DETECTION OF A PRIVATE STRAIN-SPECIFIC IDIOTYPIC ANTIGENIC DETERMINANT ON ANTIGEN-RECOGNITION RECEPTORS OF MURINE T LYMPHOCYTES

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It has recently been shown that as a rule antibodies against the same antigen have different idiotypes in unrelated individuals within the species [4, 8, 11]. The question of intraspecific analogous crossing of idiotypic antigenic determinants of antigen recognition receptors of T cells reacting to the same antigen has received much less study.

The writers previously obtained anti-CBA-anti-C57BL/6 xenogeneic anti-idiotypic (anti-receptor) sera (AIS), which specifically lysed T lymphocytes of CBA mice activated by transplantation antigens of C57BL/6 mice (T_{act}CBA-anti-C57Bl/6) in a cytotoxic test and specifically abolished the lethal graft versus host reaction in the CBA-anti-C57BL/6 direction. These AIS had no cytotoxicity for T_{act}CBA-anti-BALB/C and did not inhibit lethal homologous disease in the CBA-anti-BALB/C and C57BL/6-anti-CBA direction [3].

The aim of the present investigation was to look for public and private idiotypic antigenic determinants on T lymphocytes of different strains of mice reacting to the same allogeneic cells.

The intension was to detect specificity of binding of rabbit anti-CBA-anti-C57BL/6 AIS with murine T lymphocytes of different strains, activated by antigens of C57BL/6 mice by radioimmunoassay.

EXPERIMENTAL METHOD

Male CBA/H ($H\text{-}2^k$), C3H ($H\text{-}2^k$), AKR ($H\text{-}2^k$), A/Sn ($H\text{-}2^a$), DBA/2 ($H\text{-}2^d$), BALB/C ($H\text{-}2^d$), C57BL/6 ($H\text{-}2^b$), and (CBA × C57BL/6)F_1 ($H\text{-}2^{k/b}$) strains were obtained from the Stolbovaya nersery, Academy of Medical Sciences of the USSR.

The method of obtaining and testing the rabbit anti-CBA-anti-C57BL/6 AIS was described previously [3]. The essence of the method was that rabbits were immunized with immune CBA-anti-C57BL/6 lymphocytes and the resulting serum was absorbed with erythrocytes, liver, serum, and intact lymphoid cells of mice. Rabbit antiserum against mouse lymphocytes (ALS) was obtained by immunizing a rabbit with a mixture of thymus and lymph node cells from intact CBA mice and absorbing the immune serum thus obtained with mouse liver, erythrocytes, and serum [2]. T lymphocytes activated by allogeneic transplantation antigens (T_{act}) were obtained by the method in [10]: 1×10^8 allogeneic thymocytes were injected intravenously into recipients irradiated with a dose of 850 R, and 3 days after the injection a cell suspension was prepared from the recipients' spleens. Special experiments with a set of antistrain sera and serum against mouse T lymphocytes showed that the living cells in the recipients' spleens were T lymphocytes with the donor's phenotype. Cellular radioimmunoassay was carried out by the method described in [1]: 0.2 ml of thrice washed T_{act} (20×10^6/ml) was treated with 0.2 ml of the test rabbit serum in a dilution of 1:4-1:8. Incubation continued for 45 min, after which the cells were washed twice. The cell residue was treated with 1 ml of a solution of ^{125}I-labeled pure donkey antibodies against rabbit IgG with a protein concentration of 0.5 μg/ml, equivalent to 0.5 μCi/ml. Antibodies against rabbit IgG were generously presented by P. Z. Budnitskaya. The residue was resuspended and incubated for 60 min, after which the cells were layered from above on to inactivated nontoxic bovine serum and washed

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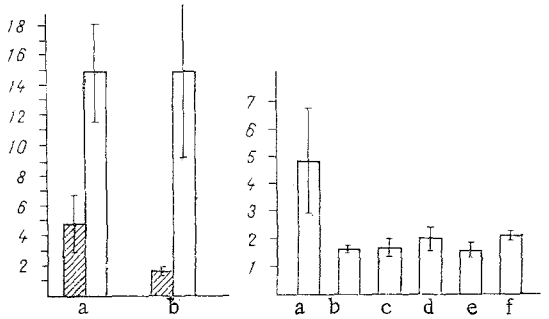

Fig. 1 Fig. 2

Fig. 1. Binding of AIS and ALW with CBA-anti-C57BL/6 T_{act} (a) and CBA-anti-BALB/C T_{act} (b). Ordinate, binding index. Unshaded columns represent binding of ALS (dilution 1:8); shaded columns binding of AIS (dilution 1:4).

Fig. 2. Binding of AIS with T_{act} from different strains of mice obtained by stimulation with antigens from C57BL/6 mice. Abscissa, different groups of T_{act}; ordinate, binding index. AIS and normal rabbit serum were used in a dilution of 1:4. a) CBA-anti-C56BL/6 T_{act}; b) DBA/3-anti-C57BL/6 T_{act}; c) BALB/C-anti-C57BL/6 T_{act}; d) A/Sn-anti-C57BL/6 T_{act}; e) AKR-anti-C57BL/6 T_{act}; f) C3N-anti-C57BL/6 T_{act}.

by centrifugation. This last procedure was repeated twice. The residue was resuspended and 1×10^6 cells were transferred to a tube for radioactivity determination on a J-counter (nuclear Chicago, USA). All manipulations were carried out at 4°C. Binding of radioactivity with T_{act} was used as the measure of binding of the test sera with the cells. For all the samples a binding index was calculated by the formula a/b, where a is the number of counts after treatment of the target cells with experimental antiserum (AIS or ALSO and b the number of counts after treatment of the target cells with intact rabbit serum.

EXPERIMENTAL RESULTS

The results of five experiments to study binding of ALS and AIS with T lymphocytes from CBA mice, activated by allogeneic cells of C57BL/6 and BALB/C mice, are given in Fig. 1. The ALS bound equally with CBA-anti-C57BL/6 T_{act} (binding index 14.8) and CBA-anti-BALB/C T_{act} (binding index 14.7). For the same target cells, the level of binding of AIS with CBA-anti-C57BL/6 T_{act} was 3 times higher. In a population of activated CBA T cells preferential binding of AIS with CBA-anti-C57BL/6 T_{act} was thus observed compared with T lymphocytes activated by a foreign antigen (BALB/C cells). The results of radioimmunoassay are in good agreement with data published previously on selective cytotoxicity of anti-CBA-C57BL/6 AIS for CBA-anti-C57BL/6 T_{act} but not for CBA-anti-BALB/C T_{act} [3]. The next step was to study binding of of AIS with T lymphocytes of different strains of mice activated by allogeneic C57BL/6 murine antigens. The results of four such experiments showed (Fig. 2) that the binding index of AIS for AKR-anti-C57BL/6, C3H-anti-C57BL/6, DBA/2-anti-C57BL/6, BALB/C-anti-C57BL/6, and A/Sn-anti-C57Bl/6 T_{act} was between 1.6 and 2.0. Meanwhile the binding index of AIS with CBA-anti-C57BL/6 T_{act} was 4.8.

It can be concluded from these findings that a private strain-specific idiotypic antigenic determinant, absent in other activated T lymphocytes tested, is evidently present in CBA-anti-C57BL/6 T_{act}. By cellular radioimmunoassay it was impossible to detect any public idiotypic antigenic determinants in these T_{act}. Little information has so far been obtained

on antigenic crossing at the level of idiotypes of antigen recognition receptors of T lymphocytes. The authors of [7] likewise found no public idiotypes in AKR-anti-C57BL/6 and SIL-anti-C57BL/6 T lymphocytes. CBA/H and C57BL/6 mice are known to have the same allele of the Mls gene (Mls^b). For that reason the reaction of CBA T cells in a CBA-anti-C57BL/6 system is directly primarily against strong transplantation antigens coded by $H\text{-}2^b$. We obtained 2.4-3.1 times more intensive binding of AIS with CBA-anti-C57BL/6 ($H\text{-}2^k$-anti-$H\text{-}2^b$) T_{act} (Fig. 2). This suggests that the gene (genes) coding the private idiotypic antigenic determinant of CBA-anti-C57BL/6 T_{act} is not a component of the H-2 complex. Facts in the literature also confirm this conclusion. Several workers have obtained proof of the linking of genes of idiotypes of antigen recognition receptors of T cells with genes of allotypes of the heavy chains of immunoglobulins and absence of linking of genes coding idiotypes with the H-2 complex [5, 6, 9]. For investigations of this type it will next be necessary to use congeneic strains of mice and strains recombinant for H-2 locus.

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