the surface of the spleen of the recipient mice on the 7th day after the injection of virus.

RESULTS

1. Blocking Activity of ESC. The antibodies (IgG) contained in the ESC of BALB/6 mice 6-8 days after injection of the virus and of C57BL/6 mice on the 10th-12th day after infection significantly blocked the cytotoxic reaction of the 19S-fraction or of whole serum taken at the peak of production of cytotoxic antibodies against group-specific surface leukemic antigen (GSSA) (Table 1). The specificity of the block test was confirmed: 1) by the complete removal of the blocking effect after exhaustion of the ESC by Rauscher leukemic cells and by cells of spontaneous leukemia of AKR mice (10^8 cells/0.1 ml), but not by the spleen cells of intact mice ($5 \cdot 10^8$ cells/0.1 ml); 2) by the absence of blocking activity of the same ESC when tested with the serum of C57BL/6 mice taken during regression of Rauscher leukemia and containing cytotoxic antibodies against type-specific RV antigen [2]. The ESC of intact mice and of C57BL/6 mice inoculated with FCA only or with RV only had no blocking properties.

2. Blocking Activity of ESA. The serum IgG obtained not later than the second week after infection, when fixed in vitro on leukemic cells possessed blocking activity against cytotoxic 19S-antibodies against GSSA, but did not reduce the cytotoxic action of antibodies against the type-specific antigen. The blocking properties of the serum 7S-fraction were completely lost after adsorption by Rauscher leukemia cells and by cells of the spontaneous leukemia of AKR mice. IgG contained in the eluates obtained at low pH values from an immunosorbent consisting of leukemic BALB/c mice and possessing the properties of specific antibodies in the indirect immunofluorescence test with Rauscher leukemia cells exhibited significant blocking activity against isologous and homologous 19S-antibodies (Table 2). The blocking effect was completely abolished after exhaustion of the ESA by BALB/c leukemic cells but it was not abolished by adsorption with spleen cells of intact mice. Eluates from cells of intact BALB/c mice, previously incubated with the sera of mice inoculated with RV+FCA, did not exhibit blocking activity in vitro.

3. Stimulation of Leukemia Formation in Vivo by Blocking Antibodies. The results of these experiments (Table 3) showed that injection of eluates of blocking antibodies isolated after adsorption in vitro on leukemic cells from the serum of C57BL/6 mice in the initial stage of Rauscher's leukemia sharply stimulated the production of colonies of leukemic cells in the recipients' spleen by comparison with the effect of

TABLE 3. Stimulation of Leukemia Production in Vivo by Blocking Antibodies (M ± m)

Materials with which BALB/6 mice receiving Rauscher virus were inoculated	Number of plaques in spleen of recipient mice (M± m)	
	experiment 1	experiment 2
ESC:		
of intact C57BL/6 mice	12,6±3,1	6,8±2,2
of C57BL/6 mice (10-12 days after injection of RV + FCA)	33,0±5,4	25,9±3,8
ESA of C57BL/6 mice (7-9 days after injection of RV+ FCA), previously adsorbed in vitro on cells of:		
intact BALB/c mice	14,5±3,6	8,0±3,0
BALB/c mice with leukemia	47,6±6,0	34,6±5,2

inoculation of eluates from cells obtained from intact BALB/c mice and incubated with the same sera. Highly significant stimulation of leukemia production also was observed in mice with developing leukemia receiving ESC compared with the results of injection of ESC into intact C57BL/6 mice.

It can be concluded from the results as a whole that in the early stage of Rauscher's leukemia, blocking antibodies of the IgG class are produced in the body of the infected mice and they can be isolated both from serum and from spleen cells. The blocking activity can be adsorbed from ESC and serum by contact with specifically antigenic leukemic cells, and it can then be detected again in eluates obtained by treating the target cells with acid buffer. The equal immunospecificity of antibodies covering the surface of leukemic cells in vivo at the beginning of the disease (IgG detectable in ESC) and of the serum 7S-antibodies, equally blocking the cytotoxicity of 19S-antibodies in experiments in vitro, and their similar biological activity show that these are identical (at least functionally) immunoglobulins responsible for the progression of the leukemic process. Antibodies isolated from the serum and from the surface of leukemic cells from animals with a developing leukemia can be used to detect the corresponding homologous group-specific surface leukemic antigen in practice as a method of immunologic control to exclude oncorna-virus contamination of tissues intended for use, in particular, for the preparation of living virus vaccines.

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