hibitory activities of these McAb: OX4 and 10.2-16 McAb inhibited T-cell proliferation by 50-60%, whereas 13/4 McAb induced approximately 40% inhibition of this response. Normal mouse IgG did not cause inhibition of the response to Ig^{a}-1b AG and PPD.

The results are thus evidence of restriction of Ig^{a}-1b-specific proliferation of August rat T-cells in vitro with respect mainly to products of the RT-1B^{c} subregion of rat MHC. It can be tentatively suggested that allotype-specific T lymphocytes recognize Ig^{a}-1b AG determinants in combination with determinants of RT-1B molecules, revealed by OX4 and 10.2-16 McAb. However, Ir-genetic control and MHC restriction of the T-cells response are linked by definition with polymorphic determinants of histocompatibility antigens. The most likely explanation of the observed inhibition of the T-cell response to Ig^{a}-1b AG is therefore steric screening of polymorphic determinants of RT-1B molecules, by these McAb, restricting the T-cell response to this antigen. A less likely explanation is modulation of the density of the surface RT-1B molecules of AG presenting cells as a result of their interaction with McAb. The results of several investigations have yielded evidence of weak modulation of surface MHC-molecules under the influence of McAb [7]. These two interpretations of the results do not contradict the general conclusion of restriction of T-cell recognition of allotypic Ig^{a}-1b determinants of serum Ig with respect to products of the RT-1B subregion of rat MHC.

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CYTOTOXIC PROPERTIES OF A CONJUGATE OF RICIN A CHAIN AND ANTIALLOTYPIC MONOCLONAL ANTIBODIES

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Immunotoxins (IT), which are conjugates of plant or bacterial toxins with antibodies, are a new class of highly specific pharmacologic agents which may be used for the oriented elimination of a particular population of target cells. Many IT so far obtained have a selective cytotoxic action on tumor cells in culture [8, 10, 13]. The use of IT in an in vitro system, i.e., direct introduction of the conjugate into the body, is particularly interesting. However, the results of such investigations have proved rather contradictory [6, 9].

The aim of this investigation was to obtain a conjugate of ricin A chain (R_A) and of monoclonal antibodies to an allelic variant of the L-chain of rat Ig(Igk-1a) and to study its ability to inhibit growth of cells carrying Ig with the 1a allotype in in vitro systems and on direct administration to rats.

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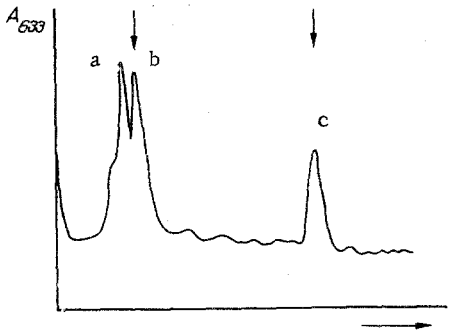

Fig. 1

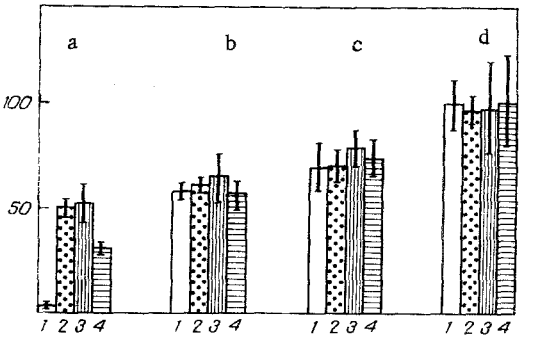

Fig. 2

Fig. 1. Densitogram of stained gel after polyacrylamide gel electrophoresis (acrylamide concentration gradient 7-22%) in a) IT containing 1 R_A molecule to 1 antibody molecule; b) modified antibodies (150 kD); c) isolated R_A (32 kD).

Fig. 2. Cytotoxic action of IT and its component parts. a) Spleen cells treated with IT (anti-1a-R_A), 65 μg; b) treated with monoclonal anti-1a-antibodies, 45 μg; c) treated with R_A, 25 μg; d) with 0.1 M phosphate buffer, pH 7.5 with 0.1 M NaCl. Cells washed off and incubated with rabbit polyclonal monospecific antibodies (1 — anti-1a, 2 — anti-1b, 3 — anti-IgG, 4 — anti-IgM) obtained as described previously [2, 3], and also with normal rabbit IgG for 20 min, and subsequently with fluorescein isothiocyanates-labeled goat antibodies to rabbit Ig (Cappel, USA). Antibodies for incubation were used in a concentration of 100 μg/ml in 50 μl PBS with 1% bovine serum albumin. After each incubation the cells were washed off 3 times with PBS. All procedures were carried out at 4°C. The preparations were examined in the ICM 405 luminescence microscope (Opton, West Germany). The experiment was carried out in three parallel repetitions. Ordinate — number of fluorescent cells among 500 counted.

EXPERIMENTAL METHOD

Monoclonal antiallotypic (anti-1a) antibodies of the IgG1, ^{k} isotype [7] were used.

The isolation of ricin and its isolated subunits and obtaining of a conjugate of antibodies and R_A, with the aid of N-succinimidyl-3-(2-pyridyldithio)-propionate and their analysis were described in detail previously [4].

Activity of the conjugate and antibodies in cell culture was determined by recording inhibition of growth of cells carrying the corresponding antigen. Splenocytes (6×10^6 cells/ml) of heterozygous F_1 rats obtained by crossing MSU females (Igk-1b-allotype) with AGU males (Igk-1a-allotype), in 1 ml of DMEM culture medium (Flow Laboratories, England) with 10% embryonic calf serum (Flow) were treated with preparations of IT, anti-1a antibodies, and R_A for 1 h at 30°C, after which the cells were washed and transferred into flasks (25 cm^2/50 ml, Nunc, Denmark). After 24 h the fraction of cells carrying IgM, IgG, and also Ig with L-chains of the 1b or 1a allotype was determined by the immunofluorescence test (IFT).

To determine the inhibitory action of IT in vivo, monoclonal anti-1a antibodies, R_A, and IT were injected into F_1 hydrids 10 days after birth, after which their cytotoxic action was determined by analyzing the levels of Ig with 1a- and 1b-allotypes. The preparations were injected intraperitoneally in a volume of 0.5 ml in PBS (0.15 M NaCl, 5 mM Na-phosphate buffer, pH 7.5). Antibodies, R_A, and IT were injected in single doses of 100, 50, and 60 μg respectively. Suppressive action of the antibodies also was determined after their injection in two doses each of 50 μg on the 10th and 12th days after birth of the rats.

For quantitative determination of allotypic variants of immunoglobulins in the serum of the quantitative determination of allotypic variants of immunoglobulins in the serum of the heterozygous rats, the radioimmunoadsorption inhibition method was used [12]. Inhibition was carried out to antiallotypic rabbit antibodies fixed to cellulose [1].

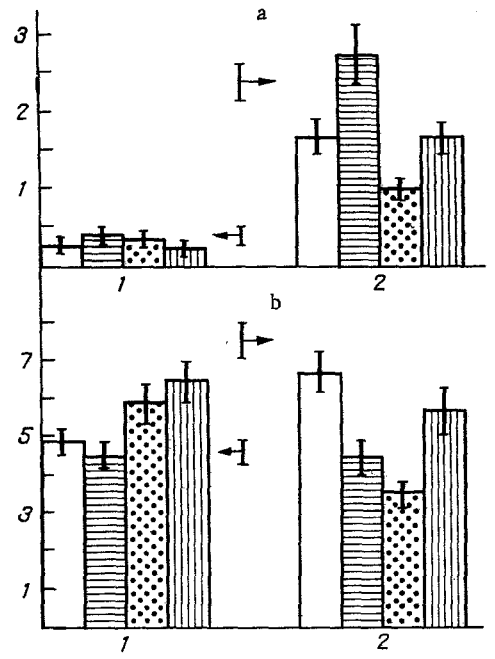

Fig. 3. Concentrations of Ig with 1a- and 1b-allotypes in serum of rats after injection of antibodies (columns from left to right: 100 μg, 50 μg + 50 μg; 50 μg R_A, and 60 μg IT). Concentration of 1a-allotype (a) and of Ib-allotype (b) determined in animals aged 30 (1) and 60 days (2). To each sample for incubation 50 μl of anti-1b-sorbent and 5 μl of anti-1a-sorbent were added. The total volume of sorbent was made up to 250 μl by sorbent with fixed bovine serum albumin. Unlabeled proteins in 0.5 ml PBS with 5% bovine serum were added to the residue of the sorbent and incubated for 1 h at room temperature. To each sample were then added 0.5 ml c of ^{125}I-IgG MSU and ^{125}I-IgG AUG in the same buffer, after which they were incubated for a further 1 h under the same conditions. Specific activity of the preparations labeled by the method in [5] was 500,000 cpm/μg. The sorbent was washed off and its radioactivity counted on a gamma-spectrometer (Minigamma 1275 or RackGamma 1270 for LKB, Sweden). The level of free sorption reached 11,000 cpm. Ordinate, Ig concentration (in mg/ml); arrow indicates Ig level in intact rats of the same age.

EXPERIMENTAL RESULTS

The qualitative composition of the IT thus obtained is illustrated on the densitogram (Fig. 1). The conjugate contained one R_A molecule to one antibody molecule. The conjugation efficiency, i.e., the percentage of antibodies bound with toxin, was 52. Thus the IT preparation (60 μg) was a mixture of conjugate (27 μg), antibodies (25 μg), and R_A (8 μg).

The cytotoxic properties of the IT and its components were determined by studying inhibition of growth of the number of antigen-carrying cells in the culture. Spleen cells of heterozygous rats were treated with IT, antibodies, and R_A, after which the composition of this cell population was analyzed quantitatively by the IFT (Fig. 2). After treatment of the spleen cells with IT in a concentration of 10^{-7} M the number of spleen cells carrying L-chains with the 1a-allotype was reduced by 21 times compared with the number of cells treated with buffer. R_A and monoclonal antibodies (anti-1a) in concentrations of $6 \cdot 10^{-7}$ and $2 \cdot 10^{-7}$ M, respectively reduced the number of cells with the Igk-1a-allotype slightly (by 1.7 times).

The very weak action of IT on cells carrying L-chains with the other allotype (Igk-1b) was virtually identical with the action of isolated antibodies and R_A.

The anti-1a-R_A conjugate thus exerts a selective cytotoxic action on target cells carrying L-chains with the 1a-allotype, but had no effect on cells carrying the other (1b) allotype.

The action of the IT obtained as described above also was tested in an in vivo system. The conjugate was injected into heterozygous (MSU × AUG) rats at the age of 10 days. It was shown that in young rats of this age the concentration of Ig with the 1a-allotype in the serum is not more than $7 \cdot 10^{-3}$ mg/ml, whereas the concentration of Ig with the 1b-allotype was 5 mg/ml. The concentration of Ig with 1b- and 1a-allotypic determinants in the serum of the young rats (aged 30 and 60 days) receiving injections of the preparations were determined by the radioimmunoadsorption inhibition test. Each group studied consisted of seven rats. The results are given in Fig. 3.

In rats aged 30 days partial inhibition of synthesis of the Igk-1a-allotypic variant was observed: 1) after a single injection of 100 μg of antibodies (anti-1a); 2) after injection of 60 μg of the IT preparation. The same decrease in content of Ig with the 1a-allotype as under normal conditions was observed in the rats of these groups 50 days after injection.

In the case of two injections of antibodies (50 μg on the 10th and again on the 12th day after birth) the serum concentration of allotypic variants of Ig in the rats was unchanged compared with normal. It will be clear from Fig. 3 that injection of $1.5 \cdot 10^{-10}$ mole (27 μg) of the conjugate reduced production of Ig with the 1a-allotype to the same degree as injection of $6.7 \cdot 10^{-10}$ mole (100 μg) of anti-1a-antibodies, i.e., IT suppresses the Igk-1a-allotype 4.5 times more effectively than the isolated antibodies composing it.

After injection of R_A into rats aged 30 days no changes took place in the concentration of Ig with 1b- and 1a-allotypes, but an appreciable decrease in the quantity of Ig nonspecific for 1b- and 1a-allotypes was observed in rats on the 50th day after injection of the preparation. The concentrations of Ig with the 1a-allotype was reduced from 2.9 mg/ml in the intact rats to 1 mg/ml in rats treated with R_A, and the concentration of the Igk-1b-allotype fell from 7.8 to 3.6 mg/ml respectively.

The results show that antiallotypic antibodies and IT containing these antibodies, if injected intraperitoneally into young rats aged 10 days, reduced the serum concentration of Ig with the 1a-allotype. The concentration of Ig with the 1a-allotype 20 days after injection of the preparation fell from 0.46 mg/ml in intact rats to 0.26 mg/ml in rats after treatment with antibodies and to 0.24 mg/ml after treatment with IT. The concentration of the Igk-1a-allotype fell to 1.7 mg/ml 50 days after injection of antibodies and IT. In intact rats at this same age the concentration of Ig with the 1a-allotype was 2.9 mg/ml. The results demonstrate the immunospecific activity of antiallotypic IT both in vitro and in vivo. Meanwhile these data indicate that IT are much less effective in vivo if their action is assessed at the serum (1a) Ig level. This may be connected with the small quantities of the conjugate reaching the target B cells in different anatomical situations.

The experimental model described above is promising for the study of the oriented action of immunotoxins in vivo on renewing and dissimulating cell populations. The problem of increasing the effectiveness of the conjugates when administered in vivo is of topical importance [11, 14] and requires further study.

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