INHIBITION OF T-LYMPHOCYTE-MEDIATED TUMOR-SPECIFIC LYSIS BY ALLOANTISERA DIRECTED AGAINST THE H-2 SEROLOGICAL SPECIFICITIES OF THE TUMOR*

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After appropriate in vivo or in vitro immunization, cytotoxic T lymphocytes (CTL) are generated which efficiently kill cells bearing particular membrane antigens in common with the immunizing cell (reviewed in reference 1). Such CTL have been most thoroughly studied in mice, employing alloimmunization with cells differing at the major histocompatibility locus, H-2. In such cases, the predominant cell surface antigens recognized by the CTL appear to be the molecules carrying the serologically defined H-2 specificities, coded for by the K and D regions of the H-2 complex (2). In other syngeneic models of cell-mediated specific cytolysis, involving lymphocyte choriomeningitis (LCM) virus- or ectromelia virus-infected cells or TNP-modified lymphoid cells, thymus-derived cells also constitute the main effector cell type. The CTL generated in these latter systems function most efficiently when virus-infected or TNP-modified target cells share identities at the H-2K or H-2D loci with the effector CTL and stimulator cells (3–5).

Another set of experimental systems in which CTL are generated and play a significant biological role is that of immunity to tumor-associated antigens (TAA) (6). The nature of the TAA which the CTL recognize is only beginning to be understood. Several recent reports indicated the existence of physicochemical and/or antigenic relationships between TAA and H-2 antigens (7, 8). These relationships, together with the genetic restrictions cited above in the generation of CTL involving products of the H-2K or H-2D loci suggested the possibility that in certain tumor systems, the TAA which are able to most effectively stimulate CTL responses might be structurally similar to, or linked with, the H-2K or H-2D molecules on the tumor surface. It has been previously demonstrated in allogeneic models that antisera specific for the appropriate H-2K or H-2D products present on a target cell could specifically block CTL-mediated lysis (1, 9). This report demonstrates that certain anti-H-2 alloantisera specific for the target tumor cells can block lysis of those target cells mediated by syngeneic tumor-specific CTL effector cells.

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Materials and Methods

Mice. The mice used in this study were DBA/2, (C57BL/6 × DBA/2)F₁, referred to as (B6D2)F₁, or (C3H/HeJ × DBA/2)F₁, referred to as (C3D2)F₁, females 8- to 14-wk old, purchased from Jackson Laboratories, Bar Harbor, Maine.

Tumors. The two tumors (P815 and SL2) used in this study were of DBA/2 origin. The P815 mastocytoma induced by methylcholanthrene and the SL2 lymphoma, which arose spontaneously were passaged intraperitoneally (i.p.) in ascites form at biweekly intervals in DBA/2 mice.

Antisera. Alloantisera were prepared by giving mice 4-8 i.p. injections of 2 × 10⁹ viable P815 or appropriate lymphocytes at 2-wk intervals and bleeding 6 to 10 days after the last injection. Sera were pooled, heat inactivated at 56°C for 30 min and stored at -70°C until use. The donor-recipient combinations used and the specificity of each serum is listed in Table 1. 0.45 ml of B10 anti-P815 was absorbed with 4 aliquots of 10 x 10⁶ spleen cells from C57BL/10 or B10.D2 mice by incubating each time for 15 min at room temperature, and 45 min at 4°C.

Media. RPMI-1640 was purchased from Associated Biomedic Systems, Inc., Buffalo, N.Y. The medium used in the cytolysis assays was termed minimal essential medium (SMEM)-10 and contains Eagle's MEM with Earle's balanced salt solution (BSS) with 2 mM glutamine, 1 x nonessential amino acids, 100 U penicillin/ml, 100 µg streptomycin/ml, and 10% heat-inactivated fetal calf serum (Microbiological Associates, Bethesda, Md.).

Immunization and Preparation of Effector Cells. Inguinal lymph nodes draining the site of subcutaneous (s.c.) tumor growth 10 days after tumor inoculation were teased, filtered through nylon mesh, washed four times, and resuspended in cold SMEM-10 to 30 x 10⁶ viable cells/ml. Normal lymph node or splenic lymphocytes were similarly prepared.

³¹Cr Release Assay. The method of preparing and ³¹Cr-labeling target tumor cells has been reported previously (10). 100 µl of labeled targets containing 15 × 10⁶ cells were placed in 10 x 75 mm round bottom glass tubes, 50 µl of SMEM-10, 1:2 dilutions of normal mouse serum (NMS) or alloantisera in SMEM-10 added, and the tubes shaken. After 15 min at room temperature, 100 µl of immune or normal lymphocytes were added, the tubes shaken, and incubated for 18 h at 37°C in a humid atmosphere of 95% air, 5% CO₂. Determination of specific ³¹Cr release in these assay tubes was carried out as described (10). Final effector to target ratio in all assays was 200:1, and final NMS or antiserum dilution 1:10 unless otherwise indicated.

Results

Previous results from our laboratory have demonstrated that CTL are the primary effector cell in preparations from the draining lymph nodes of DBA/2, (C3D2)F₁, and (B6D2)F₁ mice bearing syngeneic 10 days s.c. P815 or SL2 tumors. Fig. 1 illustrates results obtained in several experiments in which antisera were tested for inhibition of CTL-mediated tumor lysis. The data show strong (30–50%) decreases in lysis using either the B10 anti-P815 serum or serum no. 438 (anti-H-2d) for inhibition of cytolysis of either P815 or SL2 H-2d tumors. In addition, the lack of inhibition seen when (C3D2)F₁ cells, which carry both the H-2d and H-2b haplotypes, are used as effectors in the presence of antibody to the effector H-2d specificities indicates that antibodies directed only at effector cell H-2 determinants are unable to prevent target cell lysis. The activity of the congeneric serum no. 438 (anti-H-2D) demonstrates that inhibition can be mediated by sera reacting solely with antigens coded for or controlled by the H-2 complex of normal lymphoid cells. Fig. 1 also demonstrates that not all alloantisera to H-2 antigens on the tumor cell surface are able to strongly inhibit CTL activity. Thus, antiserum no. 329 (anti-H-2K) showed an inconsistent ability to provide marginal blocking of SL2 tumor cell lysis, even though titrations of complement-dependent lytic activity using this tumor antiserum combination show significant H-2K antigen on these tumor cells.
Fig. 1. Ability of various anti-H-2d alloantisera to inhibit tumor-specific CTL-mediated lysis of P815 or SL2. P values are given for each antisera in comparison to NMS.

Fig. 2. Ability of B10 anti-P815 serum to inhibit tumor-specific lysis before absorption and after absorption with B10 or B10.D2 lymphocytes. P values are given for each antiserum in comparison to NMS.

These results are extended in the data shown in Fig. 2. An aliquot of the B10 anti-P815 serum was absorbed with B10.D2 spleen cells to selectively remove antibodies directed to antigens controlled by the H-2d haplotype. The B10.D2 absorbed antiserum was completely unable to inhibit lysis.

Table I summarizes the ability of a large series of alloantisera to inhibit tumor-specific CTL activity in this system. It can be seen that antisera directed to at least the H-2Dd-coded specificities are able to mediate strong, consistent inhibition (Table I). Antisera specific for antigens controlled by other portions of the H-2 region or closely linked loci such as Tla are only weakly and inconsist-
Ability of Various Alloantisera to Inhibit CTL-Mediated Tumor Cell Lysis

The recipient/donor combinations used to prepare the antisera were: no. 001 AKR anti-C3H thymus, no. 082 (Bl0.D2 x A.Ca)F, anti-1110, no. 329 (1110 x A)F, anti-Bl0.D2, no. 423 (1110 x A)F, anti-B10.BRm, no. 426 (1110 x LP.RRI)F, anti-4R, no. 428 A.Tla- anti-A thymus, no. 438 B10 x LP.RIII)F, anti-18R, and no. 445 A.TH anti-A.TL.

-, no inhibition compared to NMS; -, slight inconsistent inhibition compared to NMS; +, modest consistent inhibition compared to NMS; and ++, strong consistent inhibition compared to NMS.

See Materials and Methods.

In data not shown, experiments were performed in which alloantisera directed to non-CTL recognized H-2 antigens on hybrid target cells were tested for inhibitory activity. CTL from C57BL/6 (H-2b) mice immunized 10 days previously with an i.p. injection of 2 x 10^7 P815 (H-2b) were tested in the "Cr-release assay using (B6D2)F1 lymph node cells as targets. In the presence of anti-H-2D^d antiserum, lysis was significantly inhibited, while the addition of a strong anti-H-2^d antiserum did not affect lysis.

Discussion

The data reported above unequivocally demonstrate that lysis of tumor targets (P815 and SL2) by syngeneic CTL can be inhibited to a large extent by alloantisera directed to antigens coded for by certain regions of the H-2 complex. The inhibitory activity of such sera is not due to antibodies elicited specifically by the TAA of the P815 or SL2 tumor cells since: (a) antisera raised by immunization with normal lymphoid cells is inhibitory; (b) absorption of antisera raised by alloimmunization with P815 so as to remove only antibodies to H-2-regulated antigens on normal cells removes all inhibitory activity; and (c) alloantisera to the P815 mastocytoma can inhibit specific syngeneic killing of...
the noncross-reactive SL2 tumor cells. In addition, the antisera apparently mediated their effects by action on the target cell surface, and not by action on the CTL itself. Finally, not all alloantisera, or even all antisera to target-borne H-2 specificities, are able to inhibit CTL activity, although the possibility that other anti-H-2 sera might show strong effects if of sufficient high titer has not been completely excluded.

These findings raise several interesting possibilities which cannot be discussed extensively in this brief report concerning the explanation for this inhibitory phenomenon. However the results we have presented, together with data on the H-2 requirements for killing in the LCM, ectromelia, hapten-derivatized, and allogeneic systems, and also reports of chemical, immunologic, and even physical correspondence between H-2 and tumor antigens favor, in our opinion, the hypothesis that the particular TAA best able to elicit CTL responses may represent altered or modified H-2 antigens. These modified H-2 antigens may coexist in the membrane with unmodified H-2, their respective concentrations held by an unknown mechanism to a constant total, but variable ratio, which is alterable by conditions of tumor growth. The reciprocal relationship between changes in amount of H-2 and tumor antigen on tumor cells passaged in vivo vs. in vitro for prolonged times is consistent with this interpretation (11). Whatever the actual relationship, this concept of tumor antigen-H-2 antigen similarity raises important questions about the function of H-2 antigens, perhaps in immune surveillance, and potential explanations for the adaptive advantage of maintaining the large number of H-2-reactive T-lymphocytes present in unimmunized animals.

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References

