SPECIFICITY, Ly PHENOTYPE, AND H-2 COMPATIBILITY REQUIREMENTS OF EFFECTOR CELLS IN DELAYED-TYPE HYPERSENSITIVITY RESPONSES TO MURINE INFLUENZA VIRUS INFECTION

By K.-N. LEUNG, G. L. ADA, and I. F. C. McKENZIE

From the Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra City, Australian Capital Territory 2601, Australia; and the Department of Medicine, The Austin Hospital, Heidelberg, Victoria 3084, Australia

There is currently much interest in the cell-mediated immune response to viral infection, which largely derives from the initial finding of Zinkernagel and Doherty (1) that cytotoxic T cells (Tc)[1] generated in mice infected with lymphocytic choriomeningitis (LCM) virus would lyse LCM-infected target cells only if effector and target cells were compatible at the K or D region in the H-2-gene complex. The findings have been extended to many other systems (2, 3). Another component of the cell-mediated immune response to viral infection is the delayed-type hypersensitivity (DTH) response. Though there are a number of recent reports of DTH responses to viral infections (4–8), the genetic requirements and antigenic properties of the effector cells (DTH T cells [Td]) have not been studied in the same detail as have Tc cells. It is generally thought that Td require I-region compatibility in cell-transfer systems (9); however, in the one example where a viral system was examined (10), K- or D-region compatibility was required for a successful transfer of DTH. The Ly antigenic specificity of the cells was not examined.

We have recently initiated a study of Td generated in mice to influenza virus. We have shown elsewhere that such reactions can be measured reliably by measuring footpad swelling or by localization in the ear of labeled mononuclear cells (11).[2] The kinetics of development and the factors affecting their production are also described. The fine specificity of the effector cells, their Ly phenotype, and their requirement for H-2 compatibility are reported in this paper. Our results with influenza virus are different, at least in respect to the last parameter, to the earlier result with LCM virus.

Materials and Methods

Mice. The various strains of inbred mice used in this work were bred at the John Curtin School (Canberra City, Australia). Mice of the same sex and age (7–12 wk) were used in each experiment. The H-2 haplotypes of the mouse strains are given in Table I.

[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; EID_{50}, egg-infectious dose(s); HAU, hemagglutinin units; LCM, lymphocytic choriomeningitis; PBS, phosphate-buffered saline; Tc, cytotoxic T cells; Td, DTH T cells.

[2] Leung, K.-N., and G. L. Ada. Production of DTH in the mouse to influenza virus: a comparison with conditions for stimulation of cytotoxic T cells. Manuscript submitted for publication.

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TABLE I
H-2 Haplotypes of Mice Used in the Experiments

Mouse strain	Haplotype			Mouse strain	Haplotype		
	K	ABJEC (I)	SGD		K	ABJEC (I)	SGD
CBA	k	kkkkk	kkk	B10.A(2R)	k	kkkkd	ddb
A.TL	s	kkkkk	kkd	B10.A(3R)	b	bbbkd	ddd
A.TH	s	sssss	ssd	B10.A(4R)	k	kbbbb	bbb
SJL	s	sssss	sss	B10.A(5R)	b	bbkkd	ddd
C3H.OH	d	ddddd	ddk	B10.AQR	q	kkddd	ddd
C3H.OL	d	ddddd	kkk	C57BL/6J	b	bbbbb	bbb

Viruses. Influenza virus strains A/WSN (H0N1), A/JAP (H2N2), A/RI (H2N2), A/PC (H3N2), and A/JAP-Bel (H2N1); B/LEE virus; and Sendai virus were grown in the allantoic cavity of 10-d-old embryonated eggs for 40–48 h. The infectious allantoic fluid was stored at −70°C until use. Virus titers are expressed as egg-infectious doses (EID_{50}) or as hemagglutinin units (HAU). The virus was purified as described elsewhere (12) and was inactivated by UV irradiation as follows: Purified virus (2×10^5 HAU/ml) was exposed to a UV light source at a distance of 15 cm for 7 min (intensity: 320 $\mu W/cm^2$). There was a complete loss of infectivity but no change in hemagglutinin activity.

Cells. Spleens were harvested 6 d after sensitization of mice with virus, and cell preparations were made by standard procedures. Secondary effector cells were generated in tissue culture as described previously (13). Lung cells were harvested 6 d after intranasal inoculation of a lethal dose (5×10^4 EID_{50}) of virus (A/WSN). Ig-negative cell fractions were prepared as described previously (14).

Antiserum. Monoclonal anti-Ly 1.1 (1147A) antibody[3] and anti-Ly 2.1 antiserum (15, 16) have been described previously. 5×10^7 Ig-negative cells from spleen were incubated with a 1: 250 dilution of anti-Ly 1.1 or a 1:8 dilution of the anti-Ly 2.1 antiserum for 30 min at room temperature, the cells were washed, resuspended in 1 ml of a 1:5 dilution of normal rabbit serum (which had been preadsorbed with 3×10^8 syngeneic mouse spleen cells), and incubated (37°C, 30 min). The cells were washed, and viability was determined. Anti-Ly 1.1 + C′ treatment killed 50% of the cells; anti-Ly 2.1 + C′ treatment killed 30% of the cells.

Measurement of DTH in mice

SENSITIZATION OF MICE. Normal mice or mice injected 48 h beforehand with cyclophosphamide (100 mg/kg)[2] were injected subcutaneously with normal allantoic fluid, infectious allantoic fluid, or UV-irradiated (inactivated) virus. Optimal doses for sensitization with infectious or inactivated virus were 10^3 and 5×10^3 HAU, respectively.

ADOPTIVE TRANSFER OF CELLS AND CHALLENGE WITH VIRAL ANTIGEN. Cell preparations were tested for their ability to transfer DTH as follows: (*a*) Primary immune spleen cells (~8×10^7) were injected intravenously, and 2–8 h later, virus (6×10^3 HAU) in 30 μl was injected into the right-hind footpad and 30 μl of phosphate-buffered saline (PBS), pH 7.4, was injected into the left-hind footpad. (*b*) Secondary (tissue culture) preparations, lung cells, or the Ig-negative fraction of primary immune spleen cells were injected directly into the footpad. In initial experiments, three groups each of three to four mice, were used to test each cell preparation. In each group, the left-hind footpad was injected with 40 μl of medium only. Virus (6×10^3 HAU) alone, cells ($2\text{–}5 \times 10^6$) alone, or virus and cells (40 μl total volume) were injected into the right-hind footpad of different groups. The results of these experiments showed that injection of virus or cells alone seldom gave an increase of swelling of 8% at 24 h and averaged 5%, so the procedure was later simplified (see below). In all experiments, unless otherwise

[3] Hogarth, P. M., and I. F. C. McKenzie. Production and immunogenetic characterization of a monoclonal anti-Lyt 1.1 antibody. Manuscript submitted for publication.

TABLE II

*Comparison of the Specificity of the DTH Response in Mice Sensitized with Infectious or Noninfectious Influenza Virus**

Strain	Sensitizing virus infectivity‡	Challenge virus	Mean increase in footpad thickness
			%
Normal allantoic fluid	NI	A/WSN(H0N1)	6.3 ± 1.3
A/WSN	I	A/WSN(H0N1)	32.8 ± 2.0§
	NI	A/WSN(H0N1)	33.6 ± 2.0§
A/RI(H2N2)	I	A/WSN(H0N1)	23.4 ± 1.6§
	NI	A/WSN(H0N1)	4.4 ± 0.8
A/PC(H3N2)	I	A/WSN(H0N1)	26.6 ± 3.3§
	NI	A/WSN(H0N1)	7.1 ± 0.8
B/LEE	I	A/WSN(H0N1)	3.1 ± 1.3
	NI	A/WSN(H0N1)	6.3 ± 1.0
A/WSN	NI	A/RI(H2N2)	2.4 ± 0.8
A/RI	NI	A/RI(H2N2)	29.7 ± 2.0§
A/PC	NI	A/RI(H2N2)	6.9 ± 1.2
A/JAP-BEL(H2N1)	NI	A/RI(H2N2)	28.1 ± 1.3§

* Groups of four to five CBA mice were injected subcutaneously with infectious or noninfectious virus; 6 d later they were challenged into the footpad with purified, UV-inactivated A/WSN virus (6×10^3 HAU). Footpad swelling was measured 24 h after virus challenge. Details are given in Materials and Methods.

‡ I, infectious; NI, noninfectious.

§ Significantly greater than control group of mice (sensitized with normal allantoic fluid only). $P < 0.001$.

mentioned, purified UV-inactivated virus (6×10^3 HAU) was used to elicit the DTH response, as it was found that injection of infectious virus per se gave higher control readings.

MEASUREMENT OF FOOTPAD SWELLING. In most experiments reported, PBS (or medium) was injected into the left-hind footpad and virus or virus plus cells were injected into the right-hind footpad. The footpad thickness was measured with a pair of calipers (H. C. Kröplin, Schlüchtern, Hessen, Federal Republic of Germany, calibrated to ± 0.05 mm), and increase in footpad thickness was calculated as the difference of readings (right-hand side vs. left-hand side), divided by the mean thickness of the feet measured before challenge (17). In transfer experiments, footpad thickness was measured 24 and 48 h after challenge. It was found that the peak always occurred at 24 h.

Statistical Analysis. Results are expressed as the arithmetic mean ± SE. The significance of differences were calculated with Student's *t* test.

Results

Specificity of the DTH Response in Mice Sensitized with Infectious or Noninfectious Virus. Previous work with mice infected with influenza virus had shown that the population of Tc generated to this antigen in spleen would lyse target cells infected with either the homologous virus or any other A-strain virus (18–20). In contrast, secondary effector Tc generated in tissue culture after exposure to UV-irradiated virus yielded potent effector cells that were specific for the homologous virus (12). It was previously shown that the DTH response to influenza A virus in mice was specific in that the response in mice sensitized with an A-strain virus could be elicited with the homologous virus but not with a B-strain virus or Sendai virus.[2] Table II compares the fine specificity of the DTH response of mice when infectious or noninfectious virus was used for sensitization and shows that: (*a*) Mice sensitized with infectious virus generate Td populations that are cross-reactive within the A strains. (*b*) Mice

TABLE III

*Effect of Anti-Ly Sera on the Ability of Immune Cells Taken from Mice Sensitized with Infectious or Noninfectious Virus to Transfer DTH**

Sensitizing virus		Cell transfer	Treatment of cells§	Injection into footpad	Mean increase in footpad thickness
Strain	Infectivity‡				
					%
A/WSN(H0N1)	I	−‖	—	Virus	31.3 ± 1.3
A/WSN	I	+	Nil	Cells	2.1 ± 1.1
A/WSN	I	+	Nil	Cells + virus	25.0 ± 1.0
A/WSN	I	+	α-Ly 1.1 + C′	Cells + virus	6.3 ± 1.3¶
A/WSN	I	+	α-Ly 2.1 + C′	Cells + virus	25.6 ± 1.6**
A/WSN	NI	−‖	—	Virus	33.6 ± 2.0
A/WSN	NI	+	Nil	Cells + virus	25.0 ± 1.3
A/WSN	NI	+	α-Ly 1.1 + C′	Cells + virus	5.5 ± 0.8¶
A/WSN	NI	+	α-Ly 2.1 + C′	Cells + virus	25.0 ± 2.2**
Nil	—	−‖	—	Virus	3.1 ± 1.8
A/PC(H3N2)	I	−‖	—	Virus	33.3 ± 2.8
A/PC	I	+	Nil	Cells + virus	26.6 ± 2.0
A/PC	I	+	α-Ly 1.1 + C′	Cells + virus	4.2 ± 1.1¶
A/PC	I	+	α-Ly 2.1 + C′	Cells + virus	28.9 ± 1.5**

* Local adoptive transfer of virus-specific DTH response was performed 6 d after sensitization of CBA mice. Details were described in Materials and Methods.

‡ I, infectious; NI, noninfectious.

§ The Ig^- fraction of day-6 immune cells was either untreated or treated with anti α-Ly + C′, and the same number of viable cells were injected into the footpad in each case.

‖ No cell transfer means sensitization and elicitation of DTH reaction occurred in the same animal.

¶ Significantly lower than the control (no treatment of cells). $P < 0.01$.

** Not significantly different from the control (no treatment of cells).

sensitized with noninfectious virus generate a Td population that is specific for the homologous virus and probably for the hemagglutinin.

Ly Phenotype of Td. The results in Table III demonstrate that the cells that, on adoptive transfer to naive mice, confer DTH activity are sensitive to anti-Ly 1.1 antibody and complement and are not lysed by anti-Ly 2.1 serum and complement. This is the case for cells from donor mice that were sensitized with either infectious or noninfectious virus.

Requirement for H-2 Sharing between Donor and Recipient Strains of Mice for Expression of DTH

SENSITIZATION OF DONOR MICE WITH UV-IRRADIATED VIRUS. In preliminary experiments, CBA($H\text{-}2^k$), C57BL/6($H\text{-}2^b$), and BALB/c($H\text{-}2^d$) mice were used as donor and recipient mice of immune cells. Transfer of DTH occurred between syngeneic but not allogeneic mice. Similarly, in studies with (BALB/c × CBA/H)F_1 mice, transfer of DTH occurred when primed cells from the F_1 were transferred into either parent, but not into another strain. Groups of experiments were then carried out to determine the region or subregion of the H-2-gene complex required for successful cell transfer. The results are shown in Table IV. In all experiments, A/WSN was used to both sensitize and elicit the reaction. Group 1 experiments show that differences in the K- and D regions do not affect the transfer of DTH. Group 2 experiments show that difference at S-, G-, or D region is unimportant but sharing at I region is required for DTH

TABLE IV

Adoptive Transfer, with Various Strain Combinations, of DTH with Cells from Donor Mice Sensitized with Noninfectious Influenza Virus

Mouse strains		H-2 regions shared	Increase in footpad thickness
Donor*	Recipient		
			%
Experiment 1			
A.TL	A.TL	All	31.3 ± 0.0
CBA	A.TL	I	30.1 ± 1.3
CBA	CBA	All	29.7 ± 1.6
A.TL	CBA	I	33.3 ± 3.9
Experiment 2			
A.TH	A.TH	All	24.3 ± 1.5
A.TL	A.TH	K and D	7.1 ± 0.8‡
SJL	A.TH	K and I	23.9 ± 1.1
A.TL	A.TL	All	26.3 ± 0.8
A.TH	A.TL	K and D	7.1 ± 2.0‡
SJL	A.TL	K	2.1 ± 1.1‡
C3H.OH	C3H.OH	All	30.5 ± 3.1
C3H.OL	C3H.OH	All except S and G	27.4 ± 2.4
C3H.OL	C3H.OL	All	31.3 ± 1.3
C3H.OH	C3H.OL	All except S and G	30.5 ± 2.0
Experiment 3			
B10.A(5R)	B10.A(5R)	All	27.1 ± 1.1
B10.A(3R)	B10.A(5R)	All except IJ	26.1 ± 1.1
B10.A(3R)	B10.A(3R)	All	25.0 ± 0.9
B10.A(5R)	B10.A(3R)	All except IJ	31.3 ± 0.0
B10.A(2R)	B10.A(2R)	All	27.1 ± 3.8
B10.A(4R)	B10.A(2R)	K, IA, and D	28.1 ± 2.2
B10.A(4R)	B10.A(4R)	All	26.1 ± 2.8
B10.A(2R)	B10.A(4R)	K, IA, and D	25.0 ± 1.3
Experiment 4			
B10.A(4R)	B10.A(4R)	All	34.3 ± 2.6
B10.A(4R)	B10.AQR	IA	33.8 ± 0.8
B10.A(4R)	CBA	K and IA	29.7 ± 1.6
B10.A(4R)	C57BL/6J	All except K and IA	4.7 ± 1.6‡

* Donor spleen cells (8×10^7) were transferred intravenously to recipient mice 6 d after sensitization. A/WSN virus was used to sensitize mice and to elicit DTH.

‡ These values are significantly lower than control values of mice that received syngeneic immune cells ($P < 0.01$).

transfer. Group 3 experiments show that difference at the IJ subregion is unimportant and that similarity at the K- and D region and the IA subregion allows transfer of DTH. The experiments in group 4 indicate that sharing at the IA subregion alone is sufficient for DTH transfer to be successful.

Taking all these results together, it is concluded that IA-subregion sharing is both necessary and sufficient for DTH to be demonstrated.

SENSITIZATION OF DONOR MICE WITH INFECTIOUS VIRUS. A similar but more limited series of experiments to those described in the previous section were carried out, with the results shown in Table V. In the first three groups of experiments, the same virus (A/WSN) was used to sensitize for, and to elicit, the DTH reaction. Again, sharing of

TABLE V

Adoptive Transfer, with Various Strain Combinations, of DTH with Cells from Donor Mice Sensitized with Infectious Influenza Virus

Mouse strains		H-2 regions shared	Increase in footpad thickness
Donor*	Recipient		
			%
Experiment 1			
CBA	CBA	All	23.4 ± 0.9
A.TL	CBA	I	23.4 ± 1.6
A.TL	A.TL	All	22.7 ± 1.5
CBA	A.TL	I	23.9 ± 2.1
A.TH	A.TL	K and D	1.1 ± 1.1‡
A.TH	A.TH	All	22.7 ± 2.0
A.TL	A.TH	K and D	3.1 ± 1.8‡
Experiment 2			
B10.A(2R)	B10.A(2R)	All	24.3 ± 2.0
B10.A(4R)	B10.A(2R)	K, IA, and D	24.4 ± 1.2
B10.A(4R)	B10.A(4R)	All	25.0 ± 2.0
B10.A(2R)	B10.A(4R)	K, IA, and D	24.3 ± 1.5
Experiment 3			
B10.AQR	B10.AQR	All	37.2 ± 1.9
B10.AQR	B10.A(4R)	IA	40.5 ± 0.8
Experiment 4			
A.TL	A.TL	All	26.6 ± 4.7
A.TH	A.TL	K and D	3.9 ± 0.8‡
CBA	A.TL	I	25.8 ± 0.8
CBA	CBA	All	27.4 ± 1.5
A.TL	CBA	I	25.8 ± 1.5
A.TH	A.TH	All	26.6 ± 1.6
A.TL	A.TH	K and D	2.4 ± 0.8‡

* Donor spleen cells (8×10^7) were transferred intravenously to recipient mice 6 d after sensitization. In experiments 1–3, A/WSN virus was used to sensitize mice and to elicit DTH. In experiment 4, mice were sensitized with A/PC virus, and DTH was elicited with A/WSN virus.

‡ These values are significantly lower than the control values of mice that received syngeneic immune cells ($P < 0.01$).

the IA subregion is necessary and sufficient for successful transfer of DTH. These results suggest that the T cell subgroup that was responsible for cross-reactivity between different A strains of virus was also I-region restricted, and an extra group of experiments (Table V, experiment 4) confirmed this.

DTH Activity of Secondary Effector Cell Populations with Enhanced Tc Activity. Because of the earlier demonstration (10) that DTH in the LCM system was transferred by cells that required K- or D-region sharing between donor and recipient, cell populations were tested that were known to contain influenza virus-specific effector T cells that required K- or D-region compatibility. Memory cells from mice primed with infectious virus were restimulated in vitro with infectious homologous virus. Such populations have high Tc activity (13). A/WSN-immune cells (1.5×10^7) prepared in this way were injected intravenously into syngeneic (CBA/H) mice, and the eliciting virus was then injected into the footpad. Measurement of footpad swelling 24–72 h after antigen injection showed a minor, nonsignificant effect compared with the control. One explanation of this result might be that cultured cells circulate relatively

TABLE VI

Effect of Anti-Ly Sera on the Ability of Secondary Immune Cells Generated in Tissue Culture to Transfer DTH

Tissue culture preparation of cells*	Treatment of cells	Injected into footpad	Mean increase in footpad thickness
			%
No cells	—	Virus	3.1 ± 0.0
Infected stimulator plus memory spleen cells	—	Cells	2.4 ± 0.8
Infected stimulator plus memory spleen cells	—	Cells + virus	35.2 ± 2.0
Infected stimulator plus memory spleen cells	α-Ly1.1 + C′	Cells + virus	7.3 ± 1.1‡
Infected stimulator plus memory spleen cells	α-Ly2.1 + C′	Cells + virus	39.1 ± 3.0§
Uninfected stimulator plus memory spleen cells	—	Cells + virus	9.4 ± 1.3

* Preparation described in Materials and Methods. A/WSN virus was used to sensitize mice and to elicit DTH.
‡ Significantly lower than the control (no treatment of cells). $P < 0.01$.
§ Not significantly different from the control (no treatment of cells).

TABLE VII

*H-2 Restriction and Ly Phenotype of Td Recovered from the Lungs of Infected Mice**

Mouse strain		H-2 regions shared	Cell treatment	Injected into footpad	Mean increase in footpad thickness
Donor	Recipient				
					%
Experiment 1					
CBA	CBA	All	—	Virus and cells	27.4 ± 1.5
CBA	A.TL	I	—	Virus and cells	29.2 ± 1.1
A.TL	A.TL	All	—	Virus and cells	30.5 ± 1.3
A.TL	CBA	I	—	Virus and cells	34.4 ± 1.5
A.TL	A.TH	K and D	—	Virus and cells	4.7 ± 1.4‡
Experiment 2					
CBA	CBA	All	Nil	Cells	2.1 ± 1.1
CBA	CBA	All	Nil	Virus and cells	31.3 ± 2.6
CBA	CBA	All	α-Ly 1.1 + C′	Virus and cells	6.3 ± 1.3§
CBA	CBA	All	α-Ly 2.1 + C′	Virus and cells	34.4 ± 1.8‖

* Viable cells were recovered from lungs of infected mice 6 d after intranasal instillation of a lethal dose (5×10^4 EID_{50}) of A/WSN virus.
‡ The value is significantly lower than the control values of mice that received syngeneic cells ($P < 0.01$).
§ Significantly lower than the control (no treatment of cells). $P < 0.01$.
‖ Not significantly different from the control (no treatment of cells).

poorly to distant areas, such as feet. To circumvent this, cells (5×10^6) with virus or virus alone were injected directly into the footpad. Feet injected with cells plus virus gave a significant swelling (Table VI) at 24 h and remained swollen until 72 h after injection. However, the effector cells in this preparation that transferred DTH were sensitive to anti-Ly 1.1 and complement and not to anti-Ly 2.1 sera and complement (Table VII) and were I-region restricted (data not shown).

The Properties of Td from Lungs of Infected Mice. Mice were intranasally inoculated with a lethal dose (5×10^4 EID_{50}) of A/WSN virus. Previous work (21) showed that after such an inoculation, very high levels of infectious virus were present in the lungs

24 h later and these increased until death ensued 7–9 d after infection. The lungs of the infected mice were removed at 6 d, a cell suspension was made (21), and 1–2 × 10^6 of these were injected with virus into the hind footpads of mice with the same or different H-2 haplotypes as the donor mice. The results in Table VII show (*a*) that I-region sharing was necessary for successful transfer of DTH, and (*b*) that the Ly phenotype of the Td from the lungs was Ly 1.1.

Discussion

Earlier papers from this laboratory described the production of Tc in mice infected with influenza virus and produced evidence that suggested that these cells were important in the recovery of mice during infection with these viruses (22, 23). Part of the evidence that Tc were the responsible effector cells was the abrogation of the protective effect when the cell preparation was treated with anti-Ly 2,3 sera and complement before transfer to syngeneic hosts and the requirement for K- or D-region sharing between the donor of the transferred immune cells and the recipient mouse. In contrast, the effector cells that effect DTH are considered to be Ly 1.1 positive and insensitive to anti-Ly 2,3 sera and complement (24). Miller et al. (9), who used the ear localization of labeled cells procedure, showed that DTH to fowl γ-globulin was I-region restricted. Weinberger et al. (25) showed that the DTH to the (4-hydroxy-5-iodo-3-nitrophenyl) acetyl and (4-hydroxyl-3-nitrophenyl) acetyl haptens in mice required at least IA-subregion compatibility, and, in a preliminary study, Asherson et al. (26) reported that in the transfer of contact sensitivity in mice to the agent oxazalone, "the left hand side of the MHC [major histocompatibility complex] was more important than the right hand side." There are two reports that DTH may be K- or D-region restricted. Vadas et al. (27) found that DTH to the hapten dinitrofluorobenzene was both I-region and K- and D-region restricted, and Zinkernagel (10) found that cells expressing DTH in the mouse to the virus, LCM, required K- or D-region compatibility. Zinkernagel (10) suggested that antigen parameters determine whether T cells are specific for "altered" I- or "altered" K- and D-region coded structures, postulating that multiplying infectious agents like viruses that actively invade cells and interfere in the genetic and metabolic pathways of the cell would alter K- and D- rather than I-region coded structures.

Because of the result with LCM and our earlier transfer experiments, which suggested that Tc were important in the recovery of mice from infection, it was necessary to check the properties of influenza virus-specific Td. In this report, we have (*a*) characterized cells transferring DTH activity both by their sensitivity to anti-Ly sera and complement and their H-2 compatibility requirements, and (*b*) compared the specificity patterns seen with infectious and noninfectious preparations of influenza virus. That infectivity does have an effect is shown clearly by the specificity of the Td generated. Injection of UV-irradiated virus yields Td that are specific for the homologous virus, and most likely for the hemagglutinin (Table II). Injection of infectious virus yields Td that are cross-reactive with the A strains of influenza virus (Table II). A similar cross-reactivity has been observed previously (28), although the results quoted were very variable. Irrespective of this difference, all cell preparations showing DTH were sensitive to the action of anti-Ly 1.1 and complement and not to anti-Ly 2.1 and complement (Table III). The genetic requirements for successful transfer of DTH are summarized in Fig. 1. In all situations tested, I-region, and not

A B J E C

K I S G D

NON-INFECTIOUS VIRUS	INFECTIOUS VIRUS TOTAL	INFECTIOUS VIRUS CROSS-REACTIVE
-	-	N D
+	+	+
-	-	-
+	N D	N D
+	N D	N D
+	N D	N D
+	N D	N D
+	+	N D
-	N D	N D
+	+	N D

FIG. 1. Summary of the genetic requirements for transfer of DTH, as described in Tables III and IV, and in the text. Shared (|||||) and unshared (▭) subregions of H-2 between donor and recipient mice. Successful (+) and unsuccessful (−) transfer. ND, not done.

K- or D-region, compatibility was necessary; for infectious and noninfectious virus, the requirement was for IA-subregion compatibility (Tables IV and V, and text).

Despite this clear pattern, the question arose whether cells expressing Ly 2,3 antigens and a known requirement for K- or D-region compatibility can transfer DTH in this system. In the experiments recorded in Table IV, the cells transferring DTH were generated after sensitization of mice with UV-irradiated virus and such are known to contain negligible Tc activity (12). In the experiments recorded in Table V, the cells transferred would have possessed Tc activity. As yet, we know of no protocol that results in the production of Tc and not Td. One of the last two experiments was designed to test the DTH potential of cell populations possessing high Tc activity, and, therefore, which contained K- or D-region-restricted and Ly 2,3-positive cells (22). These cells were produced in tissue culture but did not transfer DTH activity when injected intravenously into the recipient mice. However, they caused prolonged footpad swelling when injected directly into the footpad with virus. The responsible cells were Ly 1 positive and I-region restricted. The other experiment was to test cell populations from infected lungs that, in contrast to spleen, are known to contain high levels of infectious virus (21). Such cell populations also contain Tc activity (21), yet upon transfer into footpads with virus the observed DTH was again a result of Ly 1-positive cells that required I-region compatibility. It seems, therefore, that the property of infectivity per se does not determine the genetic requirements that the cells transferring DTH will display.

It should be emphasized that these results do not unequivocally show that Ly 2,3-positive influenza-immune cells that recognize K- or D-region gene products cannot transfer DTH; they show that the major activity is carried by Ly 1-positive cells that require IA-subregion compatibility. What then is the explanation for the difference between LCM and influenza virus infections? One obvious difference is the kinetics of the DTH reaction. The footpad swelling observed both in the nontransfer experiments and in those experiments involving transfer of influenza virus-specific immune

spleen cells reached peak values at 24 h; the value at 72 h being much reduced. In contrast, the reaction observed after transfer of LCM-immune spleen cells usually peaked at 48–72 h, and, on occasions, there was minimal swelling at 24 h (10). Although it could be postulated that migration properties of the two cell populations differed, the possibility remains that the effector cells differed in other respects apart from surface antigen and H-2-antigen recognition, such as lymphokine production.

The results in this report suggest that the influenza virus-specific Td, like Tc (18–20, 29, 30), recognize hemagglutinin and possibly another viral antigen. In fact, the two T cell subsets show some similarities in this respect. If noninfectious virus was used for stimulation, both primary Td (in vivo) and secondary Tc (in vitro) were specific for the homologous virus. If infectious virus was used for stimulation, both primary Td and Tc generated in vivo showed cross-reactivity with the A strains of virus. It seems as though the influenza viral antigens that are recognized by these effector cells may be associated on stimulator cells with both K- and D- and I-region products. The identity of the viral antigen(s) recognized by LCM effector cells is unknown, but it can be speculated that it might associate more effectively with K- or D- than with I-region products.

Summary

Delayed-type hypersensitivity (DTH) to infectious and to noninfectious (UV-irradiated) influenza A viral preparations was measured in mice by the increase in footpad swelling 24 h after injection of the eliciting virus. DTH mice sensitized with noninfectious virus was elicited only by virus that shared hemagglutinin specificity with the sensitizing virus, whereas footpad injection of a given A-strain virus (A/WSN) could elicit DTH in mice sensitized with a variety of infectious A-strain viruses, including some not sharing hemagglutinin or neuraminidase specificities. The effector T cells generated in mice sensitized with either form of virus were sensitive to anti-Ly 1.1 serum and complement, but not to anti-Ly 2.1 serum and complement. Adoptive transfer of DTH was H-2 restricted. With spleen cells from mice sensitized subcutaneously with either infectious or noninfectious virus, sharing of the IA region was both necessary and sufficient for successful transfer to occur. Cells recovered from infected mouse lungs, and secondary effector cells generated in vitro transferred DTH if injected into the footpad with the eliciting virus. The effector cells had the Ly 1 phenotype, and, in both cases, the cells were I restricted. These results contrast with earlier findings that transfer of DTH to lymphocytic choriomeningitis virus infection required K- or D-region sharing between donor and recipient. Thus, the earlier hypothesis that multiplying infectious agents such as viruses would "alter" K- or D-coded, rather than I-coded, stuctures is not generally correct.

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