

## **Brief Definitive Report**

### **A SINGLE GENETIC ELEMENT IN *H-2K* AFFECTS MOUSE T-CELL ANTIVIRAL FUNCTION IN POXVIRUS INFECTION**

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T cells play an essential role in the process of recovery from primary infection of mice with ectromelia virus, a poxvirus related to vaccinia and variola viruses (1-4), but the precise mechanisms involved and the participation of different effector T-cell subsets and ancillary cells remain to be fully elucidated. Massive liver necrosis is the major cause of death (1-3). Recovery therefore depends upon control of virus replication and spread in progressing lesions of the liver parenchyma and upon control of infection in other tissues, such as spleen, which may contribute to the cell-associated viremia (1) which in turn may initiate new liver lesions.

A number of potential mechanisms of recovery could be supplied by effector T cells. First, cytotoxic T cells could kill virus-infected cells before they support virus replication, thus halting virus spread; this is feasible since ectromelia-infected cells display antigenic changes recognized by cytotoxic T cells long before progeny virus is assembled (5). Second, recognition of foci of infection by effector T cells leads to recruitment (2, 3, 6) and activation (7) of mononuclear phagocytes which destroy virus-infected material (3). Third, effector T cells may secrete interferon at sites of infection, thus protecting other cells in the immediate vicinity (3, 8). Available evidence denies an important role for helper T cells and antibody in the normal process of recovery (1-4).

All of the mechanisms described above would require specific recognition of virus-induced antigenic patterns, since the protection conferred by immune T cells is virus specific (2). However, recent findings in a number of different systems indicate that cytotoxic T cells do not simply recognize virus-specified antigenic determinants, but that host cell gene(s) in the *H-2* gene complex are involved in producing new antigenic patterns on infected cells (9-14). Thus, T-cell-mediated lysis of ectromelia-infected target cells requires *H-2K-* or *H-2D-* region homology between donors of cytotoxic T cells and infected target cells (15). Previous experiments with inbred mice have also shown that transfer of virus clearance mechanisms triggered by effector T cells against ectromelia infection in vivo is not possible unless one *H-2* haplotype is shared by donors of T cells and virus-infected recipients (4). Since the antiviral effect is seen in recipients within 24 h of cell transfer and since  $F_1$  hybrid-parental strain donor-recipient combinations are effective, it seems that failure to transfer the effect to totally allogeneic recipients is not due to rejection of the transferred T cells, but to failure of T cells to recognize virus-induced antigenic patterns on allogeneic cells (4). We describe here a series of cell transfer experiments, using mice bearing recombinant or mutant *H-2* haplotypes, which further define the *H-2* requirement.

## Materials and Methods

*Animals.* All mouse strains were bred at the John Curtin School and used at 7–10 wk of age.

*Virus.* Virulent (Moscow) and attenuated (Hampstead egg) strains of ectromelia virus were grown (1) and titrated by plaque assay on L929 cells as described previously (1, 4).

*Immunization and Cell Transfer Procedures.* Donors of immune spleen cells were immunized intravenously (i.v.) with  $2 \times 10^4$  plaque-forming units (PFU) of attenuated virus, and their spleens were harvested 5 days later at about the peak of the effector T-cell response<sup>1</sup> (2). Cell suspensions (4) were transferred i.v. to recipients that had been infected i.v. 24 h previously with virulent virus. Antiviral effects transferred were determined by titrating virus in individual spleens (and sometimes livers) of groups of four recipients sacrificed 24 h after cell transfer (2, 4). Data from spleen and liver are essentially similar (2), but spleens are more conveniently titrated and spleen data only are given here. Statistical significance was determined by Student's *t* tests.

## Results and Discussion

Transfer of antiviral activity occurred only in donor-recipient combinations which shared either *K*- or *D*-region genes (Table I). *I*-region homology was neither sufficient nor necessary, and the remainder of the genotype seemed irrelevant. These results thus conform with previous data indicating a requirement for *K*- or *D*-region genes in expression of T-cell-mediated lysis of virus-infected or TNP-modified target cells in vitro (15, 16). Cytotoxic T cells may therefore play a central role in recovery, particularly since the kinetics of production in the spleen of T cells with in vivo antiviral function (as described here) and in vitro cytotoxicity are similar.<sup>1</sup> However, this evidence is indirect, and a firm conclusion cannot yet be drawn.

Since *I*-region homology between donors and recipients is sufficient for other T-cell functions such as helper activity (17), delayed-type hypersensitivity to nonviral antigens (18), and protection against bacterial infection,<sup>2</sup> the present data indicate that the effector T cells responsible for virus clearance, whatever the mechanisms they employ or trigger, are a subset that is defined by the requirement for operation of gene(s) in the *K* or *D* regions of the *H-2* complex.

The molecular basis for the phenomena cited above (9–16) poses important questions. Let us assume that immunocompetent, precursor T cells employ the same mechanisms of antigen recognition while interacting with infected "stimulator" cells during the process of induction of the effector T-cell response, as employed by their progeny (effector T cells) which lyse infected target cells in vitro or trigger virus clearance in vivo. Potential models for these interactions have been proposed and tested by Zinkernagel and Doherty (19). Available evidence from experiments with lymphocytic choriomeningitis (LCM) virus (19, 20), ectromelia virus,<sup>3</sup> trinitrophenyl (TNP)-modified cells (16), and minor histocompatibility antigens (12) supports the following model. T cells interact with stimulator or target cells only via receptors for antigen, but the major antigenic patterns recognized are not simply virus specified; they incorporate features dictated by *K*- or *D*-region genes. This raises questions concerning the way in

<sup>1</sup> Blanden, R. V., and I. D. Gardner. Manuscript in preparation.

<sup>2</sup> Dunlop, M. B. C., U. Kees, and R. V. Blanden. Unpublished data.

<sup>3</sup> Blanden, R. V., and T. E. Pang. Unpublished data.

TABLE I  
*H-2 Requirement for Transfer\* of Antiviral Effect by Ectromelia-Immune T Cells*

| Expt. | Immune T-cell donors |          | Virus titers in recipient spleens 24 h after cell transfer |                   |             |
|-------|----------------------|----------|------------------------------------------------------------|-------------------|-------------|
|       | Strain               | H-2 map‡ | Strain                                                     | H-2 map‡          | Titer§      |
| 1.    | BALB/c               | dddddd   | BALB/c                                                     | dddddd            | <1.30       |
|       | C3H.OH               | dddddk   | BALB/c                                                     | dddddd            | <1.30       |
|       | D2.GD                | ddbddd   | BALB/c                                                     | dddddd            | <1.30       |
|       | B10.A(2R)            | kkkddb   | BALB/c                                                     | dddddd            | 5.11 ± 0.13 |
|       | A.TFR 2              | fffsd    | BALB/c                                                     | dddddd            | 1.46 ± 0.24 |
|       | Nil                  |          | BALB/c                                                     | dddddd            | 5.12 ± 0.11 |
| 2.    | CBA/H                | kkkkkk   | A.TL                                                       | skkkkd            | 4.60 ± 0.35 |
|       | SJL/J                | ssssss   | A.TL                                                       | <del>skkkkd</del> | <1.30       |
|       | BALB/c               | dddddd   | A.TL                                                       | skkkkd            | <1.30       |
|       | Nil                  |          | A.TL                                                       | skkkkd            | 4.89 ± 0.94 |
|       | CBA/H                | kkkkkk   | CBA/H                                                      | kkkkkk            | <1.30       |
|       | Nil                  |          | CBA/H                                                      | kkkkkk            | 5.07 ± 0.30 |
| 3.    | H21                  | "ba"bbbb | H21                                                        | "ba"bbbb          | 3.32 ± 0.56 |
|       | B10.A(5R)            | bbdddd   | H21                                                        | "ba"bbbb          | 5.98 ± 0.23 |
|       | B10.A(2R)            | kkkddb   | H21                                                        | "ba"bbbb          | <1.30       |
|       | Nil                  |          | H21                                                        | "ba"bbbb          | 6.51 ± 0.06 |
|       | B10.A(5R)            | bbdddd   | C57BL/6                                                    | bbbbbb            | <1.30       |
|       | Nil                  |          | C57BL/6                                                    | bbbbbb            | 5.51 ± 0.15 |

\* Experimental procedure is described in Materials and Methods. All donors were immunized i.v. with  $2 \times 10^4$  PFU of attenuated virus. Recipients were injected i.v. with  $2 \times 10^4$  of virulent virus for experiments 1 and 2 and with  $5 \times 10^5$  PFU for experiment 3. Viable immune spleen cell doses, given i.v. to each recipient, were  $5 \times 10^7$ ,  $7 \times 10^7$ , and  $15 \times 10^7$  for experiments 1, 2, and 3, respectively.

‡ H-2 maps are for *K*, *I-A*, *I-B*, *I-C*, *S*, and *D* regions of the gene complex. *K* region of H21 is designated "ba" on the basis of evidence cited in the text.

§ Results are expressed as mean  $\log_{10}$  virus PFU per organ  $\pm$  SE of the mean in groups of four mice. The limit of detection was 20 PFU/spleen ( $\log_{10} = 1.30$ ).

|| Significantly less than control groups given no cells ( $P < 0.01$ ).

which *K*- or *D*-region genes and/or their products interact with the viral genome or its products, or other antigens such as TNP, to produce new antigenic patterns which stimulate precursors of effector T cells. It also provokes speculation as to the nature of T-cell receptors for antigen, and the scope of receptor dictionaries on various T-cell subsets.

Further characterization of the nature of the genes in *H-2K* or *H-2D* regions is relevant to these questions. We therefore investigated B6.C-*H-2<sup>ba</sup>* (H21) mice which bear a mutation that arose in the *K* end of the *H-2<sup>b</sup>* complex (21) and has now been extensively characterized. F<sub>1</sub> hybrids of H21 and another mutant of C57BL/6 reject C57BL/6 skin grafts (22), thus suggesting strongly that each mutation involved the same single genetic element. Since C57BL/6 and H21 and mice also give reciprocal mixed lymphocyte reactions (MLR), cell-mediated lympholysis (CML), and graft-versus-host reactions (GVHR), it seems that T cells recognize the antigenic pattern(s) affected by the mutation, and that *K* region was the site of change (23, 24). However, the structure of antigenic determinants recognized by B cells appear qualitatively unchanged by the mutation on the basis of sound serological evidence<sup>4</sup> (21, 25).

<sup>4</sup> McKenzie, I., G. Morgan, R. Melvold, and H. Kohn. Submitted for publication.

The data in Table I clearly show the effect of the Hz1 mutation on T-cell antiviral function in vivo. Immune T cells from B10.A(5R) mice, which reduced virus titers very efficiently in C57BL/6 mice because of shared *K* region, did not have a significant protective effect in Hz1 recipients, whereas T cells from B10.A(2R) mice operated efficiently in Hz1 recipients because of *D*-region homology. These results therefore suggest that the antigenic patterns induced by ectromelia infection, which are recognized by effector T cells, do not directly involve serologically-defined *H-2* antigenic determinants. Instead, they seem to be controlled partly by a single genetic element responsible for alloantigenic patterns recognized by T cells in such reactions as MLR, CML, GVHR, and graft rejection (23, 24). What is the nature of this genetic element? Is it a single cistron coding for a polypeptide, as the conventional view of the complementation results would suggest (22), or is it a more complex genetic unit, perhaps concerned with the saccharide portion of the *H-2* glycoprotein (26), without precedent in the multigenic elements defined thus far in prokaryotic systems? Further characterization of the virus-induced antigenic patterns recognized by effector T cells may clarify this issue.

Finally, in view of the large quantitative effects of *K*- or *D*-region genes reported here, the rational exploitation in clinical medicine of T-cell-mediated mechanisms against infections and tumors, perhaps including transfer factor (27), could well be optimized by investigation of genetic regions analogous to *K*, *D*, and *I* in the HLA complex.

### Summary

Cell transfer experiments using mice with recombinant *H-2* haplotypes were used to map the *H-2* regions which must be shared by ectromelia-immune T-cell donors and virus-infected recipients for transfer of virus clearance mechanisms in the spleen. *K*- or *D*-region genes were necessary and sufficient; *I*-region genes were not involved. The remainder of the mouse genome could be varied widely without impairing the efficacy of T-cell antiviral function, provided either a *K* or a *D* region was shared in the donor-recipient combination. A mutation in a single genetic element of the *K* region of the *H-2* complex abolished the antiviral effect of immune T-cell transfer in a donor-recipient combination which shared the *K* end.

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