MHC RESTRICTION OF ANTIALLOTYPIC T-CELL RESPONSE IN VITRO

A. Yu. Rudenskii, N. V. Gorokhovets, and V. L. Yurin

UDC 612.112.94.017.1:612.6.052

KEY WORDS: MHC restriction; T-cell recognition; Ig allotype

Immune responses of inbred rats to Ig$\varkappa$-1b-allotype type $\varkappa$ light chains of rat serum Ig are controlled by a dominant Ir-gene, linked with the main histocompatibility complex (MHC, in RT-1 rats) [2]. In a study [2] of antibody formation and generation of helper T-cells and effector T cells of delayed-type hypersensitivity in vivo it was shown that August (RT-1^c, Ig$\varkappa$-1a) rats and (WAG × August)F_1 hybrids give a marked response, whereas WAS (RT-1^n, Ig$\varkappa$-1a) rats do not respond to the Ig$\varkappa$-1b-allotype of Ig (Ig$\varkappa$-1b AG) of rats of a 3rd line (MSU/Bl). Later a system of antigen-specific T-cell proliferation in vitro to Ig$\varkappa$-1b AG was developed, in which the Ir-gene-dependent T-cell response of August and F_1 hybrid rats were demonstrated, but T cells of WAG rats did not respond to this alloantigen. As a result of the investigation of the ability of macrophages of the responding August line and the nonresponding WAG line to present Ig$\varkappa$-1b AG to immune T cells of (WAG × August)F_1 rats it was shown that only macrophages in August, and not of WAG rats can induce Ig$\varkappa$-1b-specific T-cell proliferation in vitro [1]. These data, together with the results of hybridologic analysis, which revealed RT-1-linked inheritance of antiallotypic immunoreactivity [2], are evidence of restriction of T-cell recognition of the Ig$\varkappa$-1b allotype with respect to MHC products of the responding August line of rats. A widely used approach to the study of MHC restriction of T-cell recognition is selective inhibition of antigen-specific T-cell proliferation by antibodies to MHC products.

The aim of this investigation was to study the specificity of MHC restriction of T-cell recognition of Ig$\varkappa$-1b allotypic determinants of serum Ig.

EXPERIMENTAL METHOD

Female August and WAG rats (weight 120-140 g) were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, and Fisher (RT-1^1; Ig$\varkappa$-1b) rats were bred at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The IgG_2 fractions obtained from preparations of Ig from August (Ig$\varkappa$-1a AG) and Fisher (Ig$\varkappa$-1b AG) rats by chromatography on DE-32 cellulose (Whatman) [2], and also a purified protein fraction of tuberculin PPD (from "Staaten Seruminstitut"), were used an antigen. Polyclonal WAG-anti-August alloantisera were obtained by immunizing WAG (RT-1^u) rats by skin grafting followed by repeated intraperitoneal injections of spleen cells from August (RT-1^c) rats. The specificity of the sera was tested by immunoprecipitation of surface proteins of splenic lymphocytes from August rats, labeled by the lactoperoxidase radioiodination method [3]. Analysis of proteins precipitated by the alloantiserum, by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate [5], revealed antibodies to products of RT-1 genes of the I (RT-$1A^c$) and II (RT-1B, D^c) classes. OX4 mouse monoclonal antibodies (McAb) (Serotec) to monomorphic determinants of rat RT-1B molecules were used. Mouse anti-1-A^k (10.2-16) and anti-I-E^k (13/4) McAb were isolated from the culture fluid of hybridomas 10.2-16 and 13/4 [6] on sepharose sorbent with rabbit antibodies against mouse Ig. Poly- and monoclonal antibodies to RT-1 products were recorded by indirect cellular radioimmunoassay on monolayers of rat splenic lymphocytes. For this purpose, lymphocytes isolated on a Ficoll–Hypaque density gradient (d = 1.083) were centrifuged (150g, 5 min, 4°C) in 96-well flat-bottomed polyvinyl chloride microplates (Flow Laboratories) at the rate of 10^6 cells per well. The sedimented cells were fixed in the wells with glutaraldehyde (Sigma) in a final concentration of 0.25% over a period of 7 min at 20°C. The resulting monolayers were washed and then incubated with a 1% solution of bovine serum albumin (Serva). Dilutions of anti-

Laboratory of Immunology, All-Union Research Institute of Genetics, Ministry of the Medical and Microbiological Industry of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 5, pp. 565-567, May, 1988. Original article submitted March 18, 1987.

 0007-4888/88/0005-0676$12.50

bodies to RT-1 molecules were added to the monolayers, incubated overnight at 4°C, and washed, after which ^{125}I-labeled rabbit antibodies against rat Ig (to determine rat anti-RT-1 antibodies) or against mouse Ig (to determine mouse McAb) were added to the wells. After additional incubation with the label (2 h, 20°C) the monolayers were washed and cell-bound radioactivity was determined on a "Gamma-Rack II" gamma-spectrometer (LKB, Sweden). Antigen-specific proliferation of T-cells in vitro was carried out as described previously [1], except that dilutions of antibodies and antigens in 50 µl, and also (3-4) × 10^5 immune T lymphocytes in 100 µl of culture medium were introduced into the wells of a flat-bottomed 96-well microplate (Linbro). The results were presented as a percentage of the proliferative response in the absence of antibodies:

$$\text{proliferative response (in \%)} = \frac{E_1 - C_1}{E_0 - C_0} \times 100\%$$

where E_1 denotes incorporation of ^{3}H-thymidine in the presence of Igκ-1b-AB (PPD) and antibodies (in cpm); C_1 background incorporation (without antigen) in the presence of antibodies (in cpm); E_0 incorporation in the presence of Igκ-1b AG (PPD; in cpm); C_0 the background incorporation (in cpm).

Dilutions (concentrations) corresponding to the plateau of binding with the monolayer of target cells (indirect cellular radioimmunoassay), but not inhibiting the background level of proliferation of immune T lymphocytes, were chosen as initial values for all anti-RT-1 antibody reagents.

EXPERIMENTAL RESULTS

To determine the specificity of MHC-restriction of T-cell proliferation in vitro to Igκ-1b AG, WAG anti-August alloantiserum containing antibodies to polymorphic RT-1^c determinants of molecules of classes I and II, and also McAb to determinants of class II RT-1 molecules were used. Mouse anti-I-A^k McAb (10.2-16) and anti-I-E^k McAb (13/4) cross-react with monomorphic RT-1B (homolog of molecules of I-A mice) and RT-1D (homolog of molecules of I-E mice) molecules respectively [4]. OX4 McAb are specific directly to monomorphic determinants of RT-1B molecules. The results of investigation of the inhibitory action of these antibodies on Igκ-1b-specific T-cell proliferation are given in Fig. 1. As the control, we investigated the effect of the same antibodies on PPD-specific proliferation of the same T-cell population.

It will be clear from Fig. 1 that WAG anti-August alloantiserum completely abolishes antigen-specific proliferation of T lymphocytes in vitro both to Igκ-1b AG and to PPD. The inhibitory action of the antiserum is dose-dependent. Normal WAG rat serum does not induce significant inhibition of the response either to Igκ-1b AG or to PPD. These results are evidence of restriction of the antiallotypic response of August rat T-cells, like the response to PPD, with respect to RT-1^c products and they agree with the results of a previous investigation of the Igκ-1b-presenting capacity of August and WAG rat macrophages [1].

McAb to particular products of the RT-1 complex enabled the specificity of restriction of T-cell recognition of Igκ-1b AG to be determined more precisely. It will be clear from Fig. 1 that 10.2-16 and OX4 McAb induced 70-80% inhibition, whereas 13/4 McAb determine a relatively low level of inhibition (about 25%) of the response to Igκ-1b AG. The study of the PPD-specific proliferative response (Fig. 1) revealed a different ratio between the in-

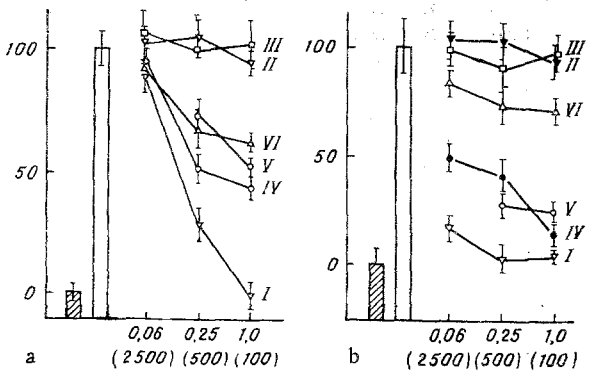

Fig. 1. Inhibition of Igκ-1b-specific (a) and PPD-specific (b) proliferation of immune August rat T lymphocytes in vitro. Abscissa, concentration of antibodies (in µg/ml, reciprocals of dilutions of sera shown in parentheses); ordinate, proliferation response (in %). Shaded columns represent background response of immune T cells in absence of antigen, unshaded columns — level of response without inhibitors and in response to stimulation of T cells by 200 µg/ml of Igκ-1b AG (a) or 30 µg/ml of PPD (b). I) WAG anti-August serum; II) normal WAG rat serum; III) normal mouse IG; IV) 10.2-16 McAb; V) OX4 McAb; VI) 13/4 McAb.

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.

LITERATURE CITED

1. A. Yu. Rudenskii and V. L. Yurin, Byull. Éksp. Biol. Med., No. 6, 719 (1986).
2. V. L. Yurin, A. Uspenskii, and I. M. Meinert, Byull. Éksp. Biol. Med., No. 1, 44 (1977).
3. V. L. Yurin and L. D. Dunaevskaya, Immunologiya, No. 4, 40 (1980).
4. J. Frelinger, L. Hood, and P. Wettstein, Transplant. Proc., 13, 1360 (1981).
5. P. Jones, V. Oi, J. Goding, and L. A. Herzenberg, Curr. Top. Microbiol. Immunol., 81, 115 (1978).
6. U. K. Laemmli, Nature, 227, 680 (1970).
7. B. Pernis, Immunol. Today, 6, 45 (1985).

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

V. A. Rakhmanova, A. G. Tonevitskii, V. V. Cherepakhin, and R. A. Bobreneva

UDC 615.275.2:547.962.3].015.46

KEY WORDS: immunotoxin; ricin A chain; antiallotypic antibodies

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.

Laboratory of Immunology, All-Union Research Institute of Genetics and Selection of Industrial Microorganisms, Ministry of the Medical and Biological Industry of the USSR. Laboratory of Immunology, All-Union Research Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 105, No. 5, pp. 568-570, May, 1988. Original article submitted April 10, 1987.

0007-4888/88/0005-0678$12.50