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on
IMMUNOSUPPRESSION

INTERNATIONAL SYMPOSIUM ON
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IMMUNOSUPPRESSION
IN ORGAN TRANSPLANTATION

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RECENT ADVANCES IN TRANSPLANTATION

Foreign H-2-Like Molecules on a Murine Tumor (MCG4): Target Antigens for Alloreactive Cytolytic T Lymphocytes (CTL) and Restricting Elements for Virus-Specific CTL

V. Schirrmacher, F. Garrido, D. Hübsch, E. Garcia-Olivares, and U. Koszinowski

WE RECENTLY reported about a sarcoma of BALB/c (H-2^d) mice that had been newly induced by methylcholanthrene and adapted to the ascites form.[1-3] This tumor, MCG4, upon transplantation into normal BALB/c mice, showed a high frequency of spontaneous regression. An H-2 typing analysis of the tumor revealed a loss of four antigenic specificities present on normal H-2^d cells (i.e., the private specificity 4 and the public specifities 3, 8, and 13) and a gain of the public specificity H-2.5, which is characteristic of foreign haplotypes (e.g., H-2^k and H-2^b). The tumor also reacted with different monoclonal BALB/c hybridoma-derived anti-H-2^k antibodies.[3]

Furthermore, regressor animals were found to have developed antibodies with high cytotoxic antitumor activity.[2] These BALB/c anti-MCG4 isoantisera recognized an alloantigen on the tumor that was very similar to H-2 antigens expressed on normal cells from mouse strains of foreign H-2 haplotypes. Lymphoid cells from strains sharing the H-2 haplotype with the tumor-bearing host (H-2^d) did not react with the MCG4 isoantisera.[2,3]

In this article we will present further evidence for the H-2 nature of the alloantigens expressed by the MCG4 tumor. The evidence derives from a typing analysis with specific cytolytic T lymphocytes (CTL): CTL from BALB/c mice sensitized against foreign H-2 molecules (e.g., H-2^k) will be shown to react specifically with the MCG4 tumor. Furthermore, anti-Sendai virus CTL from H-2^k mice will be shown to recognize H-2^k-like determinants as restricting elements on Sendai-virus-infected MCG4 tumor target cells.

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MATERIALS AND METHODS

^{51}Cr Release Cytotoxicity Test

This 4-hr assay was performed as described previously.[4]

Generation of Cytolytic T Lymphocytes

CTL against alloantigens were generated in mixed lymphoctye cultures in vitro using normal spleen cells as responders (R) and mitomycin-C-treated syngeneic or allogeneic spleen cells as stimulator cells (S). The cells were cocultured for 4–5 days in Falcon flasks at a density of 4×10^6 responder cells and 10^6 stimulator cells per milliliter. The effector cells generated were washed twice before they were tested in the cytotoxicity assay. Either tissue-culture-adapted MCG4 tumor cells or Con-A-activated lymphoblasts were used as target cells. The latter were generated in spleen cell cultures by incubation for 2 days with concanavalin A at 12 μg/ml. The blast cells were enriched by Ficoll-Isopaque centrifugation and labeled with ^{51}Cr as described.[4]

CTL against Sendai virus (SV) were generated by coculturing spleen cells from SV-immunized mice in vitro with inactivated SV.[5] Target cells were sensitized with SV antigens as described.[5]

Further details about Materials and Methods are published elsewhere.[1-3]

RESULTS

H-2 Allodeterminants on MCG4 Recognized by Specific Cytolytic T Lymphocytes

Figure 1 illustrates the results obtained in a cytotoxicity test using BALB/c anti-H-2^b CTL as effector cells and the BALB/c-derived tumor MCG4 as target. Specific lysis of tumor cells was observed with BALB/c

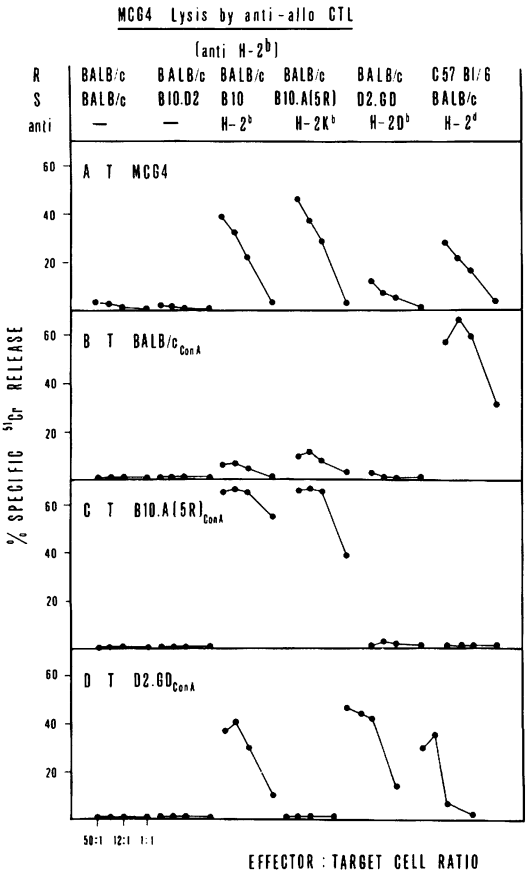

Fig. 1. Allodeterminants recognized on MCG4 by BALB/c anti-H-2^b cytolytic T lymphoctyes.

anti-B10 (anti-H-2^b) effector cells, while the negative control BALB/c anti-B10.D2 was noncytotoxic to the tumor. Effector cells with selective specificity for the K-end of H-2^b [BALB/c anti-B10.A(5R)] also lysed the MCG4 target, while those with specificity for the D-end of H-2^b (BALB/c anti-D2.GD) showed little if any antitumor cytotoxic activity. Positive control target cells were Con-A blasts from B10.A(5R) and D2.GD (Fig. 1 C and D). These targets were specifically lysed by the anti-K^b and the anti-D^b CTL, respectively, without any detectable cross-reactivity. Con-A blasts of BALB/c origin were not lysed by any of the BALB/c anti-H-2^b cytolytic T cells, while they were lysed by C57BL/6 anti-BALB/c (anti-H-2^d) CTL (Fig. 1B). MCG4 was also lysed by the anti-H-2^d CTL but to a lower extent than observed with the anti-H-2^b CTL.

Similarly, we tested the expression of H-2^k-like allodeterminants on MCG4 with anti-H-2^k CTLs (Fig. 2). BALB/c anti-H-2^k CTLs were found to have even higher cytolytic activity on MCG4 targets than the CTLs specific for H-2^b (50% ^{51}Cr release at effector:target cell ratio of 10:1 as compared to 50:1). Furthermore, CTL with specificity for either the K-end or the D-end of the H-2^k haplotype was cytolytic towards MCG4, while the selective specificity could be demonstrated on lymphoblast targets expressing the K^k (Fig. 2B) or the D^k (Fig. 2C) molecule.

H-2-Restricting Determinants on MCG4 Recognized by Allogeneic Antivirus Cytolytic T Lymphocytes

Anti-Sendai-Virus (SV) CTL were generated in B10.D2 (H-2^d), C57BL/6 (H-2^b), and

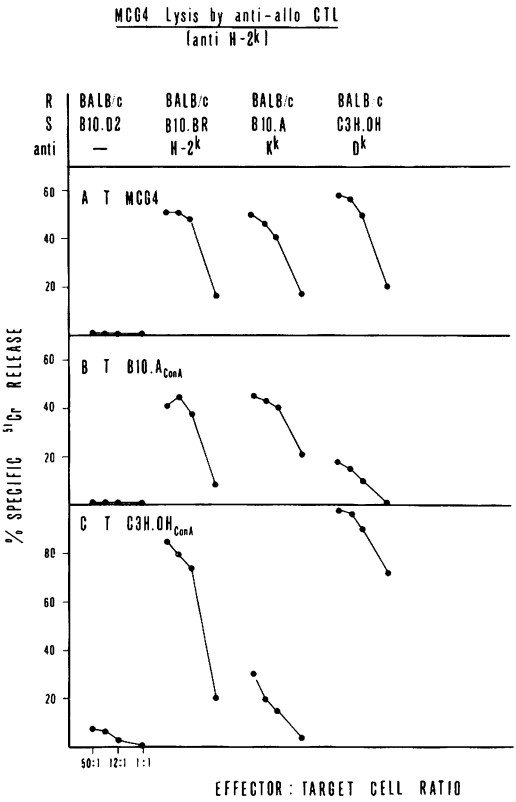

Fig. 2. Allodeterminants recognized on MCG4 by BALB/c anti-H-2^k cytolytic T lymphocytes.

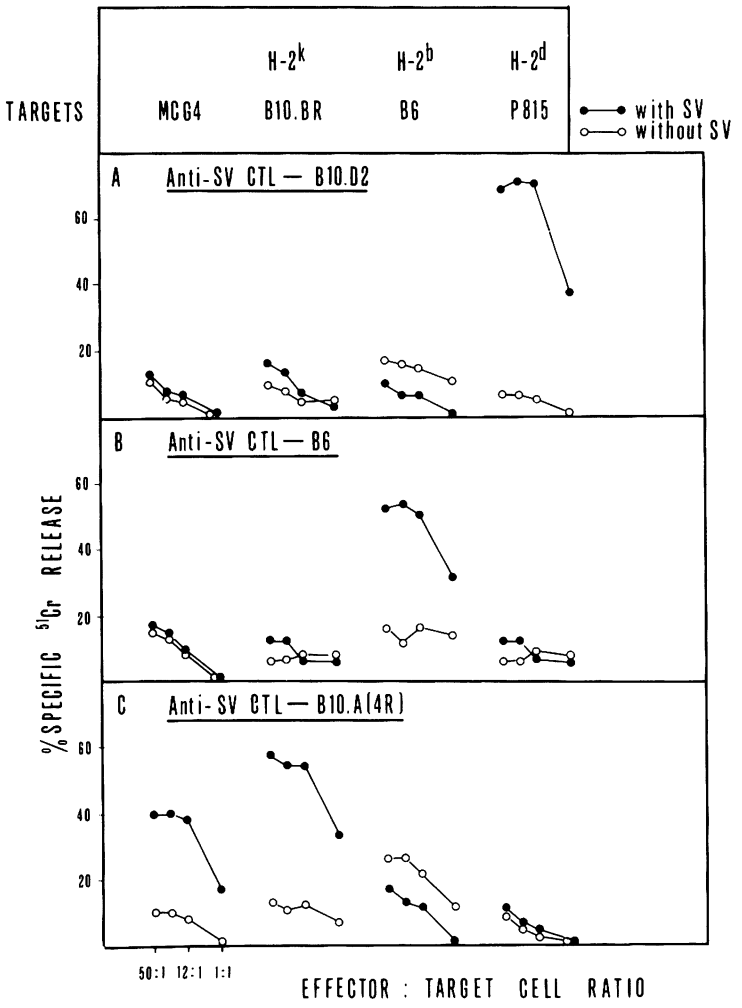

Fig. 3. H-2K^k-like restricting determinants recognized on MCG4 by allogeneic virus-specific CTL.

B10.A (4R) (H-2^{h4}) mice. They were tested for virus-specific cytolytic activity on noninfected and SV-infected MCG4, P815, C57BL/6, and B10.BR lymphoblast target cells. The results are illustrated in Fig. 3.

Anti-SV CTL from H-2^d mice specifically lysed the SV-infected H-2^d tumor target P815 but did not react with any of the other virus-infected targets, including MCG4. Anti-SV CTL from H-2^b mice specifically lysed the SV-infected H-2^b lymphoblast and none of the other targets. However, anti-SV CTL from B10.A (4R) mice (K^{k}D^d) specifically lysed the SV-infected H-2^k lymphoblast from B10.BR and the SV-infected MCG4 target. They did not react with P815-SV and only marginally with C57BL/6-SV target cells.

These results clearly demonstrate determinants on MCG4 tumor cells that can be detected in associative recognition by CTLs specific for SV and restricted by molecules coded for by the K region of the H-2^k haplotype.

This conclusion was supported by further experimental data that are not presented: Anti-SV CTL from B10.BR (H-2^k) mice specifically lysed MCG4-SV and B10.BR-SV lymphoblasts but not P815-SV, while anti-SV CTL from A.TL (K^{s}D^d) and D2.GD (K^{d}D^b)

mice lysed specifically P815-SV but not MCG4-SV.

DISCUSSION

Foreign H-2 specificities have previously been detected on various murine tumor cells by serologic or transplantation techniques.[2,6–9] According to our present concept about the genetic organization of the H-2 complex,[10–12] such determinants could not be coded for by the H-2 alleles of the tumor-bearing host. They have therefore been called "alien," "foreign," "H-2-like," or "inappropriate." Foreign H-2 specificities might indicate (1) the presence of mutated[12] or otherwise altered normal H-2 molecules, or (2) the presence of products of derepressed normally silent H-2 genes.[13,14] A distinction between these alternative hypotheses depends on a detailed biologic and chemical characterization of these molecules.

Here we present the first preliminary evidence that foreign H-2 specificities can be recognized by specific T lymphocytes sensitized against normal H-2 determinants of foreign haplotypes. This evidence is twofold: (1) H-2^d anti-H-2^k cytotoxic T lymphocytes (CTL) specifically lyse the H-2^d-derived tumor MCG4, and (2) H-2^k anti-Sendai-virus (SV) CTL specifically lyse SV-infected MCG4 tumor cells and SV-infected target cells of H-2^k origin.

With regard to the first part, namely, the typing of MCG4 with alloreactive CTL, the data indicate the presence of alloantigenic determinants normally expressed on K^k, D^k and K^b molecules. BALB/c-derived CTL against D^b molecules only reacted marginally with MCG4. BALB/c spleen cells cocultured with syngeneic H-2^d lymphoid cells did not react at all with the tumor under our assay conditions, indicating absence of "spontaneous" or "natural" antitumor cytolytic activity. Since anti-K^k and anti-D^k CTL showed only a low degree of cross-reactivity when tested on appropriate lymphoblast target cells, but reacted strongly with MCG4, it is likely that the tumor expresses both a K^k-like and a D^k-like molecule. The reactivity of H-2^d anti-K^b CTL with the tumor is most easily explained as a reactivity against a public specificity shared between K^k and K^b molecules. Such a public specificity has been serologically defined as H-2.5. Previous serologic studies have shown that MCG4 indeed expresses an H-2.5 determinant.[1,3]

The data obtained when typing for associative recognition with H-2-restricted virus-specific CTL again support our previous conclusion that the tumor MCG4 expresses H-2^k-like molecules. It should be mentioned, however, that these represent data from one, our first, experiment. Unequivocal results were obtained in that anti-SV CTL from B10.BR (K^kD^k) and B10.A(4R) (K^kD^b) mice could lyse SV-infected MCG4 tumor cells, while anti-SV CTL from B10.D2 (K^dD^d), A.TL (K^sD^s), D2.GD (K^dD^b), and C57BL/6 (K^bD^b) mice were negative. These results suggest that MCG4 tumor cells express a K^k-like molecule (possibly also a D^k molecule) that is recognized as a restrictive element by virus-specific CTL. Furthermore, since anti-H-2^b CTL reacted with the tumor, but H-2^b-derived virus-specified CTL did not, the H-2 determinants involved in associative recognition appear more restricted to particular haplotypes than the determinants recognized by alloreactive CTL. Third, as reported previously, the MCG4 tumor cells did not react in direct cytotoxicity tests with antisera against the private specificities 4, 23, and 32, which characterize, respectively D^d, K^k, and D^k molecules.[1] Thus, K^k-like molecules were detected on MCG4 in associative recognition, although they did not seem to carry the private specificity 23. This would suggest that restricting H-2 determinants recognized by specific T cells, although being very distinct, are independent of and not identical with the private serologic specificities.

All these conclusions, however, have to be taken as preliminary, since further experiments are needed to verify and substantiate our findings. As it stands at present, the MCG4 tumor seems to express predomi-

nantly an H-2^k-like alloantigen that fulfills most criteria of a true foreign H-2 molecule. H-2^d-type molecules from the strain of tumor origin seem to have a comparatively low expression.* Our data are reminiscent of Parmiani's BALB/c-derived chemically induced fibrosarcoma C-1, which has been characterized as expressing predominantly H-2^d-type molecules, but in addition, alien molecules very similar to K^k and D^k.[15]

*NOTE ADDED IN PROOF: A possible mix-up of the tumor was recently excluded by an isoenzyme analysis performed by Dr. H.-H. Krog, Copenhagen. It revealed identity of MCG4 with BALB/c.

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Discussion

Ferrone. Do you have any data from immunodepletion experiments using anti-H-2^k or antitumor antisera? Did you try any adsorption-inhibition assays in which you react H-2^k cells with your antitumor antibodies, then determine whether you have blocked adsorption of specific H-2^k antiserum?

Schirrmacher. We have preliminary data from sequential immunoprecipitation analysis using isoantiserum first, and then anti-H-2^d or anti-H-2^d first, and then isoantiserum that indicates that the foreign H-2 determinants on MCG4 are on molecules different from H-2^d. We have not yet done the experiments you suggest.

Parmiani. The tumor that you described looks very similar to the BALB/c tumor that we use. Could the H-2^k-like antigen appearing on these BALB/c tumors be due to a common origin for the BALB/c and C3H strains of mice?

Schirrmacher. That is a very interesting point, but I don't believe anyone has the answer.

Alexander. We have always found it very difficult to adapt sarcomas to grow in the ascites form. Have you tested the original sarcoma for expression of H-2^k-like antigen? Could the antigen have developed after adaptation of the tumor to the ascites form?

Schirrmacher. This tumor was induced by Dr. Garrido, and had we known it was going to be such an unusual tumor, we would have kept the original. Unfortunately, we no longer have it. He is setting up an extensive tumor-induction program in which the normal tissues of the animals will be kept for comparison. It was very difficult to get an ascites out of this solid tumor; this was the first of about 50 tumors that would grow as an ascites. We don't have any idea about the frequency with which these inappropriate alloantigens appear or whether they are due to adaptation of the cells into an ascites form. Dr. Parmiani's C-1 tumor is an example of a solid tumor expressing alien specificities.

Bortin. Is the gain or loss of antigenic specificites unique to tumor cells maintained in tissue culture or does it occur in cells from spontaneous tumors as well?

Waksal. I would say that 99% of the 30–40 spontaneous AKR tumors we have looked at do not express the alien H-2.4 specificity.

Alexander. Dr. Bortin's question is very appropriate. How much of this phenomenon is an artifact due to carrying the tumors in vitro? About 5 or 6 years ago Dr. Festenstein asked for some of our chemically induced thymomas. Dr. Garrido examined them and found all sorts of specificities; however, when we gave him some earlier passage materials, he did not find any of these specificites. These alien antigens may not be on spontaneous or recently induced tumors.

Schirrmacher. Dr. Garrido has been looking at recently induced tumors, and the situation is just as striking as on long-term passage tumors.

Waksal. Normal fibroblasts passaged in vitro long

enough will begin to show antigenic changes similar to those seen on tumor cells because of the expression of xenotropic MuLV.

Parmiani. As far as long-term culture of tumors is concerned, in our system, not only is the detection of alien histocompatibility antigens a problem, there is also a loss of original H-2 antigens.

Bonavida. In our reticulum cell sarcomas we were very concerned with whether or not these alien antigens were an artifact of culture. We have looked at about 50 spontaneous tumors, and we still can see the inappropriate specificity by immunofluorescence. In addition, animals that have the tumor produce antibodies that cross-react with the alien specificity.