GENERATION OF BOTH CROSS-REACTIVE 
AND VIRUS-SPECIFIC T-CELL POPULATIONS AFTER 
IMMUNIZATION WITH 
SEROLOGICALLY DISTINCT INFLUENZA A VIRUSES*

BY RITA B. EFFROS, PETER C. DOHERTY, WALTER GERHARD, AND JACK BENNINK

(From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104 and the 
Department of Pathology, University of Pennsylvania 19104)

Immune thymus-derived lymphocytes (T cells) generated during the course of virus 
infections of mice have, in most instances, shown predictable specificity patterns (1). 
Reciprocal exclusion of cytotoxic T-cell activity has been demonstrated for three diverse 
groups of viruses: arenaviruses [lymphocytic choriomeningitis virus (LCMV)], poxviruses 
(ectromelia and vaccinia), and paramyxoviruses (Sendai). The only aberrant finding 
is that cytotoxic T cells from mice infected with LCMV or vaccinia virus lyse 
trinitrophenyl-modified lymphocytes (2).

Virus-immune T cells also exhibit another order of specificity, which could not have 
readily been foreseen. Effector T-cell function, as measured by both in vitro and in vivo 
criteria, is recognized only when virus-infected targets share H-2K or H-2D genes with 
the mouse strain in which the lymphocytes are sensitized (3-6). Such is the case for both 
the normal physiological situation, and for radiation chimeras in which H-2-different T 
cells apparently see alloantigen as self (7-10). Either virus-immune T cells recognize both 
self (H-2) and nonself (virus), or the lymphocyte receptor is specific for some neoantigen 
determined by both host and viral genomes (11, 12).

Various speculations have been advanced to explain this H-2 restriction phenomenon 
(7, 11, 12). At one extreme is the idea that the T cell expresses two variable (V) genes, one 
specific for self-H-2 and the other for virus (12). The most drastic alternative is the 
possibility that the infectious process results in derepression of cryptic host genes, and 
reactivity is directed solely against abnormally expressed alloantigens (13). This latter 
concept implies that the T cell does not recognize virus at all. It thus becomes important 
to compare T-cell responses resulting from exposure to different viruses of similar 
molecular biology.

The influenza viruses offer an ideal experimental system for this purpose. 
Firstly, there is the possibility of using A and B strain influenza viruses, which 
bear a common host determinant (if grown in chick embryo) but are otherwise 
serologically distinct (14). Fine specificity can then be studied within the A
SPECIFICITY OF INFLUENZA-IMMUNE T CELLS

strain viruses, which express different hemagglutinin (H) and neuraminidase surface antigens but share internal ribonucleoprotein (RNP) and matrix (M) components (15). We have thus investigated T-cell responses to a variety of influenza A viruses, representing subtypes first isolated from man in 1933 (H0N1), 1957 (H2N2), and 1968 (H3N2).

Materials and Methods

Mice. CBA/J, C57BL6/J (B6), C57BL/10 (B10), B10.A(5R), B10.BR, and CBA/J × B6 F1 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.A(2R) and B10.A(4R) mice were bred in colonies maintained at the Wistar Institute by Dr. D Gotze.

Viruses. The influenza A virus strains PR8 [A/PR/8/34 (H0N1)], Bel [A/Bellamy/42 (H0N1)], AA [A/Ann Arbor/23/57 (H2N2)], and NT60 [A/Northern Territory/60/68 (H2N2)] and the influenza B strain (Blee) were originally obtained from Dr. S. Fazekas de St. Groth, Division of Animal Genetics, CSIRO, Sydney, Australia. The virus strains Hick [A/Hickox/40 (H0N1)] and HK/X31, a recombinant between PR8 and a Hong Kong strain which shows the antigenic characteristics of the Hong Kong virus (16) were supplied by the Center for Disease Control, Atlanta, Ga. and Dr. R. G. Webster, St Jude Children's Research Hospital, Memphis, Tenn., respectively. Virus stocks of high infectivity titer were grown in the allantoic cavity of embryonated chicken eggs, and stored frozen at −70°C. All such stocks contained between 1,200 and 3,000 hemagglutinating (HA) U/ml (17).

Immunization. Mice were generally immunized intraperitoneally (i.p.) with 1.0 ml of a 1:10 dilution (in phosphate-buffered saline) of allantoic fluid containing virus (100-300 HA U per mouse). In one experiment mice were anesthetized with chloroform, and given 50 µl of a 1:10,000 dilution intranasally.

Cytotoxic T-Cell Assay. The methods used are similar to those described for other viruses (18). Briefly, L929 fibroblasts (L cells) were grown in RPMI 1640 (Flow Laboratories, Inc, Rockville, Md.) containing 10% fetal calf serum and antibiotics. This medium was used throughout. Cells were trypsinized, pelleted, washed, and labeled for 1.5–2 h at 37°C with Na1Cr (New England Nuclear, Boston, Mass.) at a concentration of 250 µCi/106 cells. The cells were then pelleted again and resuspended in medium containing virus for 1 h at 37°C, washed twice in medium, and dispensed into 96-hole plates (Linbro Chemical Co., New Haven, Conn.) in 100 µl of medium to give 1.5 × 105 cells per well. The concentration used for the A strain viruses was 5.0 ml of a 1:10 dilution of stock allantoic fluid in medium per 2 × 107 cells (approximately 50 HA U per 106 cells), while a 1:300 dilution (0.5 HA U per 106 cells) was used for the Blee.

The target cells were then overlaid with the lymphocyte populations, in a further 100 µl of medium, and the assays were held overnight at 37°C in a humidified atmosphere containing 7% CO2 in air. Assay supernates (100 µl) were then removed for γ-counting. The initial assays were incubated for 16 h, but background levels of 31Cr release were found to be rather high (from 30 to 40%). All experiments are now done using a 12 h assay which gives a background, for normal spleen cells or medium, ranging from 20 to 30% of hydrolysis. The water lysis value is determined by adding 100 µl (1.5 × 105 cells) of the target cell population to 1.9 ml of distilled water, incubating this with the assay, and then measuring the number of counts present in 1.0 ml of the supernate.

All results are expressed as mean percent specific 31Cr release for replicates of three or four wells. Standard errors within the groups were reproducibly less than 5%, and generally below 2%, and are not shown for clarity of presentation of results. The formula (1) used for calculating percent specific 31Cr release is (It-Nt) × 100/Wt-Nt, where W is water lysis, t is the target, I is immune lymphocytes, and N is normal lymphocytes. Uninfected L cells were carried as controls in many experiments, but were not killed by immune spleen cells.

Lymphocytes. Immune spleen and lymph node cell populations were processed and depleted of erythrocytes as described previously (1). Viability was determined by trypan blue exclusion, and all ratios quoted in the results are adjusted to viable cell counts. Some lymphocyte preparations were depleted of B cells by passage through nylon wool columns (19), and T cells were removed by incubation with a rabbit anti-mouse brain serum [anti-T, (20)] and guinea pig complement. This serum, which has been used extensively by other workers (8), was kindly supplied by Dr. D. Gotze.
Radioimmunoassay. Unlabeled, virus-infected L cells were prepared and plated into wells as described for the cytotoxic assay. After overnight incubation they were fixed with 0.15% glutaraldehyde and were used as immunoadsorbents in a radioimmunoassay (RIA) as described by Segal and Klinman (21). This involved incubation of the immunoadsorbent with an appropriate dilution of mouse serum, followed by quantitation of the bound mouse Ig by means of 125I-labeled rabbit anti-mouse antibody.

Results

Specificity Between Type A and B Influenza Viruses and Vaccinia. Mice were immunized i.p. with large doses of influenza virus and spleen cells were assayed for effector function on virus-infected L cells. Maximal cytotoxic activity was observed at 5 days after exposure to PR8 (H0N1) or BLee, and the response was specific for the immunizing virus (Table I). Reciprocal exclusion of lytic function was also observed for PR8 and vaccinia virus (Table II). The specificity demonstrated is thus of the same order as found previously for other viruses (1).

Cross-Reactivity Between Type A Viruses. Reciprocal priming with different strains of influenza A viruses revealed a pattern of complete cross-reactivity (Table III). The different viruses varied in their immunogenic capacity: effector lymphocytes from mice given PR8, HKX31, and NT60 were the most active. However, all populations were lytic for the H0N1-, H2N2-, and H3N2-infected target cells. No clear indication was found of preference for the homologous interaction.

This result was somewhat surprising as Cambridge et al. (23) had found previously that cytotoxic lymph node cells from mice infected with influenza A viruses show specificity for the virus H antigen. Differences from the present study are that the effectors were not identified as T cells and that only one strain (A/WSN, H0N1) was used for immunization and tested on targets infected with a variety of viruses. Another possible source of discrepancy is that virus given i.p. in large quantities may be processed in an unphysiological way, with resultant generation of aberrant T-cell specificities. Cytotoxic assays were thus made using mediastinal lymph node cells from mice infected intranasally with much lower doses of virus. Again the same specificity pattern was observed, with complete cross-reactivity between the type A viruses, but reciprocal exclusion of cytotoxicity for BLee (Table IV).

All viruses used in the present study were grown in the allantoic cavity of the chick embryo. Virus particles produced in this way are known to express a chicken host component (14), which is common to influenza A and B strain viruses and normal allantoic fluid. Both the reciprocal exclusion of cytotoxicity for influenza A and B viruses and the fact that mice immunized with allantoic fluid did not generate effector capacity for either influenza virus-infected targets or for L cells previously incubated with the normal allantoic fluid (Table V) indicates that the cross-reactivity observed for A strain viruses is not due to immunization with a common antigen of chicken origin.

Also, serum antibodies detected in mice immunized by the procedure used to generate cytotoxic spleen cells did not show any significant cross-reactivity. Significant binding of antibody was recognized only for target cells infected with the virus used for immunization (Table VI). Apparently the virus-infected L cells used in this assay do not express any cell surface antigen common to PR8.
### Table I

**Specificity of Cell-Mediated Lysis for Influenza A and B Viruses**

| Immune spleen | Days after inoculation | 50:1 % ⁵¹Cr release | 100:1 % ⁵¹Cr release | PR8 % ⁵¹Cr release | BLee % ⁵¹Cr release |
|---------------|------------------------|----------------------|----------------------|---------------------|----------------------|
| PR8          | 3                      | 3                    | 15                   | 4                   | 0                    |
|              | 5                      | 48                   | 64                   | 4                   | 9                    |
|              | 7                      | 25                   | 28                   | 5                   | 5                    |
| BLee         | 3                      | 0                    | 0                    | 25                  | 36                   |
|              | 5                      | 0                    | 2                    | 32                  | 49                   |
|              | 7                      | 11                   | 7                    | 20                  | 18                   |

* B10.BR mice were inoculated i.p. with a 1:10 dilution of allantoic fluid containing influenza virus. Assays were incubated for 16 h at 37°C.

### Table II

**Reciprocal Exclusion of Cytotoxicity for Influenza and Vaccinia Viruses**

| Immune spleen | PR8 % ⁴⁰Cr release | Vaccinia % ⁴⁰Cr release |
|---------------|---------------------|-------------------------|
| PR8           | 32                  | 0                       |
| Vaccinia      | 0                   | 79                      |

* CBA/J mice were immunized i.p. 5 days previously with 250 HA units of PR8 influenza virus, or intravenously with 10⁶ TCID₅₀ of vaccinia virus (22). The vaccinia-immune population was enriched for T cells by passage through nylon wool (19). The assays were incubated for 12 h at 37°C.

### Table III

**Extensive Cross-Reactivity Between Spleen Cell Populations from Mice Immunized with Different A Strain Viruses**

| Immune spleen | PR8 % ⁴⁰Cr release | B27 H011 | AA H2N2 | NT60 H2N2 | HK H3N2 |
|---------------|---------------------|----------|---------|-----------|---------|
| PR8          | 70                  | 78       | 31      | 48        | 60      |
| Bel          | 19                  | 16       | 22      | 12        | 28      |
| Hick         | 38                  | 18       | 25      | 16        | 32      |
| AA           | 50                  | 33       | 36      | 38        | 51      |
| NT60         | 58                  | 42       | 36      | 46        | 62      |
| HK           | 62                  | 41       | 41      | 46        | 50      |

* CBA/J mice were immunized i.p. 5 days previously. The results given are for a ratio of 50 spleen cells:1 target cell. Data for Hick (H011) targets is not shown, as background levels of ⁴⁰Cr release were >70%.
TABLE IV  
Cytotoxic Activity of Mediastinal Lymph Node Cells from Mice with Influenza Pneumonia

| Mediastinal* lymph node | PR8 (H0N1) | HKX3 (H3N2) | BLee |
|-------------------------|------------|-------------|------|
| PR8                     | 30         | 51          | 0    |
| HKX31                   | 20         | 31          | 0    |
| BLee                    | 5          | 3           | 0    |

* Mice were dosed intranasally 7 days previously with 50 μl of a 10⁻⁴ dilution of stock virus. Pneumonia was most severe in those given PR8, and least marked in mice dosed with HKX31.

TABLE V  
Cross-Reactivity Does Not Reflect Immunization with Egg Antigens

| Immune population* | % ¹¹C release from L cells |
|---------------------|---------------------------|
|                     | Allantoic fluid | PR8 | HKX31 | H0N1 | H3N2 | BLee |
| Allantoic fluid     | 0                | 0   | 1     | 0    | 0    |
| PR8                 | 0                | 54  | 72    | 4    | 4    |
| HKX31               | 0                | 50  | 71    | 6    | 6    |
| BLee                | 0                | 0   | 8     | 27   | 27   |

* CBA/J × B6 F₁, mice were injected i.p. with influenza virus or a comparable (1:10) dilution of normal allantoic fluid. Assays (100:1) were incubated for 12 h at 37°C.

TABLE VI  
Lack of Serological Cross-Reactivity between Influenza A and B Viruses

| Immunizing* virus | Day of sampling | μg of antibody per ml of serum binding to:† |
|-------------------|-----------------|-------------------------------------------|
|                   |                 | L-PR8          | L-HK           | L-BLee         |
| PR8 (H0N1)        | 14              | 235            | 7              | 3              |
| PR8 (H0N1)        | 13              | 8              | 65             | 4              |
| PR8 (H0N1)        | 23              | 300            | 4              |
| HK (H3N2)         | 23              | 9              | 240            | 4              |
| BLee              | 23              | 5              | 5              | 205            |
| Normal serum      | 4               | 2              | 4              |

* CBA/J mice were immunized by the procedure used to generate cytotoxic T cells.
† Virus-infected L-cell monolayers were prepared by the technique used for the cytotoxic T-cell assay, incubated for 16 h at 37°C, and fixed with 0.15% glutaraldehyde for RIA (21).

Identity of the Cytotoxic Population. What is the nature of the effector cell in influenza-immune spleen? Cytotoxic activity was considerably enhanced by passing lymphocytes through nylon wool columns (Table VII), which tend to remove antibody-forming cell precursors (B cells) and enrich for T cells (19). The same cross-reactivity pattern was observed for the purified populations. Effector function was totally abrogated by treatment with an anti-T serum (20) and

(H0N1) and HKX31 (H3N2) viruses which is readily demonstrable by serological techniques.
SPECIFICITY OF INFLUENZA-IMMUNE T CELLS

Table VII

Effect of Nylon Wool Depletion and Treatment with Anti-T Serum and Complement

| Spleen* population         | PR8 (H0N1) | AA (H2N2) |
|----------------------------|------------|-----------|
|                            | 12 h       | 16 h      | 12 h       | 16 h       |
| PR8 immune                 | 39         | 49        | 13         | 36         |
| Nylon wool effluent        | 74         | 98        | 34         | 52         |
| Nylon wool adherent        | 35         | 38        | 14         | 33         |
| Anti-T + C                 | 0          | 0         | 0          | 3          |
| Complement alone           | 49         | 60        | 9          | 49         |
| Washed‡                    | 45         | 68        | 16         | 46         |
| AA immune                  | 30         | 48        | 11         | 32         |
| Nylon wool effluent        | 60         | 81        | 39         | 63         |
| Nylon wood adherent        | 17         | 35        | 10         | 39         |
| Anti-T + C                 | 0          | 0         | 0          | 0          |
| Complement alone           | 33         | 46        | 22         | 45         |
| Washed‡                    | 41         | 60        | 22         | 48         |

* CBA/J × B6 F1 mice were dosed i.p. 5 days previously. Immune populations were assayed at 100:1, after passage through nylon wool columns (19) or treatment with anti-T serum and complement (20). Approximately 30% of spleen cells were recovered after either treatment.
‡ Processed in parallel with the two preceding groups.

Table VIII

Immune Lysis is Maximal When Target and T Cell Share H-2 Genes*

| Mouse strain | H-2 type | % ⁵¹Cr release from HK-infected L cells (H-2*) |
|--------------|----------|---------------------------------------------|
|              | I K ABC SD | 25:1 | 50:1 | 100:1 |
| B10          | bbbbbb    | 6    | 4    | 5     |
| B10 A        | kkkkddd   | 16   | 18   | 18    |
| B10, A(2R)   | kkkkdb    | 16   | 19   | 20    |
| B10, A(4R)   | kbbbbb    | 13   | 17   | 21    |
| B10, A(5R)   | bbbbdd    | 0    | 1    | 1     |
| B10, Br      | kkkkkkk   | 21   | 28   | 38    |
| B6           | bbbbbb    | 0    | 0    | 0     |
| CBA/J        | kkkkkk    | 22   | 23   | 33    |
| CBA/J × B6 F1|          | 21   | 27   | 42    |

* Mice were immunized i.p. with HKX31 virus and killed 5 days later.

guinea pig complement. Also, specific lysis was mediated only by virus-immune spleen cells which share H-2K<sup>+</sup> or H-2D<sup>+</sup> genes with the virus-infected L cells (Table VIII), a constraint that is unique for T cells (7). It thus seems apparent that the effectors operating in these assays are cytotoxic T cells. This confirms earlier findings of Yap and Ada (24) for the A/WSN strain of influenza virus.

Evidence for Different T-Cell Subsets. Subdivision of cytotoxic T-cell specificities with respect to requirement for H-2K<sup>+</sup> or H-2D<sup>+</sup> compatibility is readily achieved by utilizing "cold-target" competitive-inhibition protocols (25). The same is true for differentiating between the effects of priming with different viruses (Fig. 1). Cross-reactive cytotoxic T-cell activity recognized for the heterologous interaction (e.g., H0N1 → H3N2) is abrogated to the same extent when
Fig. 1 Inhibition of immune spleen cell effectors (100:1) in a 12 h assay using different ratios of cold, unlabeled competitor cells. The competitors were normal L cells (N), or L cells infected with BLee, PR8 (H0N1), or HKX31 (H3N2) 4 h before the \(^{51}\text{Cr}\)-labeled targets were infected with one of these strains of influenza virus.

either H0N1 or H3N2 virus-infected, unlabeled cells are interposed between lymphocyte and target. Little inhibition is recognized when normal L cells, or L cells infected with BLee are used as competitors. In the homologous situation (e.g., H0N1 → H0N1), however, much greater inhibition is recognized with the H0N1 competitor than with the H3N2-infected cells. The converse is also true. Apparently at least two populations of immune T cells are functioning, the one being cross-reactive between different A strain viruses, the other specific for the homologous virus. This is the first time that we have been able to subdivide virus-immune T-cell specificities, other than on the basis of requirement for H-2 compatibility.

Cross-Priming. Further evidence for cross-reactivity between PR8 (H0N1) and HKX31 (H3N2) influenza virus-immune T cells was found when mice primed with PR8 were challenged 3 wk later with HKX31. A second exposure to PR8 resulted in cytotoxic activity less than that observed for primary immunization (Fig. 2). This reduction probably reflects neutralization of the input virus by antibody. Memory PR8 mice challenged with HKX31, however, generate immune spleen cells which are more lytic for both the H0N1 and H3N2 virus-infected target cells. Is cross-priming of T cells central to the "original antigenic
Fig. 2. CBA/J mice were injected i.p. with a 1:10 dilution of allantoic fluid containing PR8 (H0N1) influenza virus. These mice were then challenged (secondary) i.p. 21 days later with the same dose of PR8 or HKX31 (H3N2) influenza virus, and spleen cells were assayed after a further 5 days. Previously unexposed mice were immunized at the same time (primary). The assays were incubated for 12 h at 37°C. Specific \( ^{3} \text{H} \)Cr release caused by memory spleen cells from unchallenged PR8 memory mice was <5%, as were cytotoxic activities of all spleen populations for normal L cells.

Discussion

Recognition that there is extensive cross-reactivity in the cytotoxic T-cell response to different influenza type A viruses raises important questions concerning the role of cell-mediated immunity (CMI) in influenza. Studies with ectromelia and LCMV indicate that cytotoxic, or surveillance (26), T cells are of prime importance in elimination of virus-infected cells in vivo (4–7). Capacity to adoptively transfer effector function in these two diseases correlates closely with cytotoxic activity measured in vivo, and is subject to the same requirement for \( H-2K \) or \( H-2D \) identity between stimulator environment and virus-infected target cell, or recipient mouse.

Is this also true for influenza? If so, the fact that widespread exposure of human populations to one A strain influenza virus apparently does not protect against a new, serologically distinct pandemic strain (27) might be thought to mean that CMI plays no significant role in this disease. There is, however, some experimental evidence that mice previously infected with an H0N1 virus sup-
port decreased virus replication on subsequent challenge with an H2N2 strain (28). Other studies indicate that T cells may, as in LCMV (7), mediate immunopathological process (29, 30). Perhaps human influenza reflects both protective and immunopathological consequences of T-cell effector function. May this have been a factor in the extremely high mortality observed in young adults during the 1918 pandemic? Availability of an in vitro correlate for CMI should considerably facilitate an experimental approach to such questions (7, 18).

What are the cross-reactive T cells recognizing? One possibility is that the T-cell receptor is specific for an "altered self" determinant, perhaps an abnormally expressed alloantigen (13), which is common to cells infected with very similar viruses. An alternative is that shared virus components, such as the internal RNP and M protein, may be expressed in some way on the surface of the virus-infected cell. This is, however, thought not to occur (15). Even so, the M protein aligns on the cytoplasmic face of the plasma membrane (15). Could this induce some specific complementary modification, or rearrangement of molecules, on the outside of the lipid-protein bilayer? Such a change would not be detected by antisera directed against M protein purified from egg-grown virus (15).

Another consideration is that this is a rather acute immune response, being maximal at 5 days after primary immunization. Perhaps the specificity of the T-cell receptor is equivalent to that of an early IgM, which may be much less restricted than the late IgG used to serologically define influenza strains (14). The same mechanism [anti-idiotypic response? (31)] that regulates IgM production may also prevent further clonal expansion of effector T cells.

The fact that virus-specific T-cell populations can also be demonstrated indicates that at least part of the T-cell repertoire is directed against the virus. Perhaps we are considering a continuum of recognition. We know that a single mouse produces more than one B-cell clone specific for a given H antigen (32). The same V gene products may also be expressed on T cells (33, 34). The binding characteristics, and thus the specificity, of a secreted Ig molecule may be quite different from that of multiple recognition structures [single Ig heavy chains? (33, 34)] arranged in a stable matrix, such as the cell membrane (12). Some T-cell clones may thus be highly cross-reactive, even though free Ig is not, the degree of specificity depending (as always) on the uniqueness of the antigenic site recognized.

The central question is whether we can account for this T-cell specificity pattern in terms of known components of influenza virus. This may be possible. A range of recombinant viruses are available (14), monoclonal antisera can be generated (32), and the various virus proteins can be obtained in pure form (15). Is there any need to invoke an "altered self" concept, other than at the level of associative recognition of virus and H-2 antigen?

Summary

Specificity of cytotoxic T-cell function was investigated for a range of different influenza viruses. T cells from mice immunized with A or B strain influenza viruses, or with vaccinia virus, showed reciprocal exclusion of cytotoxicity. Extensive cross-reactivity was, however, found for lymphocyte populations from mice infected with a variety of serologically distinct influenza A viruses, though
serum antibodies did not cross-react when tested in a radioimmunoassay using comparable target cells as immunoadsorbents. This apparent lack of T-cell specificity was recognized for immune spleen cells generated after intraperitoneal inoculation of high titers of virus, and for mediastinal lymph node populations from mice with pneumonia due to infection with much less virus. The phenomenon could not be explained on the basis of exposure to the chicken host component, which is common to A and B strain viruses. However, not all of the virus-immune T-cell clones are cross-reactive. Competitive-inhibition experiments indicate that a considerable proportion of the lymphocyte response is restricted to the immunizing virus. Even so, the less specific component is significant. Also, exposure to one type A virus was found to prime for an enhanced cell-mediated immunity response after challenge with a second, serologically different A strain virus.

We thank Dr. D. Gotze for supplying both recombinant mouse strains and the anti-T serum; Dr. W. E. Biddison for advice concerning nylon wool columns; and Marge Solomon, Maureen Carey, and Michael Melino for capable technical assistance.

Received for publication 3 November 1976.

References

1. Doherty, P. C., and R. M. Zinkernagel. 1976. Specific immune lysis of paramyxovirus-infected cells by H-2 compatible thymus-derived lymphocytes. *Immunology* 31:27.

2. Starzinski-Powitz, A., K. Pfizenmaier, U. Koszynowski, M. Rollinghoff, and H. Wagner. 1976. Shared CML-determinants between virus-infected and TNP-conjugated H-2 identical target cells. *Eur. J. Immunol.* In press.

3. Blanden, R. V., P. C. Doherty, M. B. C. Dunlop, I. D. Gardner, R. M. Zinkernagel, and C. S. David. 1975. Genes required for cytotoxicity against virus-infected target cells in K and D regions of H-2 complex. *Nature (Lond.)* 254:269.

4. Doherty, P. C., M. B. C. Dunlop, C. R. Parish, and R. M. Zinkernagel. 1976. Inflammatory process in murine lymphocytic choriomeningitis is maximal in H-2K or H-2D compatible interactions. *J. Immunol.* 117:187.

5. Kees, U., and R. V. Blanden. 1976. A single genetic element in H-2K affects mouse T-cell antiviral function in poxvirus infection. *J Exp. Med.* 143:450.

6. Zinkernagel, R. M., and R. Welsh. 1976. H-2 restriction of virus-specific T-cell-mediated effector functions in vivo. I. Specificity of the T cells conferring anti-viral protection against lymphocytic choriomeningitis is associated with H-2K and H-2D. *J Immunol.* In press.

7. Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-immune effector T cells for H-2K and H-2D compatible interactions: implications for H-antigen diversity. *Transplant Rev.* 29:89.

8. Pfizenmaier, K., A. Starzinski-Powitz, H. Rodt, M. Rollinghoff, and H. Wagner. 1976. Virus and trinitrophenyl hapten-specific T-cell-mediated cytotoxicity against H-2 incompatible target cells. *J Exp. Med.* 143:999.

9. Zinkernagel, R. M. 1976. Virus-specific T-cell-mediated cytotoxicity across the H-2 barrier to altered allogeneic H-2. *Nature (Lond.)* 261:139.

10. Zinkernagel, R. M. 1976. 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. *J Exp. Med.* 144:933.
11. Blanden, R. V., A. J. Hapel, and D. C. Jackson. 1976. Mode of action of Ir genes and the nature of T cell receptors for antigen. *Immunochemistry*. 13:179.

12. Doherty, P. C., D. Götze, G. Trinchieri, and R. M. Zinkernagel. 1976. Models for recognition of virally-modified cells by immune thymus-derived lymphocytes. *Immunogenetics*. 3:517.

13. Garrido, F., V. Schirrmacher, and H. Festenstein. 1976. H-2-like specificities of foreign haplotypes appearing on mouse cells after vaccinia virus infection. *Nature (Lond.).* 259:228.

14. Schild, G. C., and W. R. Dowdle. 1975. Influenza virus characterization and diagnostic serology. In *The Influenza Viruses and Influenza*. E. D. Kilbourne, editor. Academic Press, Inc., New York. 315.

15. Choppin, P. W., and W. Compans. 1975. The structure of influenza virus. In *The Influenza Viruses and Influenza*. E. D. Kilbourne, editor. Academic Press, Inc., New York. 15.

16. Kilbourne, E. D. 1969. Future influenza vaccines and the use of genetic recombinants. *Bull. W. H. O.* 41:643.

17. Fazekas de St. Groth, S., and R. G. Webster. 1966. Disquisitions on original antigenic sin. I. Evidence in man. *J. Exp. Med.* 124:331.

18. Doherty, P. C., J. C. Palmer, and R. M. Zinkernagel. 1977. Experimental analysis of effector thymus-derived lymphocyte function in viral infections. *Methods Virol.* In press.

19. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived lymphocytes. *Eur. J. Immunol.* 3:645.

20. Rodt, H., S. Thierfelder, and E. Eulitz. 1974. Anti-lymphocytic antibodies and marrow transplantation. III. Effect of heterologous anti-brain antibodies on acute secondary disease in mice. *Eur. J. Immunol.* 4:25.

21. Segal, G. P., and N. R. Klinman. 1976. Defining the heterogeneity of anti-tumor antibody responses. *J. Immunol.* 116:1539.

22. Kozzinowski, U., and H. Ertl. 1975. Lysis mediated by T cells and restricted by H-2 antigen on target cells infected with vaccinia virus. *Nature (Lond.).* 255:552.

23. Cambridge, G., J. S. MacKenzie, and D. Keast. 1976. Cell-mediated immune response to influenza virus infections in mice. *Infect. Immun.* 13:36.

24. Yap, K. L., and G. L. Ada. 1976. Cytotoxic T cells specific for influenza virus-infected target cells. *Immunology*. In press.

25. Zinkernagel, R. M., and P. C. Doherty. 1975. 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. *J. Exp. Med.* 141:1427.

26. Doherty, P. C., and R. M. Zinkernagel. 1975. A biological role for the major histocompatibility antigens. *Lancet.* 1:1406.

27. Kilbourne, E. D. 1975. Epidemiology of influenza. In *The Influenza Viruses and Influenza*. E. D. Kilbourne, editor. Academic Press, Inc., New York. 483.

28. Schulman, J. L., and E. D. Kilbourne. 1966. Induction of partial specific heterotypic immunity in mice by a single infection with Influenza A virus. *J. Bacteriol.* 89:170.

29. Singer, S. H., P. Noguchi, and R. L. Kirschstein. 1972. Respiratory diseases in cyclophosphamide-treated mice. II. Decreased virulence of PR8 influenza virus. *Infect. Immun.* 5:257.

30. Cate, T. R., and N. G. Mold. 1975. Increased influenza pneumonia mortality of mice adoptively immunized with node and spleen cells sensitized by inactivated but not live virus. *Infect. Immun.* 11:808.

31. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol.* (Paris). 125:373.
32. Gerhard, W. 1976. The analysis of the monoclonal immune response to influenza virus. II. The antigenicity of the viral hemagglutinin. *J. Exp. Med.* 144:985.

33. Binz, H., and H. Wigzell. 1975. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigen determinants. II. Determination of frequency and characteristics of idiotype T and B lymphocytes in normal rats using direct visualization. *J. Exp. Med.* 142:1218.

34. Black, S. J., G. J. Hämmerling, C. Berek, K. Rajewsky, and K. Eichmann. 1976 Idiotypic analysis of lymphocytes in vitro. I. Specificity and heterogeneity of B and T lymphocytes reactive with anti-idiotypic antibody. *J. Exp. Med.* 143:846.
Author/s:
EFFROS, RB; DOHERTY, PC; GERHARD, W; BENNINK, J

Title:
GENERATION OF BOTH CROSS-REACTIVE AND VIRUS-SPECIFIC T-CELL POPULATIONS AFTER IMMUNIZATION WITH SEROLOGICALLY DISTINCT INFLUENZA-A VIRUSES

Date:
1977-01-01

Citation:
EFFROS, R. B., DOHERTY, P. C., GERHARD, W. & BENNINK, J. (1977). GENERATION OF BOTH CROSS-REACTIVE AND VIRUS-SPECIFIC T-CELL POPULATIONS AFTER IMMUNIZATION WITH SEROLOGICALLY DISTINCT INFLUENZA-A VIRUSES. JOURNAL OF EXPERIMENTAL MEDICINE, 145 (3), pp.557-568. https://doi.org/10.1084/jem.145.3.557.

Persistent Link:
http://hdl.handle.net/11343/257078

License:
CC BY-NC-SA