EXCLUSIVE INVOLVEMENT OF H-2D\textsuperscript{b} OR H-2K\textsuperscript{d} PRODUCT IN THE INTERACTION BETWEEN T-KILLER LYMPHOCYTES AND SYNGENEIC H-2\textsuperscript{b} OR H-2\textsuperscript{d} VIRAL LYMPHOMAS\

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Gene products coded for by the \textit{D} and/or \textit{K} regions of the major histocompatibility complex (MHC)\textsuperscript{1} must play a determinant part in the interaction between immune cytolytic T lymphocytes (CTL) and their virus-infected or hapten-modified syngeneic stimulating and targets cells, since a high level of specific cytolysis occurs only when both the CTL and the target cells possess shared H-2D and/or H-2K antigens (1-4). The same phenomenon exists in the interaction between T-killer lymphocytes and syngeneic virus-induced tumor cells. This has been demonstrated both by immunization with oncogenic viruses (5-8) and by grafts of syngeneic virus producer lymphoma cells (9-11). In murine sarcoma virus (MSV) tumors and in Friend virus (FLV) leukemias, such H-2 restrictions of CTL activity have been shown to occur during both primary in vivo response (5, 6, 9) and secondary in vitro CTL restimulation (12). Some degree of cytolysis exists also against H-2 incompatible tumor cells induced by the same viruses especially when strong responses are elicited. However quantitative experiments, with different effector to target cell ratios, demonstrate that such activity is 10-100 times weaker than that shown against H-2 compatible targets (12, 13).

Though the exact role of H-2 directed molecules in the anti-MSV and anti-FLV reactions by syngeneic CTL is unknown, the hypothesis that the reactions are directed against altered self specificities as in other systems (1-4) appears more probable than the one requiring a double contact and sometimes designated as the intimacy theory (5, 9, 10, 13). In most systems both H-2D and H-2K have been implicated (1-4) but a predominant role of H-2K in vaccinia viral infection (14) and of H-2D in other systems (11, 15-19) including tumor antigens (11) has been noted.

In the MSV tumor, we previously observed that one or the other regions of the MHC was involved depending on the H-2 haplotype (13). A more precise understanding of the respective roles of H-2D, H-2K, and possibly other molecules under the control of the MHC is necessary to determine the biological

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\textsuperscript{1} Abbreviations used in this paper: CTL, cytolytic T lymphocytes; Con A, concanavalin A; CRT, chromium release test; FLV, Friend virus leukemia; FMRG, Friend, Moloney, Rauscher, Graffi cell-surface antigen; i.m. intramuscular; i.p., intraperitoneal; MHC, major histocompatibility complex; MSV, murine sarcoma virus; MuLV, murine leukemia virus.

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significance of the H-2 restriction phenomenon. Moreover such studies could help our understanding of the nature of the structural relationship between the cellular and viral products which together contribute to the altered self-specificity.

The results reported here, obtained with mice of the H-2<sup>b</sup> and H-2<sup>d</sup> haplotypes, demonstrate that the products of only one region of the MHC are involved in the CTL/tumor target cells interaction. They are always directed by the D region with cells of the H-2<sup>b</sup> haplotype, and by the K region end of the MHC with cells of the H-2<sup>d</sup> haplotype.

**Materials and Methods**

**Mice.** 1- to 2-mo-old C57Bl/6 (B6), C57Bl/10 (B10), B10.WB (WB), B10.D2, B10.A, B10.A(2R)(2R), B10.A(4R)(4R), B10.A(5R)(5R), HTI, HTG, BALB/c, BALB/B, C3H.OH, C3H.OL, A.TH, A.TL, A.TFR2, B10.T(6R)(6R), and B10.S(7R)(7R) were obtained from our own colonies.

**Viruses.** The MSV, Moloney isolated, was maintained by regular acellular transmission in newborn B6 or BALB/c. The NB tropic FLV was maintained in vivo in adult BALB/c or in vitro in the non-Fv-1 restricted 3T3-FL line which is derived from the National Institutes of Health Swiss mice (20).

**Target Cells.** These were transplanted ascitic viral lymphomas. In the H-2<sup>b</sup> haplotype we used viral lymphomas of B6 mice: MBL2 (Moloney), RBL5 (Rauscher), FBL3 (Friend), and Gil 11 (Graffi), or BALB/B mice: HFL/B (Friend) and A13 (Tennant). The nonviral EL4 tumor was used as a negative control. In the H-2<sup>d</sup> haplotype all the viral lymphomas were of BALB/c origin: LSTRA (Moloney), BC1 (Tennant), and T2 (Friend). The nonviral DBA/2 L1210 leukemia was used as control. MBL2, RBL5, and FBL3 were originally provided by Dr. R. Herberman (National Cancer Institute, Bethesda, Md.), HFL/B by Dr. F. Lilly (Albert Einstein College, Yeshiva University, Bronx, N. Y.), A13, T2, and BC1 by Dr. B. Chesebro (National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Mont.).

**Immune Lymphocytes.** Anti-MSV immune lymphocytes were taken from the spleens of mice inoculated intramuscularly (i.m.) 10-15 days previously with 0.2 ml of a 10^-1 dilution of MSV. Local tumors appeared 5-7 days after inoculation, reached their maximum at days 7-10, and were completely rejected by day 15. Anti-FLV immune lymphocyte were obtained, only in the H-2<sup>b</sup> haplotype, from the spleens of B6 mice inoculated 10-15 days before with 0.1 ml of a 10^-2 dilution of FLV.

**The Chromium Release Test (CRT).** The CRT was performed as described previously (21) by using a 100:1 immune lymphocytes/target cell ratio and an incubation period of 6-16 h. The specific chromium release was measured in the presence of immune lymphocytes minus the chromium release in the presence of normal lymphocytes of the same line. Under our conditions a release of 4% or more is clearly significant (21). In some of the experiments the results are expressed in terms of lytic units per 10^6 effector cells. 1 lytic U was calculated according to Plata et al. (22) by using for comparison lymphoid/target cells ratios varying from 100/1 to 3/1 (see Table III).

**Concanavalin A Restimulation of Immune Lymphocytes.** This technique is described in detail elsewhere: 2.5 x 10^7 splenic lymphocytes from MSV regressor mice were cultured in 25 cm² culture flasks by using 15 ml of RPMI 1640 medium (Flopio, France) containing 5.10^-5 M of 2-mercaptoethanol and 10% of fetal calf serum. Normal syngeneic lymphocyte cultures were run in parallel as a control. Concanavalin A (Con A) was added at a final concentration of 5 μg/ml. After 7 days incubation the cells were harvested and tested in a 16-h CRT as described above.

**Immune Sera**

**ANTI-H-2<sup>b</sup>.** Different procedures of immunization were used. (a) Antisera directed against the whole H-2<sup>b</sup> were obtained from B10.D2 mice hyperimmunized against normal B10 lymphoid cells, or from BALB/c inoculated with normal BALB/B cells. In both cases an in vivo absorption step was...
used to obtain monospecific anti-H-2D^b (adsorption into 5R) or anti-H-2K^b (absorption into 4R). (b) Antisera were also prepared so that they were directed only against H-2D^b: (B10.RIII × B10.BR)^F_t, anti-B10.A(4R), or against H-2K^b products: (B10.S(9R) × B10.Htt)^F_t, anti-B10.A(5R). (c) In addition in some experiments we used antisera provided by Dr. F. Lilly (BALB/c × AKR)^F_t, anti-EL^a (anti-H-2^b), (B10.A × HTI)^F_t, anti-EL^a (anti-H-2D^b) and (B10.A × HTG)^F_t, anti-EL^a (anti-H-2K^b).

Anti-H-2^b. Antisera reacting with the whole H-2^d was obtained from B10 mice hyperimmunized with B10.D2 normal cells. Monospecific antisera were obtained in the following two combinations: (B10.AKM × A.BY)^F_t, anti-B10.A (anti-H-2D^d) and (B10.A × A.BY)^F_t, anti-B10.D2 (anti-H-2K^d).

Anti-I^a. Two anti-I^a sera were used: A.TH anti-A.TL (anti-I^a^k), and A.TL anti-A.TH (anti-I^a^k). The anti-I^a^k reacts at least with Ia.3 and Ia.15 specificities of H-2^b lymphocytes and with Ia.15 on H-2^d cells. The anti-I^a^k react with Ia.9 on H-2^d cells but does not react with any of the specificities identified on H-2^d lymphocytes.

Blocking by Anti-H-2 Sera. Target cells incubated for 20 min at 4°C with 1/2 to 1/16 diluted anti-H-2 or anti-I^a immune sera were used in the CRT, as described above, without further washing. Controls were: (a) target cells incubated with nonimmune sera from the same mouse line. (b) Target cells incubated with the immune serum and tested in the CRT with normal (nonimmune) syngeneic lymphocytes. The final activity of the immune CTL was calculated by comparing the chromium release in the presence of either immune lymphocytes or normal lymphocytes, the target cells being in both cases incubated with the immune serum.

Results

Cytolysis of Lymphoma Cells by In Vivo-Primed Anti-MSV Spleen Cells from Different H-2 Recombinant Lines

Study in the H-2^b haplotype. Tumors were induced in different inbred strains by i.m. inoculation with 1- to 2-mo-old mice with MSV. When the tumor size was maximal, at which point CTL activity is also expected to be maximal (21), spleen cells were sampled and tested in the CRT against different H-2^b lymphomas. The results were as follows: (a) effector cells from B6 or B10 mice (H-2^b) were strongly cytolytic against all six FMRG^+ H-2^b lymphomas tested (Table I). In other words the CTL of B6 or B10 mice functioned with equal efficiency regardless of whether the target lymphomas were from B6 or BALB/B origin, or induced by Rauscher, Friend, Graffi, Tennant, or Moloney viruses. (b) The same effector cells were not reactive against the nonviral EL^a^ lymphomas (not shown). (c) As previously reported (5, 13) BALB/c or B10.D2 effector cells were unable to lyse any to the H-2^b lymphoma targets. (d) A pattern of reactivity similar to that found in B6 and B10 was also detected with effector cells from HTG, 2R, 4R, and WB mice (Table I). Mice of all of these strains share some part of the D end of the MHC with the H-2^b lymphomas. This common part is limited to the D region in 2R and WB. The same situation also exists with HTG, which in addition differs from the B6 and BALB/B targets by numerous non-H-2 antigens. It has been found previously however that the non-H-2 genes do not play a role in the CTL/target cells interactions (5, 13). It must therefore be concluded that an identity limited to the D^b region is sufficient for a strong CTL activity against H-2^b viral lymphomas. (e) Anti-MSV-CTL from 5R and HTI were unable to kill any H-2^b targets, or were marginally efficient. This suggests that H-2K^b or other molecules coded by the K region are not involved in the anti-MSV CTL activity. This probably holds also for I^a antigens since the K, I, and S regions are identical in HTI effectors and in targets of BALB/B or B6 origin differences being restricted to the D region.
### Table I

**Cytolysis of H-2b Lymphoma Cells by Anti-MSV CTL* from Different Lines**

| Immune spleen cells donors | Effector cells | Percent chromium release from target cells†
|----------------------------|----------------|---------------------------------|
|                            | H-2 haplotype  | H-2 region common to effector and target |
|                            | K   I S D      | effector cells |
|                            | b b b b b b b b | all H-2 |
| C57B1/6 anti-MSV           | 40     31     31     28     25     47 |
| BALB/B anti-MSV            | 21     27     22     19     27     41 |
| B10.D2 anti-MSV            | 5      2      6      3      0      8  |
| HTG anti-MSV               | 23     25     28     18     –      22  |
| B10.WB anti-MSV            | 25     16     17     15     –      23  |
| B10.A(2R) anti-MSV         | 36     29     16     40     –      46  |
| B10.A(4R) anti-MSV         | 29     21     16     21     –      32  |
| B10.A(5R) anti-MSV         | 2      5      0      0      0      5   |
| HT1 anti-MSV               | 2      5      0      0      2      3   |
| B10.D2 anti-B10.A(3R)§     | 29     58     42     52     34     36  |
| B10.D2 anti-HTG§           | 23     35     28     42     27     30  |
|                            | d d d d d d d  | none |

* Effector to target cell ratio: 100/1, 18 h incubation.
† The mean standard errors being always between 0.4 and 2% are not given. In all cases the activity of immune CTL was calculated by comparison with the activity of normal (nonimmune) lymphoid cells of the same inbred strains. Practically no differences were found in the level of activity of the different normal lymphocytes.
§ H-2b haplotype bear the same D region specificities as H-2b.
| B10.D2 were immunized by intraperitoneal (i.p.) inoculation of 5.10⁶ B10.A(3R) normal spleen cells 6 days before, to produce anti-H-2b CTL.
One can conclude therefore that an identity of the CTL and the targets limited to the \( D \) region is both necessary and sufficient to allow lytic interactions. In all these experiments the complete and specific abrogation of the spleen cell activity by anti-Thy 1.2 serum and complement was verified showing that the effector cells are T cells (results not shown). This is in agreement with previous results obtained in the MSV system \((23, 24)\). The presence of both normal H-2\( K^b \) and H-2\( D^d \) antigens on the six FMRGI (+) lymphomas employed was shown by their sensitivity to lysis by allogeneic anti-H-2\( K^b \) CTL or anti-H-2\( D^d \) CTL.

**Study in the \( d \) haplotype.** The same type of experiments were done by using three FMRGI (+) H-2\(^d\) lymphomas (Table II). These showed that (a) BALB/B (H-2\(^b\)) CTL which were very efficient against H-2\( b \) targets (Table I and reference 13) are inactive against H-2\(^d\) cells. Identical results were found with B6 and B10-CTL (results not shown). (b) BALB/c and B10.D2 (H-2\(^d\)) anti-MSV spleen cells are strongly lytic for all three H-2\(^d\) lymphomas, without killing the nonviral H-2\(^d\) L1210 leukemia cells (not shown). (c) Among the H-2 recombinant strains, C3H.OL, C3H.OH, and HTG produce anti-MSV-CTL with a pattern of reactivity identical to that of B10.D2 or BALB/c. All of these strains share in common the \( K \) and \( I \) regions of the MHC, differences with the targets being restricted to the \( D \) or \( D+S \) regions. (d) On the contrary A.TH, A.TL, A.TFR2, HT1, 5R, 6R, and 7R which share at least the \( D^d \) region in common with BALB/c, but are different at the \( K \) end of the MHC, do not produce anti-MSV effector cells capable of lysing the BALB/c target cells. On a few occasions, A.TL anti-MSV spleen cells were found with a low degree of activity for the LSTRA (H-2\(^a\)) targets, as already reported \((13)\). This apparent discrepancy was observed more frequently and with a higher level of lysis with B10.A anti-MSV effector lymphocytes. However these exceptional reactions could not be abolished by anti-Thy 1.2 treatment of the effector cells their pattern of specificity differed from the usual anti-MSV, or anti-FLV-CTL pattern. The B10.A spleen cells from anti-MSV immune animals were found to lyse H-2\(^d\) targets in about 25% of the experiments, but in these same experiments they showed a similar degree of activity for nonviral lymphomas, and also for viral but H-2 incompatible tumors such as MBL2 (H-2\(^b\)). We therefore conclude that these reactions were directed against antigens other than those responsible for syngeneic anti-MSV reactions, and that such cytolysis being mediated by non-T cells, they are therefore not relevant to the response studied here.

On the whole it appears that T-cell-mediated cytolysis of H-2\(^d\) targets by anti-MSV spleen cells require that the effector cells possess the \( K \) end of the MHC identical to that of the target cells. It cannot be determined from these experiments if it is the \( K^d \) or the \( I^d \) regions or both which are concerned. It can however be concluded that the \( D \) region is not involved. The presence of normal nonmodified H-2\( D^d \) and H-2\( K^d \) antigens at the cell surface was verified by the demonstration that all three of the H-2\(^d\) viral lymphomas used in these experiments are killed by allogeneic anti-H-2\( D^d \) or anti-H-2\( K^d \) CTL (Table II).

**Cytolysis of H-2\(^b\) and H-2\(^d\) Viral Lymphomas by In Vitro Restimulated Anti-MSV Spleen Cells.** According to the altered self hypothesis the preceding results would indicate that the anti-MSV CTL recognizes antigens associating a viral product and the H-2\( D^b \) or the H-2\( K^d \) molecules on the lymphoma cell
## Table II

**Cytolysis of H-2^d Lymphoma Cells by Anti-MSV CTL* from Different Lines**

| Immune spleen cell donors | H-2 haplotype | H-2 region common to effector and target cells | Percent chromium release from target cells | L5T6RA (BALB/c) | BE1 (BALB/c) | T2 (BALB/c) |
|---------------------------|---------------|-----------------------------------------------|-------------------------------------------|----------------|-------------|-------------|
| BALB/c anti-MSV           | d d d d d d d  | all H-2                                       | 60                                        | 41             | 39          |
| BALB/B anti-MSV           | b b b b b b b  | None                                         | 3                                         | 0              | 0           |
| B10.D2 anti-MSV           | d d d d d d d  | all H-2                                       | 37                                        | 29             | 27          |
| C3H.OH anti-MSV           | d d d d d k k  | K to S                                        | 43                                        | 31             | 31          |
| C3H.OL anti-MSV           | d d d d d k k  | K to I-C                                      | 27                                        | 30             | 27          |
| HTG anti-MSV              | d d d d d d b  | K to S                                        | 36                                        | 30             | 24          |
| HTI anti-MSV              | b b b b b b b  | D                                             | 2                                        | 0              | 0           |
| B10.A(5R) anti-MSV        | k k k d d d d  | I-C to D                                      | 8                                         | 4              | 7           |
| B10.A anti-MSV            | s s s s s s s  | D                                             | 4                                         | 0              | 0           |
| A.TL anti-MSV             | s s s s s s s  | D                                             | 0                                         | 0              | 0           |
| A.TH anti-MSV             | s s s s s s s  | D                                             | 0                                         | -              | -           |
| B10.B(7R) anti-MSV        | s s s s s s s  | D                                             | 0                                         | -              | -           |
| B10.HTT anti-MSV          | q q q q q q q  | D                                             | 0                                         | 0              | 0           |
| B10.T6R anti-MSV          | f f f f f f f  | D                                             | 0                                         | 4              | 4           |
| ATFR/2 anti-MSV           | k k k d d d d  | I-C to S                                      | 4                                         | 3              | 4           |
| B10.A(2R) anti-MSV        | k k k d d d d  | I-C to S                                      | 4                                         | 3              | 4           |
| B10 anti-B10.A(5R)        | b b b b b b b  | None                                         | 27                                        | 20             | 18          |
| C3H anti-C3H.OL           | k k k k k k k  | None                                         | 20                                        | 22             | 16          |

* Effector to target cell ratio: 100/1, 18 h incubation.

* See footnote Table I.

§ B10 were immunized by i.p. inoculation of 5×10^7 B10.A(5R) normal spleen cells 6 days before to produce anti-H-2^d CTL.

surface. Thus, these new antigens can tentatively be called H-2D^b/MuLV and H-2K^d/MuLV (H-2D^b and H-2K^d altered by murine leukemia viruses). On the other hand, no reaction was detected against the hypothetic H-2K^b/MuLV and H-2D^d/MuLV. Because the activity of in vitro-primed CTL is relatively weak such a discrepancy might be purely quantitative. To confirm the absence of reactivity of H-2K^b and H-2D^b we restimulated in vitro the anti-MSV spleen cells obtained from those strains which were non responders when tested in vivo. To avoid the induction of an anti-H-2D^b or anti-H-2K^b reactions, restimulations of in vivo-primed spleen cells were done by culturing these cells for 7 days in the presence of Con A. As previously reported for other systems (25-27) and repeatedly demonstrated for the anti-MSV response, such a procedure yields specific reactivation of the immune CTL indistinguishable from that expected of a secondary response.

As shown in Table III, activated B6 anti-MSV-CTL obtained in this way specifically killed FMRGa (+) H-2^a targets. They remained ineffective however for FMRGa (-) H-2^d lymphoma cells and for FMRGa (+) H-2^a targets (results not shown). Similar results were obtained with 4R effector cells, whereas 5R and HTI remained ineffective regardless of the tumor tested. The absence of reactivity of HTI and 5R cells is specially significant since under the same conditions of restimulation the B6 and 4R activity was greatly increased. One can conclude that even highly stimulated spleen cells from HTI and 5R immunized with MSV do not react with the postulated H-2K^b/MuLV specificity. Similar conclusions can be derived from the experiments performed on the H-2^d haplotype. The spleen cells of BALB/c and C3H.OH which were cytolytic for H-2^d targets after in vivo
Table III

Cytotoxic Activity of Immune Spleen Cells after Con A In Vitro Restimulation

| Immune spleen cells | Days in vitro | Target cells | H-2 region common to CTL and target cells | Percent specific chromium release of target cells | LU/10^6 |
|---------------------|---------------|--------------|------------------------------------------|-----------------------------------------------|---------|
|                     |               |              | 100:1 | 50:1 | 10:1 | 3:1 | 1:1 |       |
| C57BL/6 anti-MSV    | 0 6 7         | MBL2 all H-2 | 20    | 18   | 8    | 6   | 2   | 0.3   |
|                     | 7             | LSTRA none   | 3     | 6    | 0    | 0   | 0   | 0     |
| B10.A(4R) anti-MSV  | 0             | MBL2 I-B to D| 43    | 24   | 12   | 4   | 0   | 0.7   |
|                     | 7             | LSTRA none   | 0     | 0    | 0    | 0   | 0   | 0     |
| B10.A anti-MSV      | 0             | MBL2 K to I-B| 5     | 0    | 0    | 0   | 0   | 0     |
|                     | 7             | LSTRA none   | 0     | 0    | 0    | 0   | 0   | 0     |
| HTI anti-MSV        | 0             | MBL2 K to S  | 2     | 0    | 0    | 0   | 0   | 0     |
|                     | 7             | LSTRA none   | 0     | 0    | 0    | 0   | 0   | 0     |
| A.TH anti-MSV       | 0             | MBL2 none    | 0     | 0    | 0    | 0   | 0   | 0     |
|                     | 7             | LSTRA D      | 0     | 0    | 0    | 0   | 0   | 0     |
| C3H.OH anti-MSV     | 0             | MBL2 none    | 0     | 0    | 0    | 0   | 0   | 0     |
|                     | 7             | LSTRA K to S | 18    | 14   | 9    | 0   | 0   | 0.1   |
| BABL/c anti-MSV     | 0             | MBL2 none    | 5     | 0    | 0    | 0   | 0   | 0     |
|                     | 7             | LSTRA all H-2| 25    | 12   | 7    | 0   | 0   | 0.2   |

* Spleen cells were sampled 17 days after MSV inoculation at a time when the tumors were completely rejected, about 7 days after the maximum of the primary CTL response.

† Day 0: activity of in vivo-primed nonrestimulated spleen cells. Day 6–9: activity of samples CTL in vitro cocultivated during 6–9 days with Con A.

§ Mean standard errors were always between 0.3 and 2.2%.

¶ CTL to target cells ratio.

In mice primed with Con A, effects of restimulation were studied on immune spleen cells, which were restimulated in vitro after Con A. Priming, displayed greatly increased activities after in vitro restimulation, and these activities remain virus specific, since nonviral L1210 cells are not lysed, and H-2 restricted, since the restimulated cells were unable to kill H-2b targets. On the contrary, the spleen cells of A.TH, HTI, and B10.A (5R) which were ineffective in vivo, remained so after in vitro culture with Con A.

Blocking of the Anti-MSV-CTL Activity by Monospecific Anti-H-2 Serums. It has been shown previously that incubation of tumor cells with related anti-H-2 antibodies completely and specifically abolishes their sensitivity to cytolysis by syngeneic anti-MSV-CTL (5). This observation suggests a close
topographical relationship between H-2 molecules and viral antigens, supporting the altered self hypothesis. In the present experiments advantage was taken of this phenomenon to study the nature of the altered H-2 antigens of oncornaviruses induced lymphoma cells. After incubation with monospecific anti-H-2 antibodies of different origins, the lymphoma cells were tested in the CRT. Relatively weak activities were frequent in the positive controls of these experiments and are due to the short incubation periods used in the CRT tests (6 h).

Study in the H-2^b haplotype. Lymphoma cells of B6 or BALB/B (H-2^b) were incubated with antisera reacting against the whole haplotype of the H-2 haplotype, or only against the products of the D or K regions and then exposed to syngeneic anti-MSV or anti-FLV-CTL. The results (Table IV) can be summarized as follows: (a) anti-H-2^b and anti-H-2D^b typing sera inhibit the activity of H-2^b anti-MSV or anti-FLV-CTL. (b) Anti-H-2K^b sera are completely inefficient. (c) Samples of the same anti-H-2K^b are however able to block the activity of allogeneic anti-H-2K^b CTL toward the same target cells. (d) Anti-Ia are ineffective as blocking sera. Similar results were obtained with all six H-1^b FMRGi (+) lymphomas tested (Table V). On the whole the results from the above experiments (Table IV) suggest that normal H-2K^b antigens are present on the surface of the FMRGi (+) H-2^b lymphomas and are able to react with the anti-H-2K^b allogeneic CTL. These antigens are accessible to anti-H-2K^b antibodies, since the latter can inhibit the cytolysis by allogeneic anti-H-2K^b CTL. However the H-2K^b molecules, unlike the H-2D^b, do not seem to be altered by the product of type C virus genome present at the lymphoma cell surface. Equally Ia specificities are apparently not concerned.

Study in the H-2^d haplotype. Blocking experiments were performed with the three available lymphomas of the H-2^d haplotype (Table VI and VII). In agreement with the results reported above in this haplotype the results were the following: (a) anti-H-2^d and anti-H-2K^d sera block the cytolysis of H-2^d targets by H-2^d anti-MSV-CTL. (b) Anti-H-2D^d do not block the same reaction. (c) Samples of the anti-D^d sera inhibit the cytolysis of H-2^d tumor cells by allogeneic anti-H-2D^d targets. (d) Anti-Ia are ineffective as blocking sera. Identical results were obtained with all three FMRGi (+) H-2^d lymphomas (Table VII). It can be concluded that in the H-2^d, as in the H-2^b haplotype, only one end of the MHC undergoes interaction with viral antigen, significant for cell-mediated lysis of oncornaviruses induced lymphoma cells. The involved region is different in the two H-2 haplotypes tested.

Discussion

These results obtained from the study of nine tumors show that: (a) an identity of the D region of the H-2 complex is necessary and sufficient to allow the cytolysis of H-2^b oncornaviruses induced lymphoma cells by syngeneic immune CTL. (b) An identity of the K end is necessary and sufficient when the tumor cells bear the H-2^d specificity. Blocking experiments with tumor cells preincubated with monospecific anti-H-2 sera are clearly consistent with the notion that the interaction between syngeneic effector and target cells involves exclusively the products of one region of the MHC: D^b or K^d. The H-2D^d or H-2K^b antigens however, normally represented on the H-2^d or H-2^b tumor cells are
TABLE IV
Cytolysis of MBL 2(H-2b) by Anti-MSV, Anti-FLV, and Allogeneic Anti-H-2b CTL:
Selective Effect of Specifically Directed Anti-H-2 Sera

| MBL2 targets preincubated with: | Percent specific chromium release* of the target cells in the presence of: |
|---------------------------------|---------------------------------------------------------------|
|                                 | B6 anti-MSV B6 anti-FLV BALB/c anti- H-2K b B10.D2 anti-B10.A(5R)§ |
|                                 | CTL CTL (anti-H-2KG) CTL                                    |
| No                              | 28 27 31 17                                                |
| Normal mouse serum              | 26 31 30 16                                                |
| AKR anti-C3H thymocytes         | 27 28 -- --                                                 |
| B10.D2 anti-B6                  | 0 4 2 0                                                    |
| (BALB/c × AKR/F, anti-EL4       | 2 3 -- --                                                  |
| B10.D2 anti-B6 in vivo absorbed in 4R | 27 -- 12 0                                               |
| (B10.A × HTGF, anti-EL4        | 26 28 -- --                                                 |
| (B10.BR2R × B10.HTI/F, anti-5R | 25 29 18 5                                                  |
| B10.D2 anti-B6 in vivo absorbed in 5R | 2 -- 17 15                                               |
| (B10.A × HTI/F, anti-EL4      | 0 0 -- --                                                   |
| (B10.RIII × B10.BR2R, anti-4R | 3 3 -- --                                                   |
| A.TL anti-A.TH                 | anti-Ia   29 29 -- --                                        |
| A.TH anti-A.TL                 | anti-Ia   30 29 -- --                                        |

* Effector to targets ratio 100/1, 6 h incubation, mean standard errors between 0.5 and 2%.
† The antisera had approximately equivalent titers: 1/1,000-1/2,000 in complement-dependent cytotoxicity.
§ Anti-H-2 b and anti-H-2K b immunizations were obtained by one i.p. inoculation of 5×10⁶ normal spleen cells 6 days before.
¶ Reacting at least with Ia9 on MBL2 cells.
¶¶ Reacting at least with Ia3 and Ia15 on MBL2 cells.

TABLE V
Selective Effects of Specifically Directed Anti-H-2 Sera on the Lysis of Six Different H-2b
Lymphomas by B6 Anti-MSV CTL

| Target cells preincubated with: | Percent specific chromium release of the target cells* |
|-------------------------------|--------------------------------------------------------|
| Sera                          | MBL2 (B6) Gil.11 (B6) RBL5 (B6) FBL3 (B6) HFL/B (BALB/c) A13 (BALB/c) |
| Normal mouse sera             | 16 10 15 16 18 13                                        |
| B10.D2 anti-B6                | anti-H-2 b 0 0 0 0 2 3                                    |
| B10.D2 anti-B6 in vivo absorbed in 4R | 17 13 15 13 17 21                                    |
| B10.D2 anti-B6 in vivo absorbed in 5R | 2 0 2 3 2 0                                              |

* Effector to target cells ratio 100/1, 6 h incubation.

perfectly able to function as target for allogeneic anti-H-2 CTL. These results suggest the existence of a specific interaction between a viral product and some molecule coded for by the MHC, these molecules differing with the H-2 haplotype.

The exclusive involvement of only one product of the MHC in the interaction between T-killer cells and syngeneic viral lymphomas is not the property of a given type C virus, since the nine tumors tested in these experiments were induced by five different agents: Friend, Rauscher, Moloney, Graffi, and Ten-
Table VI
Cytolysis of LSTRA (H-2^d) by Anti-MSV and Anti-H-2^d CTL Selective Effect of Specifically Directed Anti-H-2 Sera

| LSTRA preincubation with: | Percent specific chromium release* of the target cells in the presence of: |
|---------------------------|-------------------------------------------------------------------------|
| Serum: Specificity         | C anti-MSV | C3H anti-C3H.OL§ | B10 anti-B10.A(5R)§ |
| No                        | /         | 13               | 20               | 17               |
| Normal mouse serum         | /         | 15               | 25               | 20               |
| AKR anti-C3H thymocytes   | anti-Thy 1.2 | 15             | —               | —               |
| B10 anti-B10.D2            | anti-H-2^d | 0               | 0                | 2                |
| (B10.AKM × ABY) anti-B10.A| anti-H-2D^d | 16            | 27               | 0                |
| B10 anti-B10.D2 in vivo absorbed in B10.A(5R) | anti-H-2D^d | 12            | 22               | —               |
| (B10.A × ABY) anti-B10.D2 | anti-H-2K^d | 0             | 0                | 21               |
| B10 anti-B10.D2 in vivo absorbed in C3H.OL | anti-H-2K^d | 0             | 0                | —                |
| A.TL anti-A.TL¶           | anti-Ia^a  | 14            | —               | —                |
| A.TH anti-A.TL¶           | anti-Ia^a  | 16            | —               | —                |

*+, ‡, §, see footnotes *, ‡, § Table IV.
‡ No identified specificity reacting in this system.
¶ Reacting at least with Ia15 or LSTRA cells.

Table VII
Selective Effects of Specifically Directed Anti-H-2 Sera on the Lysis of Three Different H-2^d Lymphomas by C Anti-MSV CTL

| Target cells preincubated with: | Percent specific chromium release* of the target cells |
|---------------------------------|--------------------------------------------------------|
| Sera Specificity                | LSTRA | BC1 | T2 |
| Normal mouse sera               | —     | 15  | 14 |
| B10 anti-B10.D2                 | anti-H-2^d | 0  | 0  | 0 |
| (B10.AKM × ABY) anti-B10.A      | anti-H-2D^d | 16 | 15 | 16 |
| (B10.A × ABY) anti-B10.D2       | anti-H-2K^d | 0  | 2  | 0 |

* Effector to target cells ratio: 100/1, 6 h incubation.

Nant leukemia-viruses. Moreover it was observed for both anti-MSV and anti-FLV attacker cells. It must however be noted that the five agents share several biological or pathological properties, and that the tumor cells tested have in common the FMRG (+) serological specificity. One could suppose therefore that the same, or a closely related, viral protein is involved in all five cases in the interaction with the H-2 molecules. In other systems involving T-killer cells, it seems that normally both H-2D and H-2K specificity can function (1-4). In the H-2^b haplotype reactions involving the K^b antigens have been reported in response to nononcogenic viruses infections (15) or during immunization against hapten-modified syngeneic cells (16). In both cases, 5R effector cells were efficient against H-2^b targets, unlike the results described above in the MSV system. A predominant, or perhaps exclusive role of H-2D^b seems to exist, however, in the
interaction between H-2^b targets and allogeneic (but H-2 compatible) CTL reacting with minor histocompatibility antigens (17), including the H-Y specificity (18). In the H-2^d and H-2^K haplotypes both K and D region-directed molecules seem to be involved in viral (19) and haptenic (16) systems. An exclusive involvement of a D region product was described in an antitumor response (11) whereas we have found an exclusive involvement of the K end of the H-2^d haplotype in oncornavirus systems. We know, in addition, that a product of the K region is also strongly antigenic in the MSV system when H-2^k mice are tested (13). A similar exclusive involvement of the K^k specificity was reported in an anti-vaccinia viral immunization (14). One can conclude therefore that the product of the MHC involved in the self alteration may depend on the nature of the CTL-triggering factor. In some cases only one product may be concerned and this may be especially true for antitumor responses (11, and present results) making these systems particularly useful for the study of the biological significance of the H-2 restriction phenomenon.

The exclusive involvement of only one H-2 directed molecule in the CTL/target cell interaction can be explained by at least three different hypotheses: (a) several H-2 antigens are modified by the viral infection and/or transformation, but only one of them is antigenic for syngeneic T-killer cells precursors. (b) Only one H-2 product is involved because the viral proteins, or glycoproteins, have a specific relationship with this H-2 molecule. (c) The molecules involved in the interaction are not H-2 antigens but other cell surface component coded for by closely linked genes.

The first hypothesis is supported by previous observations in the trinitrophenyl-modified cell system (16). It implicates that Ir genes control the CTL generation in response to MSV or FLV infection, and that all the mice so far tested bear a nonresponder allele at the Ir gene locus directing the anti-H-2^K^b/MuLv or the anti-H-2^D^b/MuLv responses. Systematic experiments with different F1 hybrids between the nonresponder strains, for example 5R and HIT in the H-2^b experiment, and other non-H-2 inbred strains of mice might resolve this problem if one of the hybrid was able to react with the H-2^b lymphomas. Such experiments have been successful in the TNP system (16) and have allowed for the description of CTL-controlling Ir genes in the I region of the MHC. Similar experiments are now in progress in the MSV system to test further this hypothesis which cannot be definitively excluded.

The second hypothesis suggests that H-2^D^b/MuLv and H-2^K^d/MuLv do exist whereas H-2^K^b/MuLv and H-2^D^d/MuLv do not. This implies that viral products and H-2^K^d or H-2^D^d cannot interact to produce an altered self structure. The exclusive affinity of viral proteins for H-2^D^b can be supported by the recent observations that H-2^D^b antigen, to the exclusion of any other H-2 specificities, can be detected inside FLV virions produced in H-2^b/H-2^d heterozygotes (28). This observation demonstrates, that the inclusion of H-2 molecules into type C virus particles is selective rather than random. It is interesting to observe that the same association between H-2^D^b and viral structures already exists at the host cell surface in H-2^b producer cells, suggesting that H-2^D^b antigens may possibly play a specific role in viral assembly. According to this assumption one expects that H-2^K^d but not H-2^D^d antigens should be present inside the virions produced in H-2^d cells. Good evidences for the physical association between FLV antigens
and H-2D\textsuperscript{b} molecules have been also obtained by capping of the viral antigens on H-2\textsuperscript{b} tumor cells, since a partial cocapping of H-2D\textsuperscript{b} but not H-2K\textsuperscript{b} was observed (29). The latter experiment, however, does not demonstrate that the capping antigen is the same as the antigens reacting with the CTL.

The third hypothesis is that H-2 directed molecules distinct from H-2D and H-2K antigens are involved in the CTL/target cell interactions. At least two cell surface components should be discussed: Hh antigens (30) and the hypothetical product of the RFV/1 gene (31). The involvement of Hh rather than H-2 molecules cannot be ruled out since the Hh.1 specificity is coded for in the D region and the Hh.3 in the K region of the MHC. An apparent exclusive involvement of the D\textsuperscript{b} and K\textsuperscript{d} regions would be predicted in the combinations we have studied if Hh molecules were concerned since anti-Hh.1 reactions are strong against H-2\textsuperscript{b} targets and anti-Hh.3 against H-2\textsuperscript{d} and H-2\textsuperscript{k} targets, (G. Cudkowicz, personal communication). The hypothetical product of the RFV/1 gene must be also considered since this gene which maps to the D region (31) controls resistance to the FLV induced disease. Moreover it has been suggested that the RFV/1 gene acts by directing the persistence or elimination of viral antigens from the host cell surface (32), which might be achieved by the specific association of viral proteins and a normal cell surface structure. However the observation that a K end product is involved in the CTL/target cells interaction in the H-2\textsuperscript{d} haplotype does not support the idea that an RFV/1 gene product would be concerned in this case.

Summary

It was demonstrated previously that the cytolysis of murine viral lymphoma cells by anti-murine sarcoma virus (MSV) syngeneic T-killer lymphocytes was restricted by some products of the H-2 complex. The respective role of the products of different regions of the H-2 complex were studied with six H-2\textsuperscript{b} and three H-2\textsuperscript{d} lymphomas induced by five different type C viruses. They were tested in a classical chromium release test against anti-MSV T-killer cells obtained from different inbred strains of mice, including several H-2 recombinants. Tumors of the H-2\textsuperscript{b} haplotype were lysed only when effectors and target cells have in common the D\textsuperscript{b} region. On the contrary an identity limited to the K end of the H-2 complex is necessary and sufficient in the H-2\textsuperscript{d} haplotype. An in vitro restimulation of the spleen cells with concanavalin A strongly increased the activity of in vivo-primed T lymphocytes but did not provide any response for in vivo-primed but nonresponder cells. Preincubation of the tumor cells with anti-H-2 sera abolished the lysis by syngeneic anti-MSV effector lymphocytes. The same results were obtained by preincubating the H-2\textsuperscript{b} targets with anti-H-2D\textsuperscript{b}, or the H-2\textsuperscript{b} target with anti-H-2K\textsuperscript{b}. Preincubation with anti-H-2K\textsuperscript{b} or anti-H-2D\textsuperscript{d} were ineffective. These results show that the T-killer/target cells interaction in the MSV system involved some products of the H-2 complex which might be different with the various H-2 haplotypes and could possibly vary according to the antigenic specificity. A specific association of a viral product with a normal cellular structure, directed by the H-2 region during the viral budding could explain the observed results.

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