H-2 RESTRICTION OF VIRUS-SPECIFIC CYTOTOXICITY
ACROSS THE H-2 BARRIER

Separate Effector T-Cell Specificities Are Associated with
Self-H-2 and with the Tolerated Allogeneic H-2 in Chimeras*

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Increasing experimental evidence suggests that most if not all T-cell-mediated immune functions in mice are dependent on structures coded in the major histocompatibility (H-2) complex. To express an IgG immune response to soluble, chemically inert, noninfectious antigens, T-helper cells from conventional mice and precursors of antibody-forming B cells must share the I region of H-2 (1-5). Similarly, the specificity of murine T cells which are sensitized to infectious agents, chemically reactive antigens, or to alloantigens is determined by cell surface self-structures coded in the K and D regions of H-2 (6-10, reviewed in 11-13). The antigen recognized by T cells has been postulated to be "altered self", i.e., altered major transplantation antigens or structures that are coded very closely to them (altered self hypothesis). An alternative explanation is the dual recognition model. It suggests that T cells interact dually with other cells via an immunologically specific receptor for antigen and structures for self-recognition (reviewed in 3, 11, 12, 14).

The rule that H-2 compatibility is required between T cells and the cells with which they interact is apparently not absolute. T cells from zygote fusion chimeras (tetraparental mice, 15) or from irradiated F₁ bone marrow chimeras (16) can cooperate with bone marrow-derived lymphocytes (B cells) of syngeneic and the tolerated allogeneic haplotypes. Similarly, virus-immune or TNP-immune cytotoxic T cells from irradiated F₁ bone marrow chimeras can lyse infected targets of both syngeneic and tolerized allogeneic, but not of unrelated, H-2 haplotypes (17-19). This finding has been used extensively in discussing the nature of the H-2 restriction. At first glance these results suggest that the specificity of T cells is for the viral antigen alone and favor the physiological interaction model (3). However, this interpretation depends on the assumption that the same cytotoxic T cell can kill infected syngeneic as well as tolerated allogeneic targets. Therefore, it seems crucial to determine, first, whether the crossing of the H-2 barrier by H-2-tolerant virus-immune cytotoxic T cells

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reflected the absence of allogeneic inhibition or suppression, or second, whether under tolerance conditions separate T cells of one H-2 type are generated which are differentially associated with either the syngeneic or the tolerated allogeneic H-2 haplotype. To distinguish between these two possibilities, the specificity of virus immune T cells from irradiation bone marrow chimeras was investigated. The results from using cold unlabeled targets for competitive inhibition of virusespecific 51Cr release in cytotoxicity assays in vitro and from selective secondary restimulation experiments in mixed lymphocyte cultures (MLC) support the second possibility. Thus, virus-specific cytotoxicity across the H-2 barrier is also H-2 restricted.

Materials and Methods

Mice. C3H, DBA/2, B10.BR, B10.D2, C57BL/6, C3H × DBA/2 F1 were purchased from the Jackson Laboratory, Bar Harbor, Maine. B10.BR × B10.D2 F1, were bred at the Scripps Clinic and Research Foundation. Chimeras were produced according to the method of von Boehmer et al. (16). C3H × DBA/2 were irradiated with 900 rads, B10.BR × B10.D2 F1 with 950 rads. 24 h later these mice were transfused with a total of 2 x 10^7 AKR anti-0 C3H + complement (C')-treated bone marrow cells. Mice were reconstituted either with bone marrow cells from one parent alone or with equal amounts of cells from both parents. Their drinking water was supplemented with antibiotics for 2 wk. Of about 70 chimeras, 60% survived and 20% died during the first 10-12 days after reconstitution.

Virus. The WE strain of lymphocytic choriomeningitis virus (LCMV) and the WR strain of vaccinia virus (a gift from Dr. W. K. Joklik, Duke University, Durham, N. C.) have been described (20-22). Mice were immunized with 5 x 10^6 plaque-forming units (PFU) of LCMV or 1 x 10^7 of vaccinia virus, and spleens were harvested 7 and 6 days later, respectively.

Cell Lines. The cell lines used have been described previously (18, 20). The persistently LCMV-infected L929 cells were originally obtained from Dr. M. B. A. Oldstone, Scripps Clinic and Research Foundation.

Antisera. Anti-H-2 sera were produced by hyperimmunizing B10.BR mice with B10.D2 spleen cells and vice versa. Incubation of appropriate target cells at 5 x 10^7/ml with 1:2-1:30 diluted antisera (30 min, 4°C) and with a selected unabsorbed rabbit C' (1:6, 30 min, 37°C) lysed >97% of the relevant spleen cells, but did not lyse syngeneic or unrelated spleen cells (18). AKR anti-0 C3H was from Bionetics, Kensington, Md., (cat. 8301-01, lot no. 231-61-6), used at 1:10 (30 min, 4°C) and with rabbit C' (1:6, 30 min, 37°C) it lysed 42% of the spleen cells specifically.

MLC In Vitro and 51Cr Release Assay. Primary alloreactive cytotoxic T cells were generated in vitro as described by Lafferty et al. (23). Secondary in vitro MLC were performed according to Plata et al. and Dunlop and Blanden (24, 25). Minimal essential medium supplemented with 10% heat-inactivated fetal calf serum, nonessential amino acids, and antibiotics (complete medium) (Flow Research Laboratories, Rockville, Md.) was used with 3 x 10^-4 M β-2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.). The 51Cr release assays and the methods used for competitive inhibition with unlabeled cold targets have been described in detail (7, 26).

Results

Characterization of Chimeras. The following criteria indicated that lymphoreticular chimerism was completely established in the irradiated bone marrow reconstituted chimeras. At sacrifice, none of the normal or infected mice showed macroscopic signs of ongoing graft versus host disease. Lymphocytes from P1 → F1, P2 → F1, and P1 + P2 → F1 chimeras were able, in MLCs, to generate cytotoxicity against an unrelated third H-2 haplotype but not against either of

1 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; MLC, mixed lymphocyte cultures; PFU, plaque-forming units.
the parental (P) H-2 types. Thus, chimeric lymphocytes were specifically unresponsive to the tolerizing H-2 haplotype (Table I). Reconstitution of the chimeras was complete in all cases tested, since the anti-H-2k and H-2d antisera plus C’ treatments caused lysis of virtually all spleen cells from C3H → C3H × DBA/2 F1, and from DBA/2 → C3H × DBA/2 F1, cells, respectively. The unrelated anti-H-2 serum did not cause significant lysis. The tetraparental F1 chimeric spleen cells were all lysed by anti-H-2k and anti-H-2d antisera combined; each alone lysed about 50% of the cells (Tables II and III).

**Virus-Specific Cytotoxic T Cells From Chimeras Lyse Across the H-2 Barrier.** Vaccinia-virus immune spleen cells of one parental haplotype from all P1 (H-2k) → F1 (H-2k×d) or P2 (H-2d) → F1 (H-2k×d) chimeras lysed syngeneic and tolerated parental allogeneic virus-infected target cells but not uninfected targets or the unrelated third party infected target cells of H-2b type (Table II, Exp. 1). This activity was anti-0-sensitive (Table II, Exp. 2). Exactly the same results were obtained with F1 chimeras that had been reconstituted with 14-16-day-old fetal liver cells of one parental H-2 type (data not shown).

Tetraparental F1 bone marrow chimeras (P1 + P2 → F1) infected with vaccinia virus or LCMV generated anti-0-sensitive cytotoxic T cells of both parental H-2 types, and each of these T-cell types lysed both infected parental type targets but not normal targets or cells of unrelated H-2 types (Table II, Exp. 3; Table III).

**Specificity of Chimeric T Cells.** The specificity of chimeric virus-immune effector T cells was first determined by competitive inhibition experiments in vitro. 31Cr release from infected syngeneic H-2k or tolerated allogeneic H-2d targets caused by LCMV-immune cytotoxic T cells from C3H (H-2k) → C3H × DBA/2 F1, (H-2k×d) could be inhibited competitively only with unlabeled infected targets that were H-2 compatible with the labeled target. Thus, unlabeled cold LCMV-L cells inhibited 31Cr release from LCMV-L 929 cells by C3H → C3H × DBA/2 F1, immune spleen cells completely, but had little effect on lysis of labeled LCMV-J774. Cold LCMV-J774 had little effect on lysis of labeled LCMV-L929 cells, however, competed with labeled LCMV-J774 to about 70% (Table IV, Exp. 1). With higher ratios of infected competitor cells added (6:1), the inhibition of 31Cr release was even more evident (Table IV, Exp. 2). These results indicate the presence in these chimeras of at least two (probably four) sets of T-cell specificities associated with either the syngeneic or the tolerated allogeneic H-2 type. Only cold targets, which were compatible with both immune spleen cells and labeled targets, competed similarly in the specific lysis by C3H or BALB/c LCMV-immune spleen cells (Table IV, Exp. 1). These inhibitions were less pronounced than for the chimeras because the cytotoxic activity was greater than that of the chimeras. Therefore competition by threefold excess of cold targets was not sufficient to inhibit 31Cr release by more than 40–45%.

Further evidence for the presence of at least two separate T-cell specificities in chimeras that lysed either the syngeneic or alternatively the tolerated allogeneic-infected target cells, but not both, was obtained from secondary restimulation of memory T cells in vitro (Table V). LCMV-immune spleen cells from C3H → C3H × DBA/2 F1, mice, which were infected 4-wk previously, were restimulated in vitro with infected macrophages from the syngeneic (H-2k) or infected J774 cells of the allogeneic (H-2d) parents at a ratio of 8:1 for 4 days. Cytotoxic activity was restimulated predominantly against the LCMV-target which was
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**Table I**

| Responder lymph node cells (H-2 haplotype) | Stimulator spleen cells | Percent \(^{51} \text{Cr} \) release from target cells at lymphocyte to target ratios§ |
|------------------------------------------|------------------------|---------------------------------------------------|
|                                          | EL4 (b) | L929 (k) | P815 (d) |
| C57BL/6 (b) | C57BL/6 | 19| 19 | 12 | 10 | 11 | 10 |
| C3H | 20 | 20 | 96¶ | 27 | 14 | 12 |
| BALB/c | 24 | 19 | 24 | 15 | 101|| 29 |
| C57BL/6 (b) | C57BL/6 | 27 | 19 | 12 | 10 | 11 | 10 |
| C3H | 20 | 20 | 96¶ | 27 | 14 | 12 |
| BALB/c | 24 | 19 | 24 | 15 | 101|| 29 |
| C3H × DBA/2 F1 (k × d) | C57BL/6 | 102¶ | 30 | 13 | 11 | 22 | 12 |
| C3H | 27 | 21 | 18 | 12 | 18 | 14 |
| BALB/c | 26 | 23 | 18 | 15 | 20 | 13 |
| C57BL/6 (b) | C57BL/6 | 29 | 20 | 19 | 14 | 11 | 11 |
| C3H | 20 | 20 | 96¶ | 27 | 14 | 12 |
| BALB/c | 24 | 19 | 24 | 15 | 101|| 29 |

* C3H × DBA/2 F mice were irradiated with 900 rads and 1 day later reconstituted with 2 × 10^7 viable anti-4-treated bone marrow cells of one or of both parents. Mice were sacrificed 2 mo later, and mesenteric lymph nodes were used in MLC.

† MLC were done as described by Lafferty et al. (23). 2 × 10^6 responders and 4 × 10^6 850 rads irradiated stimulator cells were cultured for 5 days in 24 flat-bottomed well tissue culture plates (Linbro Chemical Co., New Haven, Conn.) with 3 × 10^{-5} M β-2-mercaptoethanol in complete medium.

§ \(^{51} \text{Cr} \) release assays used 5 × 10^4 target cells/well mixed with 7.5 × 10^4 or 1.5 × 10^4 viable cells from MLC.

|| Means from triplicates. SEM were 0.5-1.3% for EL4, 0.7 to 2.0% for L929, and 0.6 to 2.0% for P815.

¶ Significantly greater than other values (P < 0.001).

**Discussion**

These results demonstrate, one, that vaccinia virus or LCMV-infected tetraparental P1 + P2 → F1 chimeras generate T cells of one or the other parental H-2 haplotype, and each set of these T cells can lyse across the H-2 barrier infected target cells of the other tolerated parental H-2 type. Two, virus-infected irradiation bone marrow chimeras generate cytotoxic T cells of one parental H-2 type and of at least two separable H-2-restricted specificities; one is specific for infected syngeneic targets, the other for infected tolerated parental allogeneic target cells of the tolerated parental H-2 specificity. Third, virus immune T cells from chimeras are not lytic against infected target cells of an unrelated H-2 type.

Therefore, one can conclude first, that the requirement to recognize H-2 is unidirectional in that only the T cells must recognize the same altered H-2 to which they have been sensitized. This conclusion is supported by the evidence
Table II
Vaccinia Virus-Specific Cytotoxic Activity in Spleens From F, Irradiation Chimeras

| Immune spleen cell donors (H-2 haplotype) | Antiserum treatment + C" (% lysed) |         |         |         |
|------------------------------------------|-----------------------------------|---------|---------|---------|
|                                          | Vaccinia infected                  | Vaccinia uninfected | Vacccinia infected | Vaccinia uninfected |
|                                          | L929 (a)                           | P815 (d) | C57BL/6 (a) |         |
| Experiment 1 DBA/2 (d) →                 | None                               | (11)    | 48      | 16      | 83      | 20      | 34      | 35      |
|                                          | C57 x DBA/2 F, (k x d)             | Anti-H-2" | 444**   | 14      | 825**   | -       | -       | -       |
|                                          | Anti-H-2*                          | (20)    | 20      | -       | 20      | -       | -       | -       |
| C3H (k) →                               | None                               | (20)    | 505     | 15      | 815     | 21      | 38      | 38      |
|                                          | C3H x DBA/2 F, (k x d)             | Anti-H-2" | (96)   | 25      | 23      | -       | -       | -       |
|                                          | Anti-H-2*                          | (21)    | 55**    | 0       | 785**   | -       | -       | -       |
| C3H (k)                                  | None                               | (16)    | 71      | 17      | 21      | 20      | 36      | 37      |
|                                          | Anti-H-2"                          | (97)    | 28      | -       | -       | -       | -       | -       |
|                                          | Anti-H-2*                          | (17)    | 606**   | 0       | -       | -       | -       | -       |
| Normal C3H                               |                                    |         | 15      | -       | -       | -       | 33      | 36      |
| BALB/c (d)                               | None                               | (12)    | 13      | 15      | 815     | 18      | 37      | 38      |
|                                          | Anti-H-2"                          | (13)    | -       | -       | 844**   | -       | -       | -       |
|                                          | Anti-H-2*                          | (95)    | -       | -       | 14      | -       | -       | -       |
| Normal BALB/c                            |                                    |         | 14      | 16      | 13      | 15      | 35      | 37      |
| C57BL/6 (b)                             |                                    |         | 17      | 18      | 15      | 18      | 845     | 35      |
| Medium                                   |                                    |         | 13      | 15      | 13      | 15      | 37      | 39      |
| Experiment 2 C3H (k) →                  | None                               | (15)    | 706     | 28      | 925     | 37      | -       | -       |
| C3H x DBA/2 F, (k x d)                  | Anti-H-2"                          | (>96)   | 27      | -       | 35      | -       | -       | -       |
|                                          | Anti-H-2*                          | (17)    | 709**   | 0       | 901**   | -       | -       | -       |
|                                          | AKR-anti-θ                         | (54)    | 27      | -       | 37      | -       | -       | -       |
|正常 C3H                                 | C3H normal AKR serum               | (17)    | 745**   | 0       | 905     | -       | -       | -       |
| Experiment 3 B10.BR (a) + B10.D2 (d) →  | None                               | (14)    | 864     | 42      | 724     | 28      | 31      | 33      |
| B10.BR x B10.D2 F, (k x d)              | Anti-H-2"                          | (>96)   | 42      | 42      | 23      | 29      | -       | -       |
|                                          | Anti-H-2*                          | (52)    | 645     | 41      | 495     | 29      | -       | -       |
|                                          | Anti-H-2"                          | (60)    | 709     | 42      | 455     | 28      | -       | -       |
| C57BL/6 (b)                             | none                               | (14)    | 44      | 43      | 27      | 30      | 911     | 34      |

* F, chimeras were used 4-10 wk after reconstitution. Mice were infected i.v. with 1 x 10^7 PFU of WR vaccinia virus and sacrificed 6 days later.
† Single cell suspensions of spleen cells (5 x 10^8/ml) in minimal essential medium were incubated with anti-H-2 sera at a 1:2 dilution with anti-θ sera of a 1:10 final dilution for 30 min at 4°C. Rabbit C" was added at a 1:8 final dilution for 30 min at 37°C. Cell viability was determined by trypan blue exclusion. Cells were washed and resuspended according to their viability before antiserum treatment and assayed at 30 total spleen cells to 1 target cell.
‡ 1 Cr release assay was performed as described. Incubation time was: Experiment 1, 6 h at 37°C; Experiment 2, 7 h; Experiment 3, 12 h.
§ Means of triplicates. SEM were 0.8 to 2.2 for L929, 1.6 to 2.5 for P815, and 0.6 to 2.5 for C57BL/6 macrophages.
∥ Statistical significantly greater than normal spleen control (P < 0.01).
** Not different from untreated control.
†† ‑, not tested.

that anti-H-2 sera directed against K- and D-region products of the target cell alone can inhibit lysis (27, 28), and that target cells which do not express H-2 but are infected with virus are not lysed by T cells (R. M. Zinkernagel and M. B. A. Oldstone, unpublished). Second, it is not the simple lack of allogeneic or other suppressive effects by allogeneic target or spleen cells which does not permit
**H-2 Restriction of Cytotoxicity Across H-2 Barrier**

### Table III

**LCMV-Specific Cytotoxic Activity in Spleens From Tetraparental F₁ Irradiation Chimeras***

| Immune spleen cell donors (H-2 haplotype) | Antiserum treatment† + C’ (% lysed) | Percent ³¹Cr release from target cells§ | L929 (k) | J774 (d) |
|-----------------------------------------|-----------------------------------|----------------------------------------|---------|---------|
|                                         |                                   |                                        | LCMV Uninfected | LCMV Uninfected |
| C3H (k) + DBA/2 (d) → C3H × DBA/2 F₁, (k × d) | None (21) | 47§ | 28 | 73§ | 37 |
|                                          | Anti-H-2k (69) | 42§ | −** | 67§ | − |
|                                          | Anti-H-2d (66) | 44§ | − | 69§ | − |
|                                          | AKR anti-θ (60) | 29 | − | 33 | − |
|                                          | Normal AKR serum | 45§ | − | 67§ | − |
| C3H × DBA/2 F₁, (k × d)                  | None (13) | 50§ | − | 87§ | 38 |
|                                          | Anti-H-2k (>96) | 28 | − | 38 | − |
|                                          | Anti-H-2d (>96) | 27 | − | 37 | − |
|                                          | Anti-H-2b (15) | 48§ | − | 80§ | − |
|                                          | AKR anti-θ (62) | 27 | − | 38 | − |
| Normal C3 + D2 → C3H × DBA/2 F₁         | None | 28 | 24 | 36 | 37 |
| Normal C3H × DBA/2 F₁                   | None | 28 | 25 | 35 | 36 |
| Medium                                  |         | 30 | 24 | 33 | 34 |

* One chimera (10 wk) was infected i.v. with 5 x 10⁵ PFU of WE LCMV and sacrificed 7 days later.
† Single cell suspensions in minimal essential medium (5 x 10⁶/ml) were treated with antisera as in Table II.
§ ³¹Cr release assay was done at a viable lymphocyte to target ratio of 30:1 for 12 h at 37°C.
|| Means of triplicates. SEM were 1.1 to 2.0 for L929 and 1.4 to 2.9 for J774.
†† Significantly greater than controls, P < 0.01.
** − , not tested.

lysis of infected targets of the tolerized H-2 type across the H-2 barrier. Third, the H-2 restriction is in fact absolute since the virus-immune chimeric cytotoxic T cells that can cross the H-2 barrier cannot lyse syngeneic infected target cells or vice versa.

The H-2 restriction phenomenon thus does not result from a requirement for compatibility at H-2K or D, but reflects the specificity of any cytotoxic T cells for viral antigen to which they have been sensitized (10, 11) and for any self or tolerated K- or D-coded cell surface structure. Virus-immune T cells from conventional H-2k mice can only lyse infected H-2k target cells because they have been sensitized exclusively against virus-associated H-2k and have never encountered viral antigen associated with H-2d or H-2b. In H-2k → H-2k×d F₁ chimeric mice H-2k lymphocytes accept H-2k as well as H-2d as "normal" self cell surface markers. During infection they are thus exposed to, and distinct and separate T cells are sensitized to, viral antigen associated with H-2k and viral
TABLE IV
Separate Sets of T Cells of One H-2 Haplotype From F1 Bone Marrow Chimeras Are Specific for Altered Self H-2 and For Altered Alloantigen Demonstrated by Cold Target Competition Experiments In Vitro

| Immune spleen* cells (H-2 haplotype) | Antiserum* + C' treatment | Unlabeled† LCMV-infected competitor target cells (H-2 haplotype) | Percent ‡Cr release from LCMV-target cells§ |
|-------------------------------------|---------------------------|---------------------------------------------------------------|------------------------------------------|
|                                    |                           |                                                               | L929 (k)                                 |
|                                    |                           |                                                               | J744 (d)                                 |
|                                    |                           |                                                               | C57BL/6 (b)                              |
| Experiment 1                        |                           |                                                               |                                          |
| C3H (k)                             |                           |                                                               |                                          |
| C3H × DBA/2 F1 (k × d)              | C' control None           | 42 ||| 78 | - |||
|                                    | Anti-H-2d None            | 28 43 -                                                        |
|                                    | Anti-H-2k None            | 40 | 72 | - |
|                                    | None None                 | 45 | 82 | 34 |
|                                    | None 3 × LCMV-J774 (d)    | 43 | 52 | **-|
|                                    | None 3 × LCMV-L929 (k)    | 28 | 80 | - |
|                                    |                           |                                                               |                                          |
| BALB/c (d)                          | None None                 | 29 | 76 | 36 |
|                                    | 3 × LCMV-J774 (d)         | - 61 | **-|
|                                    | 3 × LCMV-L929 (k)         | - 74 | - |
|                                    | Anti-H-2d None            | - 77 | - |
|                                    | Anti-H-2k None            | - 41 | - |
|                                    |                           |                                                               |                                          |
| C3H (k)                             | None None                 | 79 | 44 | 33 |
|                                    | 3 × LCMV-J774 (d)         | 71 | - | - |
|                                    | 3 × LCMV-L929 (k)         | **- | - | - |
|                                    | Anti-H-2d None            | ~- | - | - |
|                                    | Anti-H-2k None            | 78 | - | - |
|                                    |                           |                                                               |                                          |
| Normal C3H                          | None None                 | 28 | 39 | 34 |
| C57BL/6 (b)                         | None None                 | 32 | 45 | 86 |||
|                                    |                           |                                                               |                                          |
| Experiment 2                        |                           |                                                               |                                          |
| C3H (k)                             |                           |                                                               |                                          |
| C3H × DBA/2 F1 (k × d)              | C' control None           | 57 | 56 | - |
|                                    | Anti-H-2d None            | 35 | 19 | - |
|                                    | Anti-H-2k None            | 56 | 54 | - |
|                                    | None None                 | 58 | 57 | 42 |
|                                    | None 6 × LCMV-J774 (d)    | 54 | 20 | **-|
|                                    | None 6 × LCMV-L929 (k)    | 38 | 55 | - |
|                                    |                           |                                                               |                                          |
| C57BL/10 (b)                        |                           | 44 25 76 | - |

* Mice were infected i.v. with 5 × 10⁶ PFU of WE LCMV and sacrificed 7 days later. For antiserum treatment see Tables II and III and Materials and Methods.
† Cold target competition experiments were performed as described originally by Ortiz de Landazouri and Herbermann (26) and as used previously in this system (7). A threefold excess of unlabeled LCMV-infected target cells was added to the labeled monolayers and mixed with the added spleen cells.
‡ ⁵¹Cr release assays were performed at a 10:1 lymphocyte to target ratio for 12 h at 37°C.
§ Means of triplicates. SEM were 1.1 to 2.5 for L929 cells, 1.5 to 2.7 for J774, and 1.2 to 2.8 for C57BL/6 macrophages. Percent release by immune cells on uninfected target cells were for Exp. 1: 26–29 on L929, 38–43 on J774, 32–36 on C57BL; Exp. 2: 35–42 on L929, 20–23 on J774, 38–41 on C57BL. C57BL immune cells caused release from normal targets which was not different from the release on infected targets.
¶ Significantly greater than relevant control values (P < 0.05).
** Significantly smaller than when cold H-2 incompatible LCMV targets were added (P < 0.05).
†† Not tested.
Selective Secondary Restimulation In Vitro of Distinct LCMV-Immune T Cells Specific Either For Altered Self or For Altered Alloantigen

| Spleen cells* restimulated in vitro | LCMV-infected stimulator cells (H-2 haplotype) | Percent \(^{31}\text{Cr} \text{ release from target cells}$ |
|------------------------------------|-----------------------------------------------|--------------------------------------------------|
|                                    | LCMV-infected | LCMV Uninfected | LCMV Uninfected |
|                                    | $\text{LCMV-immune}$ | $\text{Normal}$ | $\text{LCMV-immune}$ | $\text{Normal}$ |
| C3H $\rightarrow$ C3H × DBA/2 F\(_1\) (k) | C3H M\(_k\) (k) | 60\(\%\) | 40 | 38 | 31 |
| C3H × DBA/2 F\(_1\) (k × d) | C3H M\(_k\) (k) | 34 | 34 | 47 | 41 |
| Normal C3H × DBA/2 F\(_1\) (k × d) | C3H M\(_k\) (k) | 5\(\%\) | 39 | 50 | 31 |
| C3H $\rightarrow$ C3H × DBA/2 F\(_1\) (k) | J774 (d) | 32 | 34 | 10\(\%\) | 33 |
| C3H × DBA/2 F\(_1\) (k × d) | J774 (d) | 31 | 33 | 48 | 35 |
| Normal C3H × DBA/2 F\(_1\) (k × d) | J774 (d) | 31 | 33 | 52 | 38 |
| Normal C3H × DBA/2 F\(_1\) controls | | 32 | 37 | 34 | 29 |
| Medium | 30 | 34 | 35 | 30 |

* Spleen cell donors were infected with $5 \times 10^2$ PFU of WE LCMV and sacrificed 4 wk later. Immune or normal spleen cells, $4 \times 10^6$/well, were cocultivated with $5 \times 10^5$ LCMV-infected cells (peritoneal cells or J774 cells infected for 24 h with LCMV) in complete medium containing $3 \times 10^{-5}$ M mercaptoethanol for 4 days at 37°C.

\(\%\) \(^{31}\text{Cr} \) release assay was done at a viable lymphocyte to target cell ratio of 10:1 for 12 h at 37°C.

\(\%\) Means of triplicates. SEM were 1.0 to 2.1 for L929 and 1.3 to 2.8 for J774.

| Significantly greater than on uninfected targets or than normal spleen cell stimulated in vitro and assayed on infected targets ($P < 0.01$). |

antigen associated with H-2\(^d\). One can extrapolate from experiments with conventional heterozygote F\(_1\) and H-2 recombinant mice (8) that P\(_k\) (H-2\(^k\) → F\(_1\) (H-2\(^k\) × d) chimeras probably have four sets of T-cell specificities, one each associated with the syngeneic and allogeneic tolerated K and D specificities.

The results presented here are compatible with the idea that separate cytotoxic effector cells from chimeras are specific for altered self and alternatively for altered tolerezed alloantigen. This interpretation implies that the H-2K and D structures are not only mandatory because they may be the sites at which cell wall damage measurable by \(^{31}\text{Cr} \) release is made easiest, but also because they are an essential part of the sole antigenic entity seen by T cells expressing clonally a single immunological receptor (altered self hypothesis).

Is there another explanation for the H-2 restriction of virus-specific cytolyis across the H-2 barrier? Does H-2-associated specificity of effector T cells reflect specificity for altered self or, alternatively, the need for a dual recognition one for viral antigen and one for a K- or D-coded self-marker (6–11), or is the answer somewhere in between these extremes? Stated differently, is the distinction made by the T cell between uninfected and infected target cells a process that involves active recognition of both infected and uninfected cells or recognition...
only of infected targets (29). A question of general importance, which unfortunately cannot yet be answered because all the available data concerns effector T cells only, is whether dual or physiological recognition may be essential at the T-cell induction level.

The apparently general H-2 restriction of virus-immune T-cell specificity even for chimeric T cells, therefore, could also be explained with the dual interaction model. It assumes that T cells recognize normal self (but do not react with normal self). Therefore in irradiation bone-marrow chimeras, interaction structures for the tolerated alloantigens, as well as self, but not for unrelated alloantigens, differentiate during the regeneration process of the lymphoreticular system (14). As pointed out earlier, the dual interaction model would demand the existence of two separate clonally expressed recognition systems on virus-immune cytotoxic T cells, one immunologically specific for viral antigen and one for self recognition separately for each K or D structure present (7). The evidence presented here and the results from testing conventional F1 and H-2 recombinant mice (7) are compatible with this dual recognition model only if interaction structures for self or tolerated alloantigen are also expressed clonally and on separate T cells for each K or D structure, together with the generally accepted clonally expressed immunologically specific receptors. T cells specific for viral antigen which express the physiological interaction structure for tolerated allogeneic H-2K do not express the structures for syngeneic H-2K or the syngeneic or allogeneic H-2 self-structures.

Because of the fact that virus-immune T cells from some of the mutant mice do not lyse infected target cells of wild-type H-2 specificity (20), it is unlikely that the physiological interaction structures are coded for by the same genes as the H-2K and H-2D loci. Therefore, the appropriate genes would have to be either closely linked to these loci or not be located in the K or D regions of H-2 or not within H-2 at all. This alternative hypothesis is thus a model for T-cell recognition involving simultaneously two distinct, clonally expressed receptors that are not identical with the now known H-2 gene product and may not be an H-2 gene product at all. The cell structure that is recognized is a K- or D-region product for cytolytic interaction, probably an I-region-coded structure for helper T-cell interactions. The frequency of clonal expression of recognition units for antigen and for the self-marker would have to differ vastly in that the frequency of T-cell clones expressing self-interaction receptors would be much greater.

The double clonal, dual recognition system implies that T-cell-mediated cell damage which results in target cell death and ^{31}Cr release is made through the H-2K and H-2D self-marker. Therefore activity against alloantigens may be explained most simply as follows. Because the K and D structures are the sites for self-T-cell-mediated cell wall damage the recognition of them by a single immunological receptor is sufficient for lysis. Double recognition is not needed because the immunological receptor against alloantigen hits the crucial structure directly. In contrast, the self-recognitive structure alone cannot deliver the lytic signal in a closed syngeneic system. The self-T cells possessing immunological receptors against self-K or D structures, i.e. self-reactive T cells, must have been eliminated constantly, probably in the thymus (29).

Why has it proven impossible so far to generate virus-immune or TNP-specific
cytotoxic T cells across the H-2 barrier by cocultivating lymph node cells of H-2d with virus-infected or TNF-modified allogeneic H-2k lymphoid stimulator cells? The interaction of T cells from conventional mice with viral antigens on allogeneic cells is unlikely to trigger measurable T-cell activity for viral antigen on the allogeneic cell, first, because activity against alloantigens is more easily triggered and therefore supersedes (e.g., because of additional I-region differences and because alloreactivity requires only one immunologically specific interaction without self-interactions) and, second, because the combination of interaction structures for alloantigens and recognition structure for virus is rare or absent. This imbalance would change under conditions of tolerance. Here interaction structures for self and tolerated alloantigen could occur with about the same frequency. The possibility that the self-recognitive structure and the immunological receptor recognizing this K or D structure as alloantigen are probably identical has been discussed elsewhere (30). The question is open whether these two clonally expressed recognition units are combined in one receptor comprising two chains, which recognize a complex between self plus viral antigen, or whether the two receptors are separate entities. The second possibility seems less likely. Since multiple self-recognition alone or multiple recognition of viral antigen alone is of much lesser avidity than when both are recognized simultaneously, it is probable that the two receptors of a dual recognition system would have to be linked somehow. Such a composite receptor model is functionally not distinguishable from the altered self model.

Some of these possibilities have been discussed previously but were at that time regarded as a less likely explanation for the H-2 restriction phenomenon than the altered self hypothesis (8, 10, 11, 20). However, the modified double clonal dual recognition model is a viable alternative which fits the available data well, particularly the results with the H-2 mutants and chimeras in the virus models and probably explains better the evidence from minor histocompatibility antigen (31) and H-Y antigen (32) system, alloreactivity, and the recent observation that the idiotypic specificity of T-cell receptor and antibodies is similar or identical for a given antigen (33-36).

The reformulated dual recognition model stresses the fact that self-recognitive structure for one H-2K or H-2D product and immunological receptor for viral antigen are linked but independently, clonally expressed on virus-immune cytotoxic T cells. Furthermore, as appears probable from these chimera experiments and experiments with the H-2 mutants, and as we have discussed elsewhere, it seems likely that the gene (probably emerging by somatic mutation) coding for the self-recognitive structure for e.g. self-Kk is identical with the immunological receptor against alloantigen Kk.

Only the biochemical and immunochemical characterization of both the self-recognitive and the immunological T-cell receptor(s) or receptor parts or alternatively of the antigens recognized by it/them will confirm or disprove one or the other of the hypotheses.

Summary

During infection with lymphocytic choriomeningitis or vaccinia virus, F1 irradiation chimeras reconstituted with bone marrow cells from or both parents
generate cytotoxic T cells which can lyse infected targets across the H-2 barrier. However, activity of chimera T cells is H-2 restricted as shown by cold target competition experiments and selective restimulation of a secondary response in vitro; T cells of H-2<sup>k</sup> specificity which lyse tolerated infected H-2<sup>d</sup> target cells do not lyse infected H-2<sup>k</sup> or unrelated target cells and vice versa. Therefore, H-2 restriction of virus-specific cytotoxic T cells probably does not reflect need for like-like self-interactions for lysis to occur.

The specificity of virus immune T cells is thus determined by the H-2K and H-2D specificities present in the infected animal and which are probably recognized unidirectionally by T cells. The results are compatible with the idea that T cells are specific for "altered self" or "altered alloantigen," i.e., a complex of cell surface marker and viral antigen. Alternatively, explained with a dual recognition model, T cells may possess two independently, clonally expressed receptors, a self-recognizer which is expressed for one of the syngeneic or tolerated alloge-neic K or D "self" markers, and an immunologically specific receptor for viral antigen.

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