CYTOTOXIC AND BLOCKING ANTIBODIES IN SERUM AND SPLEEN CELL ELUATES OF MICE WITH RAUSCHER LEUKEMIA DEPENDING ON ITS COURSE

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Functional antagonism of cytotoxic and blocking humoral antibodies belonging to the same class of IgG and directed against type-specific antigen of Rauscher virus was found. Progression of leukemia in C57BL/6 and BALB/c mice inoculated with Rauscher virus and Freund's complete adjuvant was shown to be coupled with the production of 7S immunoglobulin (IgG) antibodies blocking in vitro the cytotoxic effect of 19S antibodies against the group-specific surface antigen of mouse leukemias, and later blocking also 7S antibodies against type-specific antigen. Regression of leukemia in C57BL/6 mice is connected with the cessation of production of both types of blocking antibodies. Complete resistance of C57BL/6 mice to the leukemogenic action of Rauscher virus is brought about immunologically by the production of cytotoxic humoral antibodies against type-specific antigen in the total absence of production of blocking antibodies.

KEY WORDS: Rauscher virus; mouse leukemia; antitumor antibodies.

The object of this investigation was to make a comparative study of the dynamics of activity of cytotoxic and blocking antibodies detected in the serum and spleen cell eluates (SCE) in Rauscher's disease following different types of course: 1) in reversible (regressive) leukemia of C57BL/6 mice produced by injection of Rauscher virus (RV) together with Freund's complete adjuvant (FCA), 2) in irreversibly progressive, but relatively slowly developing leukemia of C57BL/6 mice inoculated with RV-FCA before the age of one month, 3) in rapidly progressive FCA-stimulated leukemia of BALB/c mice.

In conjunction with investigations of the humoral immune response of C57BL/6 mice infected with RV only and remaining completely resistant to the leukemogenic action of the virus, these data will contribute to the analysis of the role of immune responses in the pathogenesis of leukemia.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 and BALB/c mice (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR). Four basic groups of animals were used in the experiments: 1) to produce progressive Rauscher leukemia in C57BL/6 mice, FCA (Difco, USA) was injected intraperitoneally in a dose of 0.1 ml before the age of 30 days, and 6 days later this was followed by intravenous inoculation with RV (whole plasma of leukemia BALB/c mice). The titer of the RV preparation was 10^4-10^5 plaque-forming units/0.2 ml. 2) Regressive leukemia was obtained by infecting C57BL/6 mice at the age of 2-2.5 months with FCA and leukemic plasma in a dilution of 1:10. 3) Some adults C57BL/6 mice were inoculated with RV only. 4) BALB/c mice aged 2 months were injected with FCA in the same dose and with RV in a dilution of 10^{-2}.

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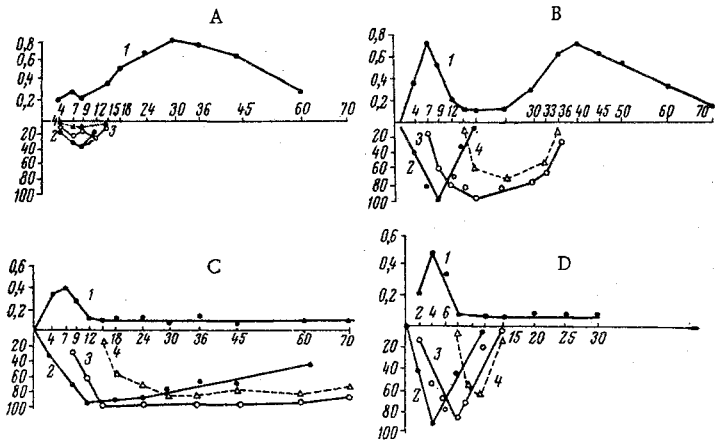

Fig. 1. Dynamics of activity of cytotoxic and blocking antibodies in mice inoculated with Rauscher virus (RV): A) C57Bl/6 mice inoculated with RV only; B) C57BL/6 mice inoculated with RV-FCA, with the regressive form of Rauscher's leukemia; C) C57BL/6 mice inoculated with RV-FCA, with the irreversibly progressive form of Rauscher leukemia; D) BALB/C mice inoculated with RV-FCA, with the progressive form of Rauscher leukemia. Ordinate: cytotoxic index of serum antibodies shown above abscissa (1); blocking of cytotoxic reaction (%) of 19S antibodies against group-specific surface antigen by antibodies from 7S fraction of serum (2) and IgG eluted from spleen cells (3) below abscissa; blocking of cytotoxic reaction (%) of 7S antibody against type-specific antigen by eluates from spleen cells (4). Abscissa: days after injection of Rauscher virus.

In the cytotoxic test, the dynamics of activity of the 19S and 7S fraction obtained by gel filtration on Sephadex G-200 and of the whole sera of the experimental mice was determined. The SCE were prepared by the method described in a previous communication; the presence of antibodies of the IgG class (7S type) was monitored selectively in the indirect immunofluorescence test with monospecific rabbit antibodies against mouse IgG. The blocking activity of the 7S antibodies in the sera and SCE obtained from mice at different times after infection was determined in the block test [2] on the basis of the reduction in cytotoxicity of the same standard sera from C57BL/6 mice of the regressive class, taken at the height of the first and second peaks of their cytotoxic activity.

EXPERIMENTAL RESULTS

Experimental data reflecting the dynamics of activity of the cytotoxic and blocking antibodies in mice with the four different types of course of Rauscher leukemia are illustrated in Fig. 1.

C57BL/6 Mice Infected with RV without Adjuvant (Fig. 1A). A gradual increase in cytotoxic activity of the serum was observed, up to a maximum in the 4th-6th week after infections. Only at the very earliest stages did the cytotoxic antibodies belong to the IGM (19S) class, and later they were of the IGG (7S) class. As was shown previously (1), these antibodies are directed against type-specific antigen (TSA) of the FMR group. The presence of blocking antibodies could not be detected in either the serum or the SCE in the early stage of the immune response, but in later stages the SCE had slight cytotoxic although no blocking activity.

C57BL/6 Mice Inoculated with RV-FCA with Regressive Rauscher Leukemia (Fig. 1B). The direct cytotoxic test showed the presence of two types of immunologic activity in the sera of the "repressor" mice: a transient first peak due to 19S antibodies, and a second peak, longer in duration, in which initially antibodies of both 19S and 7S type possessed cytotoxic activity, but later only IGG. The antibodies of the second peak had the same specificity as the cytotoxic antibodies of mice infected with RV only. The block test revealed antibodies blocking the cytotoxic reaction (CTR) of the standard serum of the first peak (or its 19S fraction) from the 7th to the 12th day after infection in the 7S fraction of the sera. Later no blocking activity of the serum IGG could be determined, but this was not the result of cessation of their synthesis at this time: blocking antibodies of the same specificity were found in SCE for a considerable time (from

the 2nd to the 5th week after infection). Antibodies blocking CTR of the serum of the second peak could not be determined in the 7S fraction of the sera of the "repressor" mice, but blocking antibodies of this type could be found in SCE from the 3rd to the 5th week after injection, i.e., the short period between the two peaks and at the beginning of the second peak of cytotoxic activity of the sera.

C57BL/6 Mice with Irreversibly Progressive Leukemia (Fig. 1C). Only the 19S fraction of sera taken in the early stage of the disease (4th-9th day after infection) possessed a cytotoxic action. 7S Antibodies blocking the CTR of the serum of the first peak were found in the serum of the "progressor" mice from the end of the first week after inspection; they persisted for a long time, and disappeared only shortly before death of the mice from leukemia. Antibodies blocking the CTR of the serum of the first peak were found in the serum of "progressor" mice starting from the third week after infection. SCEs taken in the early stage of the disease blocked only the CTR of sera (19S antibodies) of the first peak, but those taken after 18-24 h also blocked the CTR of the 7S antibodies of the second peak.

BALB/c Mice with FCA-Stimulated, Rapidly Progressive Leukemia (Fig. 1D). The specific humoral immune response of BALB/c mice inoculated with RV-FCA differed from that of "progressor" C57BL/5 mice by the short duration of the period during which: a) cytotoxic 19S antibodies could be detected in the serum (from the 3rd to the 6th day), 2) antibodies blocking CTR of the 19S fraction of the early isologous serum or serum of the first peak from C57BL/6 mice were found in the 7S fraction of the sera and in SCE not later than the 6th-10th day after infection. Only in isolated experiments were the SCE taken on the 10th-12th day able to block the CTR of the second peak from the "progressor" mice. In the later stages, evidently because of the rapidly increasing virus-induced immunodepression of the serum and SCE, no activity was found.

The information on the role of humoral immune factors in the pathogenesis of the various types of response of the animals to injection of leukemia virus described above can be reduced to the following basic principles.

1. Complete (genetically determined) resistance of the C57BL/6 mice to infection with RV is determined immunologically by at least two factors: 1) the production of cytotoxic humoral antibodies (IgG) neutralizing the virus and aimed against TSA of the FMR group; 2) the complete absence of production of blocking antibodies.

2. Temporary depression of the resistance of C57BL/6 mice with the development of leukemic changes, subsequently undergoing "spontaneous" regression, is associated with a combination of several immunologic phenomena. Progression of leukemia coincides with the period of production of blocking antibodies, whereas its regression is connected with their disappearance. In the initial stage, corresponding to rapid progression of the leukemic changes, an immune response develops against group-specific surface antigen (GSSA), including the production of cytotoxic antibodies of the 19S type and 7S antibodies blocking their action. Both disappear quickly from the circulation, but the blocking antibodies are still found on the surface of the spleen cells, a fact that may be connected with the increase in the number of antigen-containing cells absorbing all humoral antibodies on themselves. In the intermediate phase of stabilization of the process, when progression of the leukemic changes is slowed down, the production of another type of blocking antibodies (IgG), aimed against the type-specific RV antigen, also is observed. Their presence in the serum, at a time when it also contains nonblocking (cytotoxic) antibodies of the same class, can be deduced only from indirect evidence: the 7S fraction of the serum taken at this period possesses neither cytotoxic nor blocking activity, whereas indirect immunofluorescence revealed antibodies in them; this suggests that a mixture of cytotoxic and blocking antibodies is present in the circulation. This conclusion is fully confirmed by the discovery in SCE at this period of IgG blocking CTR of 7S antibodies taken at the height of the second peak of immunologic activity. The phase of regression is accompanied by the gradual but complete cessation of production of both types of blocking antibodies and with a sharp rise in the cytotoxic activity of IgG against type-specific antigen. A similar phenomena of disappearance of the blocking effect of the sera against cytotoxic immune lymphocytes during the period of spontaneous regression of the tumor has been described in mice with Moloney sarcoma [3].

3. Complete suppression of the resistance of C57BL/6 mice with irreversible progressive leukemia is connected with persistence of production of anti-GSSA blocking antibodies which, as the experiment shows, can be found for a long time both on the blocked spleen cells and also circulating in the blood stream, probably in the form of an antigen-antibody complex. The discovery of antibodies blocking the cytotoxicity of antitype-specific antigen in eluates from spleen cells, despite their absence in the serum of the "progressor" mice, was completely unexpected. This "preventive" production of blocking antibodies may per-

haps inhibit competitively the synthesis of cytotoxic humoral factors of the same specificity, or alternatively these antibodies may be able to block the cytotoxic effect of the immune lymphocytes. A similar phenomenon, the appearance of antibodies blocking antitype-specific antigen, is sometimes detectable also in BALB/c mice before the development of complete immunodepression. In addition to the functional antagonism established previously between antibodies of identical (or very close) specificity, but belonging to different classes of immunoglobulins (cytotoxic IGM and blocking IgG directed against GSSA), a phenomenon of functional antagonism is also found between cytotoxic and blocking antibodies belonging to the same class of IgG and directed against type-specific antigen.

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