Brief Definitive Report

SUPPRESSION OF CELL-MEDIATED IMMUNITY BY STREET RABIES VIRUS*

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We recently reported (1) that a strong specific cell-mediated cytotoxic (CMC) response was generated in mice inoculated with a strain of attenuated rabies virus, or with an inactivated rabies virus vaccine. This response was dependent on thymus-derived lymphocytes (T cells), and was severely depressed by the prior inoculation of mice with anti-rabies antibody. We have now attempted to reproduce this CMC response using several other attenuated and virulent (street) strains of rabies virus. The level of T-cell effector function was found to be directly correlated with survival. Furthermore, concurrent infections with street rabies virus and influenza virus suppressed the development of a primary CMC response specific for influenza virus.

Materials and Methods

Mice. C57BL/6 (*H-2*^{b}) and B10.A (*H-2*^{a}) inbred mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, and were used when 8–12 wk old. The clinical status of virus-infected mice was assessed by daily examination, and by weighing at regular intervals.

Virus Preparations. The clone-purified Flury high egg passage (HEP) and the ERA strains of attenuated rabies virus were propagated in monolayers of baby hamster kidney (BHK) cell cultures (2). Infectivity of virus stocks, determined by plaquing in agarose-suspended BHK S13 cells (3), was $10^{8.6}$ plaque-forming units (PFU)/ml for HEP-BHK virus, and $10^{8.4}$ PFU/ml for ERA-BHK virus. A suspension of salivary glands from a naturally infected wild red fox (courteously supplied by Dr. L. Andral, Centre d'Études sur la Rage, Malzeville, France) was used as a source of street rabies virus (AF-SG); the infectivity of this virus stock was $10^{6.8}$ LD_{50}/ml as determined by intracerebral (i.c.) inoculation in 5-wk-old ICR mice.

The HK (HKX31, H3N2) and PR8 (A/PR/8/34, HON1) strains of influenza A virus were propagated in the allantoic cavity of chick embryos. The hemagglutinating (HA) activity of both virus preparations was 2,000 HA U/ml (4). Mice were immunized intraperitoneally (i.p.) with 1.0 ml of a 1:10 dilution of allantoic fluid.

Antibody Determination. Virus-neutralizing antibody (VNA) was measured by the rapid fluorescent focus-inhibition technique in BHK cell cultures (5).

Cytotoxicity Assays. The cytotoxic activity of immune lymphocytes was assessed using methods previously described (1, 6). Briefly, single cell suspensions were made from pools of three spleens and added to ^{51}Cr-labeled normal or virus-infected target cells. The assays were incubated for 12 h (for influenza virus) or for 16 h (for rabies virus) at 37°C in 5% CO_2/95% air at 100% humidity. The targets used were MC57G (*H-2*^{b}) and L929 (L cell, *H-2*^{k}) fibroblasts, and mouse neuroblastoma cells (MNB, *H-2*^{a}).

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Results

Groups of C57BL/6 mice were inoculated i.c. with 100 PFU of HEP, 100 PFU of ERA, or 1,000 LD_{50} of street virus. No signs of disease were apparent after infection with HEP. Transient neurological signs occurred after exposure to ERA but, despite a 30% drop in body weight between 7 and 11 days postinfection (p.i.), all mice survived for the 60-day observation period. Mice given street virus showed evidence of neuronal dysfunction at 8 days p.i., were paralyzed 2 days later and died on day 12 p.i. The first clinical evidence of disease was a 20% loss of body weight between days 5 and 6 p.i. Mice had lost as much as 40% of their original weight at time of death, and spleen size was decreased proportionally.

The kinetics and magnitude of antibody production were similar for all three viruses, and could not be correlated with the severity of disease (Fig. 1). Significant cytotoxic T-cell responses were generated after nonfatal infection with HEP and ERA. Lysis was maximal on day 7 in both infections (Fig. 1). However little, if any, CMC response was seen in mice given street virus. Development of fatal rabies is thus directly associated with defective T-cell responsiveness. Similar results were observed after inoculation of mice with 10^6 or 10^4 PFU of HEP and ERA viruses and with 10 LD_{50} of AF-SG street virus and after inoculation of three other virulent strains of rabies virus (data not shown).

To determine if this depression of CMC by street virus was specific for rabies, B10.A mice were injected i.c. with 1,000 PFU of HEP virus or 1,000 LD_{50} of street virus. Rabies-infected mice and normal controls were then inoculated i.p. 48 h later with 200 HA U of the HKX31 strain of influenza virus. The mice were killed on day 7 p.i. at the peak of the influenza-specific T-cell response (6). Prior exposure to HEP apparently did not modify CMC resulting from inoculation with HK, whereas little, if any, effector function occurred in mice given street virus (Fig. 2). The influenza-immune T cells (6) mediated lysis of target cells infected with the PR8 type A influenza viruses (Fig. 2 b), but there was no cross-reactivity of CMC between influenza and rabies (Table I).

Furthermore, the T-cell response resulting from immunization with influenza virus was suppressed by street rabies virus given as late as 1 day after exposure to PR8 (Table I). Secondary CMC resulting from cross-challenge with two different influenza A viruses (6) was, however, unaffected by concurrent infection with street rabies virus (Table II).

Discussion

Experiments with poxviruses and arenaviruses have shown that development of an inflammatory process and clearance of virus in vivo depend on the same subset of T cells that causes CMC in vitro (7–9). Intracerebral injection of street rabies virus does not induce the development of cytotoxic T cells, generates little inflammation (10), and leads to a uniformly fatal neurological disease. Conversely, the attenuated HEP and ERA rabies viruses are not lethal for C57BL/6 mice and are associated with a strong CMC response. It thus seems likely that fatal rabies infection reflects defective operation of the cell-mediated immunity (CMI) protective mechanisms (11).

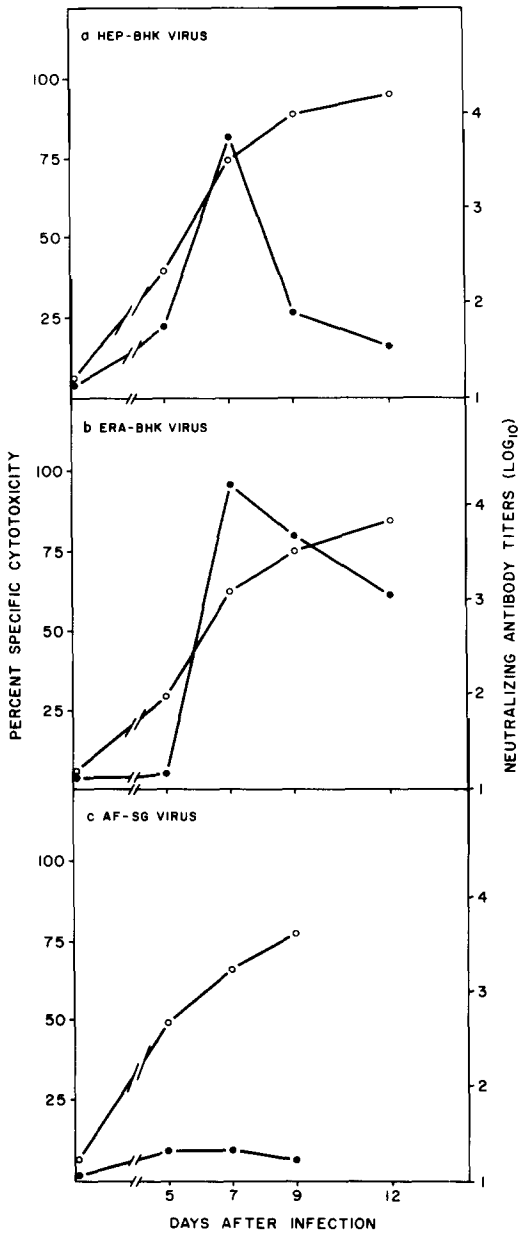

FIG. 1. Development of CMC (●) and VNA (○) in C57BL/6 mice infected i.c. with 100 PFU of HEP-BHK virus (a), 100 PFU of ERA-BHK virus (b), or 1,000 LD_{50} of AF-SG street virus (c).

Several possible explanations can be made for this failure of street rabies virus to induce cytotoxic T cells. All strains of rabies virus (both virulent and attenuated) multiply mainly in neurons of the central nervous system (12). Perhaps the failure of CMI responsiveness after i.c. street rabies virus infection may occur because the immune system is not sufficiently exposed when street

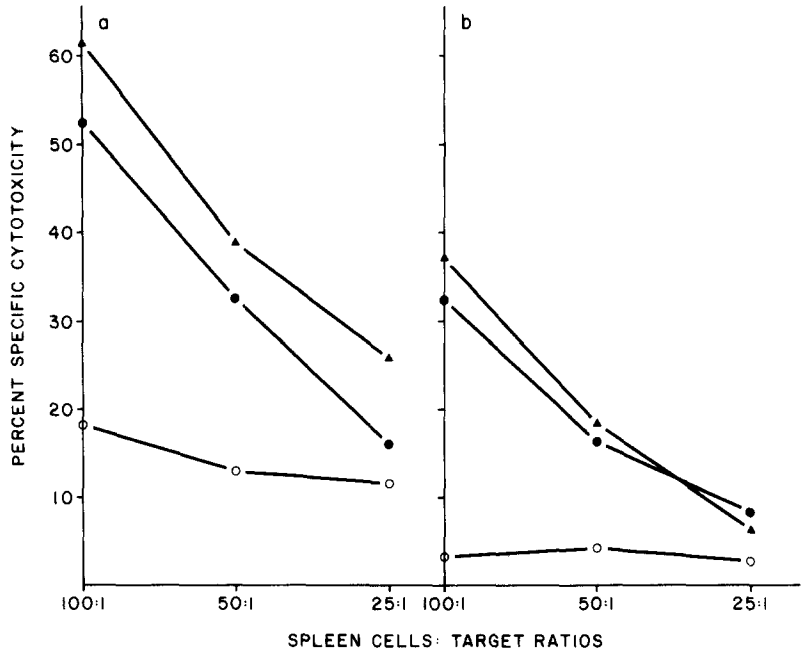

FIG. 2. Suppression of the influenza-immune T-cell response by concurrent infection with street rabies virus: B10.A mice were infected i.c. with 1,000 PFU of HEP-BHK (●) or 1,000 LD_{50} of AG-SG street virus (○). 2 days later rabies virus-infected mice and uninfected control mice (▲) received i.p. 200 HA U of HK strain of influenza virus. After a further 5 days, spleen cells of all mice were tested for CMC response against HK (a) or PR8 (b) influenza virus-infected L cells.

TABLE I

The Immunosuppressive Effect of Street Rabies Virus Given at Different Times Relative to Inoculation of PR8 Influenza Virus

Day of* rabies inoculation	Viruses given			% ^{51}Cr release from virus-infected L cells		
	HEP rabies	Street rabies	PR8 influenza	Influenza		ERA rabies
				PR8	HK	
−2	−	+	−	0	0	5
−2	−	+	+	10	11	6
−1	−	+	+	6	10	NT‡
0	−	+	+	9	12	1
+1	−	+	+	14	18	NT
0	−	−	+	50	44	0
−2	+	−	+	58	41	20
−2	+	−	−	0	0	23

* The times given are relative to inoculation of CBA/J mice with influenza virus (day 0).
‡ NT, not tested.

virus multiples in the brain, and the virus thus "sneaks through" T-cell surveillance (13). Alternatively, infection with street virus may not cause antigenic changes on the cell surface that are recognized by cytotoxic T cells.

The above mechanisms, however, fail to explain the depression of primary T-cell responsiveness to concurrent infection with an unrelated virus, the in-

TABLE II

Exposure to Street Rabies Virus Does Not Suppress the Secondary Cytotoxic T-Cell Response to Influenza A Viruses

Day of* rabies inoculation	Viruses given			% ^{51}Cr release from virus-infected cells	
	HEP rabies	Street rabies	PR8 influenza	PR8	HK
−4	+	−	+	62	82
−4	−	+	+	58	74
−2	−	+	+	23	42
0	−	+	+	90	85
0	−	−	+	77	97

* The times given are relative to inoculation of B10.A mice with HK influenza virus. Spleen cells were assayed (75:1) on day 3. The mice had been primed with A/Ann Arbor/1957 (H2N2) influenza virus 10 days previously.

fluenza virus. Interferon levels are no higher after infection with virulent than attenuated rabies virus (14, 15). Perhaps the answer lies in the fact that spleen size is greatly reduced in mice exposed to rabies street virus. This does not occur with the ERA or HEP strains. Does this reflect some defect at the level of stimulator cells, even though there is evidence that rabies virus does not replicate in spleen (16) and the rabies-specific antibody response is normal? The secondary influenza-specific T-cell response, which is unaffected by concurrent infection with street virus, probably requires a much smaller antigenic stimulus (17). Are we considering some general physiological effect of virus-induced neuronal dysfunction? Elucidation of the underlying mechanisms may allow manipulation of the immune response to benefit the host.

Summary

Mice lethally infected with street rabies virus failed to develop cytotoxic T cells specific for rabies virus-infected target cells, whereas high levels of cell-mediated cytotoxicity (CMC) were generated after nonfatal infection with the attenuated high egg passage (HEP) or ERA rabies virus strains. Furthermore concurrent infection with street, but not with HEP, rabies virus suppresses development of a primary (but not a secondary) CMC response specific for influenza virus. No cross-reactivity is found between effector T-cell populations from mice immunized with HEP or with influenza virus. It thus appears that street rabies virus, which is not known to replicate in the cells of immune system, induces some general defect in the primary CMC lymphocyte response, though restimulation of memory T-cell populations is unimpaired and there is no defect in antibody formation. Development of fatal rabies may reflect the operation of this selective immunosuppressive mechanism.

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