H-2 COMPATIBILITY REQUIREMENT FOR
T-CELL-MEDIATED LYSIS OF TARGET CELLS INFECTED
WITH LYMPHOCYTIC CHORIOMENINGITIS VIRUS

Different Cytotoxic T-Cell Specificities are Associated with Structures
Coded for in H-2K or H-2D

By ROLF M. ZINKERNAGEL AND PETER C. DOHERTY

(From the Department of Microbiology, The John Curtin School of Medical Research, Australian
National University, Canberra City, A. C. T. 2601, Australia)

Compatibility at the H-2-gene complex is essential for interaction of sensitized
thymus-derived lymphocytes (T cells) and target cells infected with either
lymphocytic choriomeningitis (LCM) virus or with ectromelia virus. This
constraint has been demonstrated both in vitro (1–4), by the use of 51Cr-release
assays, and in vivo in adoptive transfer experiments (5, 6). Similar restrictions
have also been described for T-cell-mediated lysis of trinitrophenyl-modified
spleen cells (7), for the T-cell helper effect (8–10), for the transfer of cell-
mediated immunity to Listeria monocytogenes (11) and for the proliferation of
sensitized guinea pig lymphocytes exposed to antigen-pulsed macrophages (12).
Furthermore, in vitro cell-mediated lysis of Rous sarcoma virus-transformed cells
appears to be restricted in the same way (13).

Two mutually exclusive hypotheses have been proposed to explain these phenomena (1,
2, 14). Firstly, that genes in the H-2-gene complex, perhaps located in the Ir region, code
for products involved in recognition of other somatic cells by T cells. This implies the
existence of a dual recognition system, requiring both presence of structures involved in
physiologic interaction and an antigen-specific T-cell receptor. The second possibility is
that cell surface components specified in the H-2K or H-2D regions are involved in antigen
presentation. T cells are sensitized to "altered-self," either modified H-2 antigens or
structures coded for by the H-2-gene complex that are not normally expressed on the cell
surface, or to some complex of viral and H-2 antigens.

Evidence presented here supports the second hypothesis, by establishing that
distinct cytotoxic T-cell specificities are associated with each parental haplo-
type in F1 mice, or with either the H-2K or H-2D region as demonstrated with
H-2 recombinant strains.

Materials and Methods

Mice. CBA/H, BALB/c, CBA/H × BALB/c F1, and A/J mice were from colonies at the Australian
National University, Canberra City, Australia. The C3H.0H mice were originally obtained from

1 Abbreviations used in this paper: FCS, Fetal calf serum; L cells, L929 fibroblasts (H-2b); LCM,
lymphocytic choriomeningitis; LU, lytic units (19); P-815, DBA/2 mastocytoma cells (H-2b).
T CELLS SPECIFIC FOR ALTERED H-2K OR H-2D

Doctors D. C. Shreffler and C. S. David, Department of Human Genetics, the University of Michigan Medical School, Ann Arbor, Mich., and were then bred locally. The H-2 genotypes (15) of the mouse strains used throughout these experiments are shown in Table I.

**Immunization.** 7- to 10-wk old mice were injected intracerebrally (i.c.) with 300 LD₅₀, or intravenously (i.v.) with a mean lethal dose (LD₅₀) of the WE3 strain of LCM virus.

**Target cells.** C3H (H-2k) mouse L929 fibroblasts (L cells) were grown in Eagle's minimal essential medium (F-15; Grand Island Biological Co., Grand Island, N. Y.) plus 10% fetal calf serum (FCS) in 250-ml Falcon plastic tissue culture flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). When confluent, the monolayer was infected with supernates of a 10% suspension of LCM-infected guinea pig lung for 1 h at 37°C. After one wash with 20 ml of prewarmed medium the cells were incubated for 24 h at 37°C. After trypsinization, normal or infected L cells were transferred into spinner flasks, at a density of 2-5 × 10⁶/ml, and maintained as spinner cultures for a further 24 h before being used as ^⁵¹Cr-labeled targets, or as unlabeled competitor cells.

Mastocytoma cells of DBA/2 (H-2d) (P-815) origin were cultured in Dulbecco's modified Eagle's medium (H-16, GIBCO) plus 10% FCS. LCM-infected P-815 cells were maintained as a continuously infected line.

**Peritoneal Macrophages.** Peritoneal macrophages were obtained from mice killed by cervical dislocation. The abdominal skin was reflected and 10-15 ml of cold (4°C) Puck's saline A (16) were injected vigorously into the abdominal cavity, aspirated, and reinjected twice more. Between 2 × 10⁴ to 2 × 10⁶ viable large nucleated cells were recovered from each mouse. These cells were plated at a density of 2 × 10⁴ large cells per well of 96-hole tissue culture trays (Linbro Chemical Co., New Haven, Conn.). Cell losses during infection, ^⁵¹Cr labeling, and washings left about 5 × 10⁵ cells per well.

**Cytotoxicity Assay.** The assay for measuring T-cell-mediated virus-specific ^⁵¹Cr release was performed, with some modification, as described previously (17). LCM virus-infected or normal target cells were labeled with ^⁵¹Cr and washed. Target cells in suspension were dispensed in 50-μl aliquots, containing 5 × 10⁴ cells, into individual flat-bottomed wells of 96-hole plastic trays.

Targets were overlayed at a ratio of 20:1, except where otherwise stated. After incubation for 8-10 h at 37°C, the supernates were pipetted and the remaining ^⁵¹Cr was recovered by water lysis. Results are expressed as the mean ± SEM percent ^⁵¹Cr release for four replicates (17). The cytotoxic activity per spleen was calculated by determining the total amount of arbitrary lytic units (LU), defined as numbers of nucleated spleen cells necessary to specifically lyse 33% of the target cells in one well (19).

Competing unlabeled target cells were added, at appropriate concentrations, in 50-μl vol to wells containing ^⁵¹Cr-labeled targets. Spleen cells were then added in a further 200 μl, using a tuberculin syringe fitted with a 26 gauge needle to achieve good mixing.

---

**Table I**

H-2 Genotypes of the Mouse Strains Used (15)

| Strain          | K | IA | IB | IC | Ss-Slp | D |
|-----------------|---|----|----|----|--------|---|
| BALB/c          | d | d  | d  | d  | d      | d |
| CBA/H           | k | k  | k  | k  | k      | k |
| CBA/H × BALB/c  | k | k  | k  | k  | k      | k |
| A/J             | d | d  | d  | d  | d      | d |
| C3H.OH          | d | d  | d  | d  | d      | k |

---

[1428]
Results

Assay on \( F_1 \) and \( H-2 \) Recombinant Target Cells. Compatibility of immune spleen cells and target cells at one \( H-2 \) haplotype, or at \( H-2K \) or \( H-2D \), was sufficient for the lytic interaction to occur (Table II). Providing that this degree of identity was achieved, presence of foreign \( H-2 \) specificities on T cells or target cells did not cause impairment of cytotoxicity, establishing that the requirement for \( H-2 \) compatibility in no way reflects allogeneic inhibition (18).

Relative Cytotoxic Activity Generated by \( F_1 \) and \( H-2 \) Recombinant Mice. The capacity of immune \( F_1 \) and \( H-2 \) recombinant spleen cells to lyse \( H-2^* \) or \( H-2^d \) virus-infected targets was determined firstly as LU (19), secondly as LU per spleen, and thirdly as percent of cytotoxic activity for syngeneic \( H-2^* \) or \( H-2^d \) combinations (Table III). Comparisons of absolute LU values can only be made within the same target system, as the P-815 are apparently more readily lysed than are the L cells. In both systems T cells from \( F_1 \) mice showed from 70 to 100% of the activity of parental strain lymphocytes, depending on the method of comparison. Recombinant T cells were relatively more effective when there was compatibility at \( H-2D \) (Tables I and III), an observation indicating that there may be at least two specificities of cytotoxic T cells, those associated with the \( D \) end being the more potent.

Cold Target Competition In Vitro. Further evidence for more than one specificity of cytotoxic T cells in LCM-immune mice was found from cold target competitive inhibition experiments in vitro (20). The cytotoxic activity of immune T cells for \({}^{14}Cr\)-labeled, virus-infected target cells could only be inhibited specifically by mixture with unlabeled, syngeneic virus-infected cells (Fig. 1). Syngeneic normal cells, or virus-infected allogeneic cells, had no effect.

### Table II

| Mouse strain | Spleen cells| \( ^{14}Cr \) release from target macrophages |
|-------------|------------|---------------------------------------------|
|             |            | CBA/H LCM Normal | BALB/c LCM Normal | CBA/H x BALB/c F\(_1\) LCM Normal | A/J LCM Normal |
| CBA/H       | Immune     | 61.0±1.3 | 28.3±1.1 | 34.5±2.4 | 36.4±1.3 | 55.1±0.8 | 31.9±0.9 | 57.5±1.2 | 37.9±2.5 |
|             | Normal     | 31.4±2.1 | 27.5±0.6 | 38.4±3.3 | 34.6±0.7 | 34.7±2.8 | 31.3±2.5 | 36.3±0.3 | 38.0±2.2 |
| BALB/c      | Immune     | 35.2±1.4 | 32.2±1.3 | 68.0±2.8 | 35.2±1.9 | 71.7±2.0 | 29.3±0.4 | 69.3±1.9 | 38.2±1.2 |
|             | Normal     | 34.0±1.5 | 31.9±1.3 | 36.8±2.6 | 38.9±1.6 | 36.9±3.3 | 31.5±2.5 | 33.6±1.2 | 38.4±1.4 |
| CBA/H x BALB/c F\(_1\) | Immune     | 56.4±1.5 | 29.2±2.0 | 69.9±2.7 | 39.6±2.3 | 73.1±1.2 | 29.3±0.4 | NT | NT |
|             | Normal     | 33.4±1.3 | 28.7±2.1 | 33.3±3.3 | 37.5±2.5 | 35.8±2.6 | 31.6±1.7 | NT | NT |
| A/J        | Immune     | 50.0±2.8 | 32.4±2.2 | 67.8±0.2 | 32.5±0.6 | 63.0±1.0 | 31.4±0.7 | 68.7±4.8 | 38.7±2.3 |
|            | Normal     | 28.5±0.7 | 31.2±0.4 | 43.8±0.4 | 39.8±3.7 | 36.8±1.5 | 29.3±2.3 | 31.4±4.8 | 40.1±1.7 |

* Peritoneal macrophages were infected 12 h after plating and assayed 24 h later.
† Spleen cells were overlayed at 30:1 for 9 h at 37°C. Mean ± SEM of four replicates.
‡ Donor mice were infected i.v. with 2,000 LD\(_{50}\), WES 9 days previously.
§ Significantly greater than control values (\( P < 0.001 \)).
T CELLS SPECIFIC FOR ALTERED H-2K OR H-2D

**Table III**

| Mouse strain     | Total N cells per spleen | H-2K LCM target | H-2D LCM target |
|------------------|--------------------------|-----------------|-----------------|
|                  |                          | LU†            | LU†            | CBA/H (H-2K) activity | LU†            | LU†            | BALB/c (H-2D) activity |
| CBA/H            | $4.2 \times 10^7$        | 5.5            | 77             | 100             | $>10^6$      | <5             | <2               |
| BALB/c           | $6.8 \times 10^7$        | $>10^6$        | <7             | $<9$            | $2.0 \times 10^6$ | 340             | 100              |
| CBA/H x BALB/c   | $3.0 \times 10^7$        | $5.5 \times 10^4$ | 55             | 72              | $1.1 \times 10^4$ | 268             | 79               |
| A/J              | $3.7 \times 10^3$        | $1.1 \times 10^4$ | 35             | 46              | $1.6 \times 10^4$ | 237             | 70               |
| C3H. OH          | $6.1 \times 10^7$        | $1.0 \times 10^5$ | 59             | 77              | $4.0 \times 10^5$ | 153             | 45               |

* All mice were sampled on day 7. The kinetics of the cytotoxic T-cell response is comparable in different mouse strains (3).

† LU, i.e., number of nucleated (N) cells necessary to specifically lyse 33% of the LCM-infected targets (19).

---

**Fig. 1.** Competitive inhibition with unlabeled targets, LCM-P-815 (●), normal P-815 (○), LCM-L cells (△), or normal L cells (▲), added at various ratios of the LCM-specific $^{51}$Cr release (*) by 7 day immune CBA/H (H-2K) and BALB/c (H-2D) spleen cells assayed on (a) LCM-L cells (H-2K) and (b) LCM-P-815 (H-2D). Spontaneous $^{51}$Cr (⊙) release was not significantly different from that observed for normal target cells.

Competition was most apparent when cold targets were at from three to nine times in excess. Use of higher concentrations resulted in nonspecific steric hindrance.

Cytolytic capacity of immune spleen cells from H-2K/d F1, mice was inhibited only when the competing virus-infected cells were syngeneic with the target cells (Fig. 2). Presence of excess H-2K/d virus-infected mastocytoma cells caused no specific decrease in killing of H-2K/k virus-infected L-cells, and the converse was also true. This finding is most readily interpreted as indicating that there are at least two specificities of LCM-immune cytotoxic T cells in H-2K/d F1, mice, each associated with altered-self characteristics of one parental H-2 type (14).
Essentially similar results were recorded for recombinant mice (Table I and Fig. 3). Cytotoxic activity of immune lymphocytes for H-2K or H-2D compatible virus-infected targets was specifically inhibited only when there was H-2 identity between target and competing cells, and was not diminished by presence of

Fig. 2. Competitive inhibition with various ratios of unlabeled LCM-P-815 (●), or with LCM-L cells (▲), of the LCM-specific ⁵¹Cr release by 7 day immune CBA/H × BALB/c F₁, (H-2<sup>µ-µ</sup>) spleen cells (*) assayed on LCM-L cells (H-2<sup>µ</sup>) or LCM-P-815 (H-2<sup>µ</sup>) target cells.

Fig. 3. Competitive inhibition by different ratios of unlabeled LCM-L cells (▲) or LCM-P-815 (●) of the LCM-specific ⁵¹Cr release from labeled LCM-L cells or LCM-P-815 by 7 day immune A/J (H-2<sup>K<sup>µ</sup>/D<sup>µ</sup></sup>) or C3H.OH (H-2<sup>K<sup>µ</sup>/D<sup>µ</sup></sup>) (*) spleen cells.
virus-infected cells compatible with the T cell at the irrelevant H-2 locus. Again, these experiments indicate that there are at least two specificities of cytotoxic T cells in recombinant mice, one associated with H-2K and the other with H-2D.

Selective Proliferation In Vivo. Evidence for cytotoxic T cells of more than one specificity was also found from in vivo experiments. The system used has been described previously (14). Briefly, donors are killed at 7 days after i.v. inoculation with 2,000 LD50 of WE3 LCM virus. Recipients are irradiated (850 R) 24 h before adoptive transfer, dosed with 106 LD50 of WE3 LCM virus 18 h later, inoculated with 106 immune donor lymphoid cells, and cytotoxic activity in pooled spleen and lymph nodes determined after a further 3 days. Donor cells (14) proliferate in the recipient throughout this interval, as shown by failure of irradiated (850 R) spleen cells to multiply further (Table IV). Replication depends on continued exposure to histocompatible, virus-infected recipient cells.

### Table IV

Failure of Irradiated (850 R) LCM-Immune CBA/H × C57BL F1, Immune T Cells to Show Cytotoxic Activity at 72 h after Transfer to Irradiated, Virus-Infected CBA/H Recipients

| Cell population         | Treatment | Infected   | Normal   |
|-------------------------|----------|------------|----------|
|                         |          | %          | %        |
| Before transfer*        | None     | 53.9 ± 3.2 | 15.3 ± 1.2 |
|                         | 850 R    | 54.6 ± 4.2 | 14.7 ± 2.1 |
| 72 h after transfer‡    | None     | 98.2 ± 1.9 | 18.8 ± 1.9 |
|                         | 850 R    | 19.1 ± 0.5 | 16.9 ± 3.1 |
| Control LCM immune‡     |          | 93.9 ± 2.3 | 18.4 ± 0.9 |
| Control normal spleen   |          | 18.9 ± 1.4 | 17.7 ± 1.7 |

* Assayed for 4 h at 37°C.
‡ Assayed for 15 h at 37°C.

Presence of alloantigens, in an F1 donor or recipient, has no inhibitory effect (5, 14), providing one haplotype is shared.

T cells from 7 day immune F1 (H-2k/d) mice readily lyse virus-infected targets of both parental H-2 types (Table II). However passage through H-2k recipients results in selective proliferation of lymphocytes with significant cytotoxic activity only for H-2k virus-infected fibroblasts (Table V). The converse applies after transfer through H-2d recipients. These results are best explained as representing preferential multiplication of cytotoxic T cells of two distinct specificities, each associated with one parental H-2 type (14).

Similar observations have also been made for T cells from recombinants (Table I). Spleen cells from immune A/J or C3H.OH mice lyse both H-2k and H-2d virus-infected targets (Fig. 3). Passage through H-2k recipients selects a population(s) of T cells which preferentially kill H-2k targets, i.e., specific for altered H-2K in the A/J mice or altered H-2D in the C3H.OH mice (Table V).
TABLE V

Cytotoxic T-Cell Activity in Lymphoid Tissue of Irradiated LCM-Infected Recipients Dosed with LCM-Immune Spleen Cells 3 days Previously*

| Recipients | L-929 | P-815 |
|------------|-------|-------|
|            | Infected | Normal | Infected | Normal |
| Donors     |       |       |       |       |
| CBA/H × BALB/c F₁ | BALB/c | 21.2 ± 1.2 | 18.5 ± 1.2 | 78.0 ± 1.2 | 26.9 ± 3.1 |
| CBA/H × BALB/c F₁ | CBA/H | 84.9 ± 0.9 | 25.5 ± 2.1 | 29.8 ± 0.8 | 22.7 ± 2.1 |
| C3H. OH     | CBA/H | 48.3 ± 0.3 | 21.9 ± 1.2 | 25.8 ± 1.5 | 23.8 ± 3.1 |
| A/J         | CBA/H | 63.3 ± 1.1 | 21.0 ± 1.1 | 32.9 ± 1.5 | 28.9 ± 3.3 |
| Controls    |       |       |       |       |
| BALB/c immune |       | 24.9 ± 1.0 | 18.6 ± 0.6 | 70.2 ± 3.9 | 20.5 ± 1.0 |
| BALB/c normal |       | 18.6 ± 1.5 | 16.5 ± 0.6 | 19.9 ± 0.9 | 23.6 ± 0.6 |
| CBA/H immune |       | 86.7 ± 1.6 | 22.7 ± 2.9 | 23.8 ± 0.6 | 23.6 ± 0.7 |
| CBA/H normal |       | 18.3 ± 0.5 | 23.8 ± 1.4 | 22.1 ± 3.2 | 21.6 ± 1.1 |
| Medium      |       | 18.6 ± 0.5 | 17.8 ± 0.7 | 18.7 ± 0.3 | 22.4 ± 2.7 |

*Donor mice were injected i.v. with 2,000 LD₅₀ of WE3 LCM virus and killed after 7 days. Recipient mice were irradiated (850 R) 24 h previously and dosed with 10⁶ LD₅₀ of WE3 LCM 6 h before cell transfer. Activity of donor cells in Table III and Figs. 1-3.

**Target cells were overlayed at a ratio of 30:1 and incubated for 10 h at 37°C. Means ± SEM of four replicates.

Discussion

LCM-immune mice apparently possess cytotoxic T cells of at least two broad specificities, associated with either H-2K or H-2D. The evidence for this is derived from a variety of separate approaches. Genetic mapping studies, using recombinant mice, have rigorously shown that T cells and virus-infected targets need be compatible only in the region of either the H-2K or the H-2D locus.¹ The same is true for the ectromelia model.² Identity at Ir-Ss/Slp is neither essential, nor is it sufficient, for successful interaction to occur.² It must be stressed that there is no indication that this H-2 compatibility requirement for cytotoxic T-cell killing of virus-infected cells reflects presence of a physiological interaction mechanism coded for by genes in the Ir region (9, 10, 12, 21).

Even so, existence of two separate self-recognition systems, specified by genes in either the H-2K or H-2D regions, may still be invoked, e.g., in this case the observation that compatibility at H-2D results in greater killing could indicate that the physiological interaction mechanism coded for at the D end is more effective. The apparent separation of two distinct T-cell specificities in F₁ mice, one associated with either parental haplotype, might be explained by allelic exclusion in this hypothetical physiological interaction mechanism (22, 23).

¹Blanden, R. V., P. C. Doherty, M. B. C. Dunlop, I. D. Gardner, R. M. Zinkernagel, and C. S. David. 1975. Genes required for T-cell-mediated cytotoxicity against virus-infected target cells are in the K and D regions of the H-2-gene complex. Manuscript submitted for publication.
Such an argument cannot, however, be applied to the comparable results obtained for H-2 recombinant mice, as they are homozygous. It would thus be necessary to postulate that only one, or other, of the self-recognition structures coded for at H-2K or H-2D could be expressed in any one T cell, i.e., some form of H-2K or H-2D receptor exclusion. Furthermore, the results of the selective proliferation experiments indicate that any such exclusion is clonal.

This complex mechanism is, to our knowledge, without precedent. We therefore favor the simpler postulate that the T cell is sensitized to altered self (1-3, 14), the self antigens involved being coded for at, or near to, either H-2K or H-2D. Altered self is interpreted broadly as reflecting either short- or long-range virus-induced modification of H-2 antigens, perhaps associated with the H-2 private specificities or products of genes linked closely to them. Such a concept accommodates recognition of alloantigens, or of changes induced by viruses, intracellular bacteria or chemicals, within the same model (14). Furthermore, it is in general agreement with Bodmer's modification (24) of Jerne's argument for generation of immunological diversity (25). In the present model all T-cell specificities may be considered to have arisen by somatic mutation from genes originally coding for recognition of histocompatibility antigens.

The altered-self concept is also in accord with Burnet's immunological surveillance theory, broadened somewhat to include response to infectious agents as an essential part (26). Viruses and bacteria may, in evolutionary terms, be considered as potent selective forces, affecting young animals particularly. The T-cell-mediated response to such agents is, in the great majority of cases, protective. The realization that T cells in virus-infected mice are sensitized to either altered H-2K or H-2D thus provides a reason for gene duplication in the H-2-gene complex (27). The overall T-cell response is, as numerically analyzed here, considerably augmented by the presence of two distinct cytotoxic T-cell specificities. An identical argument may be used to explain selective advantage of heterozygosity of the H-2-gene complex, four specificities are more effective than two. The extreme polymorphism of histocompatibility antigen systems (24) may thus, on a purely mechanistic basis, be considered to have developed in response to selective pressure exerted by infectious agents.

Summary

Use of syngeneic, allogeneic, F1, and H-2 recombinant mice has shown that animals injected with lymphocytic choriomeningitis (LCM) virus generate T cells which are cytotoxic for H-2K or H-2D compatible, but not H-2 different, virus-infected target cells. Three separate lines of evidence are presented which indicate that these immune T cells are sensitized to "altered-self," the self antigens involved being coded for in the H-2K or H-2D regions. Firstly, cytotoxic activity associated with mutuality at H-2D is greater than that observed when T cells and targets are identical at H-2K. Secondly, lysis mediated by immune T cells from F1 or H-2 recombinant mice is specifically inhibited only by presence of unlabeled, virus-infected cells that are H-2 compatible with the targets. Thirdly, LCM-immune F1 and H-2 recombinant T cells inoculated into irradiated, virus-infected recipients proliferate only to kill target cells that are H-2 compatible with both the donor and the recipient.
All of these experiments establish that there is a dissociation of T-cell activities between parental haplotypes in F, mice, and between H-2K and H-2D in recombinants. It would thus seem that there are at least two specificities of LCM-immune T cells in homozygotes, associated with either H-2K or H-2D, and four specificities in F, hybrids. The significance of these findings, with respect both to gene duplication and to the marked polymorphism in the H-2 system, is discussed.

We wish to thank Dr. M. H. R. MacDonald for helpful discussions, Doctors D. C. Shreffler and C. S. David for the original breeding pairs of C3H.OH mice, and Miss Gail Essery for her capable technical assistance.

Received for publication 3 February 1975.

References

1. Zinkernagel, R. M., and P. C. Doherty. 1974. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature (Lond.). 248:701.
2. Doherty, P. C., and R. M. Zinkernagel. 1974. T cell-mediated immunopathology in viral infections. Transplant. Rev. 19:89.
3. Doherty, P. C., and R. M. Zinkernagel. 1975. H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. J. Exp. Med. 141:502.
4. Gardner, I., N. A. Bowern, and R. V. Blanden. 1974. Cell-mediated cytotoxicity against ectromelia virus-infected target cells. III. Role of the H-2 gene complex. Eur. J. Immunol. 4:63.
5. Doherty, P. C., and R. M. Zinkernagel. 1975. Capacity of sensitized thymus derived lymphocytes to induce fatal lymphocytic choriomeningitis is restricted by the H-2 gene complex. J. Immunol. 114:30.
6. Blanden, R. V. 1975. Mechanisms of cell-mediated immunity in viral infection. In Proceedings of the Second International Congress of Immunology. Brighton, England. L. Brent and J. Holborow, editors. 2(4):17.
7. Shearer, G. M. 1974. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Eur. J. Immunol. 4:527.
8. Kindred, B., and D. C. Shreffler. 1972. H-2 dependence of co-operation between T and B cells in vivo. J. Immunol. 109:940.
9. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. J. Exp. Med. 137:1405.
10. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. The H-2 gene complex determines successful physiologic lymphocyte interactions. Proc. Natl. Acad. Sci. U. S. A. 70:2624.
11. Zinkernagel, R. M. 1974. Restriction by the H-2 gene complex of the transfer of cell-mediated immunity to Listeria monocytogenes. Nature (Lond.) 251:230.
12. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J. Exp. Med. 138:1194.
13. Wainberg, M. A., Y. Markson, D. W. Weiss, and F. Donjanski. 1974. Cellular immunity against Rous sarcomas of chickens. Preferential reactivity against autoch-
thonous target cells as determined by lymphocyte adherence and cytotoxicity tests in vitro. Proc. Natl. Acad. Sci. U. S. A. 71:3565.

14. Zinkernagel, R. M., and P. C. Doherty. 1974. Immunological surveillance against altered self by sensitized thymus derived lymphocytes in lymphocytic choriomeningitis. Nature (Lond.). 251:547.

15. Shreffler, D. C. and C. S. David. 1974. The H-2 major histocompatibility complex and the I immune response region: genetic variation, function, and organization. Adv. Immunol. In press.

16. Blanden, R. V., and R. E. Langman. 1972. Cell-mediated immunity to bacterial infection in the mouse. Thymus-derived cells as effectors of acquired resistance to Listeria monocytogenes. Scand. J. Immunol. 1:379.

17. Zinkernagel, R. M., and P. C. Doherty. 1974. Characteristics of the interaction in vitro between cytotoxic thymus-derived lymphocytes and target monolayers infected with lymphocytic choriomeningitis virus. Scand. J. Immunol. 3:287.

18. Hellström, K. E., and G. Möller. 1965. Immunological and immunogenetic aspects of tumor transplantation. Prog. Allergy. 9:158.

19. Cerottini, J. C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. Adv. Immunol. 18:87.

20. Ortiz de Landazuri, M., and R. B. Herberman. 1972. Specificity of cellular immune reactivity to virus-induced tumors. Nat. New Biol. 238:18.

21. Katz, D. H., and B. Benacerraf. 1974. The function and interrelationship of T cell receptors, Ir genes and other histocompatibility gene products. Transplant. Rev. In press.

22. Pernis, B., G. Chiappino, A. S. Kelus, and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissue. J. Exp. Med. 122:853.

23. Gell, P. G. H. 1967. Restriction on antibody production by single cells. Cold Spring Harbor Symp. Quant. Biol. 32:441.

24. Bodmer, W. F. 1972. Evolutionary significance of the HL-A system. Nature (London.). 237:139.

25. Jerne, N. K. 1971. The somatic generation of immune recognition. Eur. J. Immunol. 1:1.

26. Burnet, M. 1970. Immunological Surveillance. Pergamon Press (Australia) Pty. Ltd., Sydney, Australia.

27. Klein, J., and D. C. Shreffler. 1971. The H-2 model for the major histocompatibility systems. Transplant. Rev. 6:3.