COMPARATIVE ANALYSIS OF THE SPECIFICITY OF POLYCLONAL AND MONOCLONAL ANTIALLOTYPIC ANTIBODIES AND OF ALLOTYPE-RECOGNIZING T CELLS IN RATS

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Ir-genetic control of immunoreactivity is easily discovered by analysis of the immune response to antigens of limited structural heterogeneity [8]. The authors demonstrated previously Ir-genetic control of the immune response of inbred rats to the alternative (Igκ-Ib) allotype of serum Ig (Igκ-Ib AG) [2], and also investigated the Ir-genetic control and restriction of MHC recognition of Igκ-Ib AG in a system of antigen-specific proliferation of T lymphocytes in vitro [1, 10]. These data, together with information on the structural basis of Igκ-I allelic polymorphism [7] (allelic differences can be reduced to 11 amino-acid substitutions in constant domains of L-chains of κ-type) suggest that the Igκ-Ib allotype is represented by a small number of antigenic determinants, recognized by T and B lymphocytes.

The aim of this investigation was to study the diversity of allotypic Igκ-Ib determinants, detectable by polyclonal and monoclonal antibodies (PcAB and McAB, respectively, and to undertake a comparative analysis of the specificity of T and B lymphocytes which recognize Igκ-Ib AG.

EXPERIMENTAL METHOD

Female August rats (RT-1^c; Igκ-Ia; weight 120-140 g) from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, and Fisher rats (RT-1^l; Igκ-Ib) reared at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used.

IgG_2-fractions obtained from preparations of Ig from August (Igκ-Ia AG) and Fisher (Igκ-Ib AG) rats by chromatography on cellulose DE-32 (Whatman) [2], and also purified protein fraction (PPD) of tuberculin (Staaten Serum Institute) were used as antigens.

Radioiodination of the proteins was carried out with the aid of chloramine T [2]. To isolate the antibodies, sorbents based on Sepharose 4B (Pharmacia), obtained by the method in [4], were used. Anti-Igκ-Ib PcAB were obtained from adjuvant-induced ascites fluids of hyperimmune August rats [2]. The McAB were isolated on sorbents with immobilized affinity-purified rabbit antibodies to rat Ig from the culture fluid of anti-Igk-Ib hybridomas. Anti-$I\text{-}A^k$ McAB were isolated similarly from the culture fluid of hybridoma 10.2-16 [8] on sorbents with rabbit antibodies to mouse Ig.

Anti-Igκ-Ib PcAB and McAB were determined by PEG-precipitation of ^{125}I- labeled Fab-fragments of IgG_2 (Igκ-Ib) [2]. This method also was used to select clones for hybridoma production. Anti-Igκ-Ib hybridomas were obtained as a result of hybridization of spleen cells of August rats immune to Igκ-Ib AG, and of mouse myeloma X63.Ag8.653 cells followed by cloning of the hybrid cells by the limiting dilutions method [6]. The monoclonal nature of the resulting antibodies was established by isoelectric focusing [2].

Competitive solid-phase radioimmunoassay was carried out as follows: Igκ-Ib-aminocellulose immunosorbent (0.4 mg protein to 1 ml of sorbent, 10 mg cellulose to 1 ml) was diluted 200 times with a suspension of aminocellulose (10 mg/ml) in order to achieve 50% fixation of the ^{125}I-anti-Igκ-Ib PcAB to the antigenic sorbent. To 200 µl of a suspension of this diluted sorbent, containing 5 mg/ml of bovine serum albumin and 0.5 mg/ml of normal August rat IgG, 20 µl of a solution (0.5 mg/ml) of unlabeled anti-Igκ-Ib McAB, PcAB, or rabbit antibodies to rat Ig was added, and the mixture was incubated for 1 h at room tempera-

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TABLE 1. Inhibition by Allotypic Anti-Igκ-Ib McAB of Interaction of ^{125}I-Anti-Igκ-Ib PcAB with IgG (Igκ-Ib), Immobilized on Aminocellulose

Parameter	Inhibitor				
	without inhibitor	anti-Igκ-Ib PcAB	C3B9 McAB	3-53 McAB	rabbit PcAB to rat Ig
Binding of ^{125}I-anti-Igκ-Ib PcAB with sorbent, cpm	23 000	600	900	1000	21 500
Inhibition, %	0	97,4	96,1	95,6	6,6

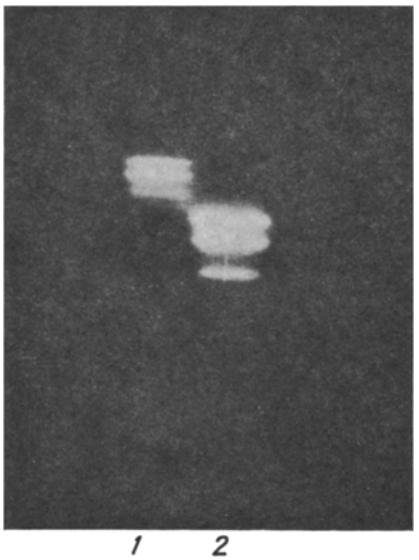

Fig. 1. IEF of monoclonal anti-Igκ-Ib antibodies with development with ^{125}I-labeled Fab (Igκ-Ib). 1) C3B9 McAB; 2) 3-53 McAB.

ture. Next ^{125}I-anti-Ig -Ib PcAB (20 µl, 90,000 cpm) was added, and after further incubation (1 h, room temperature) the samples were washed three times by centrifugation (2000g, 3 min). Binding of ^{125}I-anti-Igκ-Ib PcAB with the Igκ-Ib AG-sorbent was determined on a Gamma-Rack II gamma-spectrometer (LKB, Sweden).

Antigen-specific proliferation of T lymphocytes in vitro was carried out as described previously [1] with the following modifications: 1) as antigen-presenting cells (APC), instead of peritoneal macrophages we used antigen-loaded adherent spleen cells of nonimmune August rats; 2) 10^5 Igκ-Ib AG or PPD-APC and antibodies in different dilutions were introduced into 50 µl of culture medium in each well of a 96-well micropanel, and $(3-4)\cdot10^5$ immune T lymphocytes also were added to 100 µl of medium. The results are presented in the form of indices of stimulation (IS) of ^{3}H-thymidine incorporation by T cells:

$$IS = \frac{\text{incorporation in experiment (cpm)}}{\text{incorporation in control (cpm)}} \quad (M \pm m)$$

EXPERIMENTAL RESULTS

Immunization of August rats with Igκ-Ib AG induced the formation of serum antibodies in high concentrations (up to 3.3 mg anti-Igκ-Ib PcAB/ml) [2]. To study the diversity of the antigenic determinants recognized by these antibodies, rat antiallotypic McAB C3B9 and 3-53 were obtained. The results of isoelectric focusing (IEF) of the C3B9 and 3-53 antibodies,

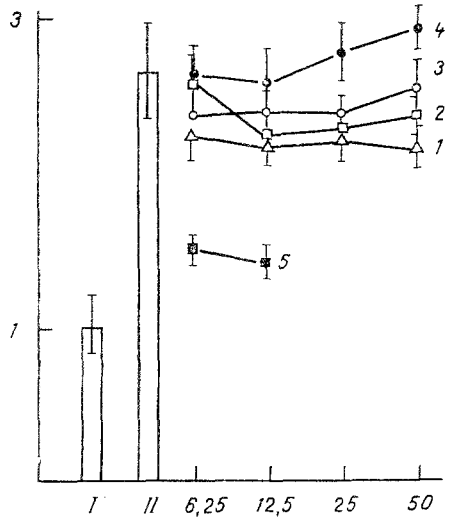

Fig. 2. The effect of anti-Igκ-Ib antibodies on Igκ-Ib-specific proliferation of immune T lymphocytes of August rats in vitro. Abscissa — concentration of antibody (μg/ml); ordinate — IS. I) Response to APC, loaded with Igκ-Ia AG; II) response to APC, loaded with Igκ-Ib AG. 1) anti-Igκ-Ib PcAB; 2) 3-53 McAB; 3) IgC from August rats; 4) C3B9 McAB; 5) 10.2-16 McAB (positive control).

revealed with ^{125}I-Fab (Igκ-Ib), are shown in Fig. 1. Differences in the IEF-spectra of these antibodies, and also the fact that they belong to the same subclass (IgG_1), indicate that C3B9 and 3-53 McAB are products of different clones of B lymphocytes. Since each type of McAB recognizes one antigenic determinant, determination of the level of inhibition of interaction of ^{125}I-anti-Igκ-Ib PcAB with Igκ-Ib AG with the aid of unlabeled McAB can serve as a method of estimating the diversity of serologic Igκ-Ib determinants, detected by PcAB. The results of such an analysis, using the competitive solid-phase radioimmunoassay technique on an aminocellulose sorbent, are given in Table 1.

It will be clear from Table 1 that both McAB induce virtually complete abolition of binding of ^{125}I-anti-Igκ-Ib PcAB with the Igκ-Ib AG sorbent, comparable with unlabeled anti-Igκ-Ib PcAB. Under these circumstances the rabbit antibodies to August rat Ig had no inhibitory action.

Thus within the limits of resolving power of the competitive inhibition method, anti-Igκ-Ib PcAB and McAB are directed toward the same dominant allotypic determinant.

To establish whether Y lymphocytes recognize this single serologically determinable determinant of the Igκ-Ib allotype, we studied the effect of anti-allotypic PcAB and McAB to the proliferative response of T lymphocytes in vitro on Igκ-Ib AG. It was shown previously that T lymphocytes recognize Igκ-Ib AG on the surface of APC in association with rat MHC molecules (RT-1) [1, 11]. In experiments with antigen-specific stimulation of immune T lymphocytes in vitro, not soluble Igκ-Ib AG, but APC loaded with Igκ-Ib AG were used. As Fig. 2 shows, neither PcAB nor McAB specific for the Igκ-Ib allotype affected the level of the proliferative response of the T lymphocytes over a wide range of concentrations. Meanwhile McAB 10.2-16, which cross-react with the monomorphic determinant of class II rat MHC molecules (RT-1B), have a marked inhibitory action. This effect is evidence of the MHC restriction of T-cell recognition of Igκ-Ib determinants with respect to RT-1B (1A) molecules of the major histocompatibility complex.

During the study of competition of anti-allotypic PcAB and McAB with anti-Igκ-Ib T lymphocytes for Igκ-Ib AG determinants on the surface of APC, the complete absence of cross-reactivity of anti-Igκ-Ib T lymphocytes and antibodies was thus found. This result is evidence of a difference in the antigenic determinants of the Igκ-Ib allotype, recognized by T and B lymphocytes, and is in agreement with the results of similar investigations conducted with other protein antigens of nonimmunoglobulin nature [5, 10]. Most probably the Igκ-Ib AG determinants recognized by T cells in association with RT-1B molecules are formed as a result of processing of native domains of κ-chains in APC. Further analysis of T- and B-cell recognition of allotypic Igκ-Ib determinants with the use of κ-chain fragments (Igκ-Ib), and also of synthetic peptides, will help to establish the structure of these "T-cell" determinants and the principles of Ig processing. On the whole, the features of T-cell recognition of allotypic determinants described in this paper may prove to be characteristic also of the T-cell response to other antigenic Ig determinants, including the idiotypic kind.

LITERATURE CITED

1. A. Yu. Rudenskii, Byull. Éksp. Biol. Med., No. 6, 719 (1986).
2. V. L. Yurin and L. D. Dunaevskaya, Immunologiya, No. 4, 40 (1980).
3. R. W. Chesnut et al., J. Immunol., 128, 1764 (1982).
4. P. Cuatrecasas, Nature, 228, 1327 (1970).
5. J. Ellner et al., J. Immunol., 118, 2053 (1977).
6. T. J. McKeam et al., Monoclonal Antibodies, ed. by R. H. Kennet et al., New York (1980).
7. R. S. Nezlin and O. V. Rokhlin, Contemp. Top. Mol. Immunol., 5, 161 (1976).
8. V. Oi et al., Curr. Top. Microbiol. Immunol., 81, 115 (1978).
9. W. E. Paul, Fundamental Immunology, ed. by W. E. Paul, New York (1984)., pp. 451-469.
10. E. M. Shevach, Fundamental Immunology, ed. by W. E. Paul, New York (1984), pp. 71-108.
11. V. L. Yurin et al., Fed. Proc., 45, 499 (1986).