

Age-Associated Changes in Fecal Excretion Patterns of Strain 93 Chick Embryo Lethal Orphan Virus in Chicks

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Oral inoculation of chickens with strain 93 (chick embryo lethal orphan) virus produced a subclinical infection of the gastrointestinal tract. The pattern of fecal virus excretion in birds infected at 4 or more weeks of age (adult pattern) differed from that in chicks infected when newly hatched (juvenile pattern). By comparison with the juvenile pattern, the adult pattern was characterized by lower peak titers of fecal virus, earlier decline in virus titer, and shorter duration of excretion. Quantitative studies on hatchmates infected at various ages showed that these characteristics are established sequentially with age: lower peak titers were observed in birds infected at 2 to 4 days of age; early decline in titer was first observed in birds infected at 14 days of age; and curtailed excretion was observed in birds infected at 21 days of age. Possible mechanisms operative in the autosterilization process are discussed.

The effect of age on the outcome of viral infection has been studied in a number of systems utilizing the parameters of disease, death, and persistence of infection (13-15, 19, 23). Considering that the majority of natural infections with common viral agents are acute and present little overt disease, a model was developed to study host response in a more typically benign virus infection.

Strain 93 avian adenovirus, antigenically indistinguishable from chick embryo lethal orphan (CELO), EV-89, GAL 3, and GAL 4 viruses (3), is a common infectious agent of the domestic fowl (9). Natural exposure (21) and experimental oral challenge (7) produce an acute, subclinical infection of the upper and lower gastrointestinal tract which resembles human infection with the adenoviruses and enteroviruses. In a previous report (7), qualitative differences were noted in the fecal excretion pattern in newly hatched birds infected with strain 93 as compared to birds infected at 6 weeks of age.

As part of a study of the mechanisms operative in the autosterilization of infection, systematic quantitative studies of fecal excretion were undertaken in hatchmates infected at ages ranging from newly hatched to 43 days of age. Reproducible age-associated differences were observed in the peak titers of virus excretion, the time postinfection at which the first marked de-

cline in virus titer occurred, and the time at which infectious virus excretion terminated. The ages at which these changes occur suggest hypotheses as to the mechanisms involved.

MATERIALS AND METHODS

Virus. Strain 93 virus at the 9th to 15th passage levels in primary chick kidney cells was used. Chicks were fed a standard challenge dose of 0.1 ml of undiluted tissue culture fluid containing approximately 10^7 plaque-forming units (PFU).

Animals. Laboratory-hatched white Leghorn chicks were used in all but one experiment, in which Vantress cross chicks were used. Uninfected birds were maintained in isolation from infected birds until the time at which they were challenged.

Virus titration. Fecal cultures were obtained by cloacal swab and processed as previously described (6). Virus was quantitated by plaque assay of serial dilutions on primary chick kidney monolayers (2) grown in 60-mm plastic petri dishes. After inoculation in triplicate, plates were incubated for 2 hr in 5% CO₂-air atmosphere and overlaid with 7 ml of medium composed of Hanks balanced salt solution with 0.5% lactalbumin hydrolysate, 10% newborn calf serum, 1% Eagle's vitamins, 0.75% Ionagar (Oxoid), and antibiotics. Two milliliters of neutral red overlay, 1/20,000, was added on day 6, and plaque counts were made on day 7. Titers of less than 1 PFU/ml were assigned the value of 1 PFU/ml for calculation of the geometric mean titer.

Serum neutralizing antibody. Titration of serum

antibody was performed in tube neutralization tests (6) using primary chick kidney cell cultures. Serial dilutions of serum were mixed with a constant virus dose of 100 TCID₅₀. All sera were examined in a single test which included a virus titration and a standard positive serum titration.

RESULTS

In three experiments, newly hatched chicks were infected with strain 93 virus, and hatchmates were infected at 28, 35, or 43 days of age. Figure 1 presents typical data from one of these experiments which illustrate what will henceforth be termed the juvenile and adult patterns of excretion. In general, the juvenile pattern is characterized by maximum fecal titers of $10^{4.5}$ to $10^{6.0}$ PFU/ml between the days 4 and 7, a decline or damping (10 to 60-fold) of virus excretion between postinfection days 7 and 9, and the persistence of low-titered excretion beyond postinfection day 14. The adult pattern is characterized by lower maximum titers in the range of $10^{2.5}$ to $10^{4.5}$ PFU/ml on day 4 or 5, damping between day 5 and day 7, and the earlier and more abrupt cessation of infectious virus excretion by day 14.

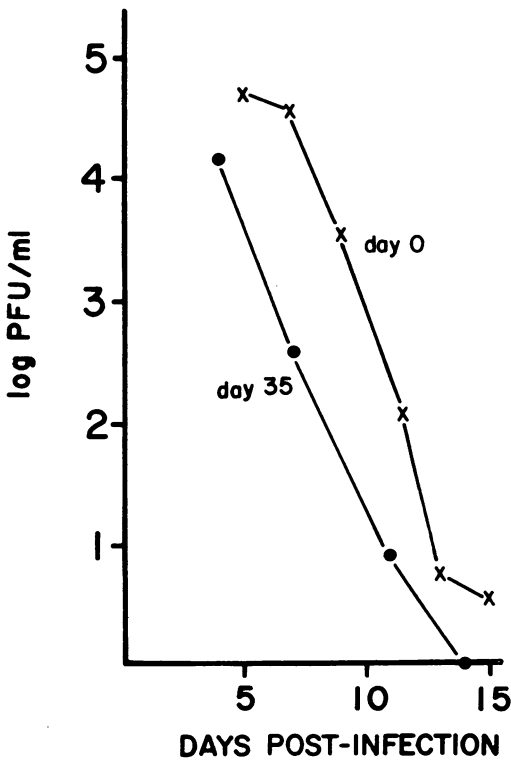


FIG. 1. Geometric mean titers of fecal virus after oral inoculation of newly hatched (day 0) and 35-day-old chickens with strain 93 virus.

To determine the age at which these differences were established, six groups of hatchmates were infected when newly hatched or at 7, 14, 21, 28, or 35 days of age. Each group was cultured every other day from day 5 through day 13 postinoculation. The results are shown in Table 1 and Fig. 2.

Decreased titers of fecal excretion are apparent in all groups infected at 7 days of age or later. In two similar experiments (Table 2), it was established that this characteristic of the adult pattern is established in chicks infected as early as 2, 3, and 4 days of age.

The adult pattern of damping between days 5 and 7 postinoculation appears from Table 1 and Fig. 2 to be established in birds infected at 14 days of age. In younger birds, the first marked decline in fecal titer (group I, 52-fold; group II, 65-fold) occurs between 7 and 9 days postinoculation. A similar tendency is seen in experiment 2, Table 2, in which mean titers in birds infected at 0 and 4 days remained relatively stable from day 5 to 7, and a decline of 10 to 20-fold occurred between days 7 and 9. By comparison, in birds infected at 14 days and later (Table 1, Fig. 2), declines in titer ranging from 22-fold (group V) to 62-fold (group IV) are seen between day 5 and day 7 postinoculation.

The third feature of the adult pattern, namely the earlier and more abrupt cessation of infectious virus excretion in older birds, has been noted previously (7). In two experiments in which chicks were infected when newly hatched and hatchmates were infected at 35 and 43 days of age, 18 of 25 younger birds were excreting virus on day 15 postinoculation while none of 13 of the older birds was excreting on day 14. In the older birds, virus was rarely recovered after day 11 postinoculation, whereas fecal cultures from the younger birds were intermittently positive through day 18. In the present experiment, the adult characteristic of curtailed excretion appeared to be first established in birds infected at 21 days of age (Table 1, Fig. 2).

Serum neutralizing antibody response was studied in group VI infected at 35 days of age. Sera drawn just before infection were negative at 1/4 dilution. Seven days postinoculation, antibody was detectable in one bird (titer 1/4), and by 14 days all five birds had responded with titers ranging from 1/5.6 to 1/90 (geometric mean 1/26).

It may be noted that the experiments shown in Fig. 1 and 2 and Table 1 were performed with chicks hatched from eggs obtained from a CELO-free flock (SPAFAS, Inc.) and were devoid of detectable anti-CELO neutralizing antibody at hatching. In the remaining experiments utilizing chicks from open flocks, the low levels of

TABLE 1. *Fecal excretion of strain 93 virus after oral inoculation of chicks at 0 to 35 days of age*

Group	Age at infection (day)	No. of chicks	Titer at day postinfection				
			5	7	9	11	13
I	0	6	5.26 ^a	5.02	3.30	1.47	0.45
II	7	7	3.45	2.96	1.15	1.43	0.63
III	14	6	3.36	1.73	1.43	0.15	0.85
IV	21	6	3.79	2.00	0.23	0.00	0.11
V	28	5	4.18	2.83	ND	ND	0.00
VI	35	5	4.04	2.34	ND	ND	0.00

^a Log geometric mean; PFU/ml.

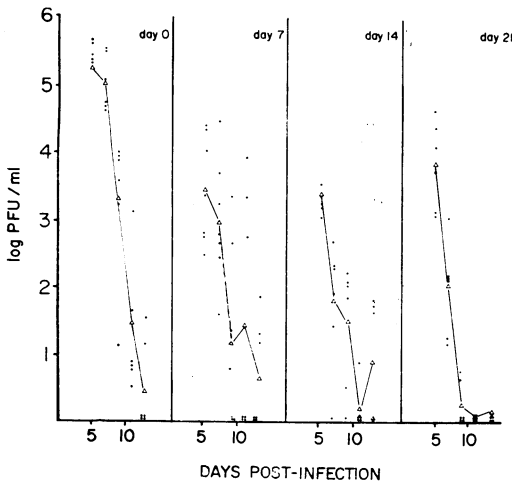


FIG. 2. *Fecal excretion of strain 93 virus after oral inoculation of chicks aged 0, 7, 14, and 21 days; (Δ) geometric mean.*

maternal anti-CELO antibody present in most newly hatched birds did not appear to affect the virus excretion pattern.

DISCUSSION

Age has long been recognized as one of many factors which affect host response to virus challenge. Most studies in which the effect of age has been demonstrated have dealt with artificial host-virus combinations and parenteral inoculation. In general, these studies have shown that newborn or infant animals cope less effectively with viral challenge than more mature animals whether the indicator be disease or persistence of infection. Most natural virus infections, however, result neither in severe disease nor, apparently, in persistent infection but are relatively benign and self-limited. For that reason, it was of interest to examine systematically and quantitatively the relationship of age to infection in a host infected by a natural route with one of its

TABLE 2. *Fecal excretion of strain 93 virus in chicks infected orally at 0 to 4 days of age*

Expt	Age at infection (day)	No. of chicks	Titer at day postinfection			
			5	7	9	11
1	0	7	5.70 ^a	4.80	ND	ND
	2	7	3.75	3.47	ND	ND
	3	8	2.55	1.73	ND	ND
2	0	8	4.70	4.59	3.57	2.08
	4	9	3.66	3.36	2.10	1.57

^a Log geometric mean; PFU/ml.

natural viral parasites. The intent was to provide a basis for the further study of those mechanisms which result in sterilization of a viral infection of the common and benign type.

The age-associated differences in fecal excretion patterns observed in chicks infected with strain 93 virus are summarized in Table 3. Lower peak titers of fecal virus were noted in birds infected as early as 2 to 4 days of age as compared to newly hatched chicks. It seems unlikely that the lower titers are due to a decrease in the number of susceptible cells, in this case primarily cells of the ileal epithelium (8) since the effect was not progressive with age after day 7. The apparent sequestering of virus in high titer within the ceca of birds infected when newly hatched but not in older birds has been observed in experiments performed in this laboratory (*to be published*). The lower titers observed in older birds may be a nonspecific phenomenon resulting from physiological changes in the gut occurring during the first posthatching day.

The damping of virus excretion between days 5 and 7 postinfection, characteristic of the adult pattern, appears to be first regularly established in chicks infected at 14 days of age. The initiation of damping by the simple exhaustion of susceptible cells is unlikely considering the rapid turn-

TABLE 3. Summary of characteristics of adult and juvenile patterns of fecal excretion after oral inoculation with strain 93 virus

Characteristic	Pattern		Age at which adult pattern is established (days)
	Juvenile	Adult	
Maximum virus titer (PFU/ml)	10 ^{4.6} -10 ⁶	10 ^{2.5} -10 ^{4.8}	2-4
Damping (days post-infection)	7-9	5-7	14
Elimination of infectious virus (days postinfection)	11-18	9-11	21

over of epithelial cells (12) and the increase in villus size with age (5). By analogy with human poliovirus infection (16), damping could be initiated by the local production of neutralizing antibodies in the gut wall, although in a study of human echovirus type 6 infection it appears that damping preceded the appearance of specific secretory immunoglobulin A (IgA) in the feces (20). An immunoglobulin with the characteristics of mammalian secretory IgA has not been characterized for fowls, but the work of Leslie et al. (*in press*) suggests that an analogous system involving primarily 7S immunoglobulins does exist in the gut wall of the chicken. Kincaid and Cooper (17), in a study of the ontogeny of immunoglobulin-containing cells in the chicken, were first able to observe 7S-containing cells in the wall of the tonsilla caecalis 4 days posthatching. The predominance of 7S-containing cells over IgM(μ)-containing cells reached a ratio of 8:1 by 2 weeks of age, and this ratio was maintained thereafter. The age at which this ratio was attained would appear to coincide with the age at which the adult damping pattern was observed to be established in the present study.

The third manifestation of the adult pattern is the earlier and more abrupt cessation of low-titered infectious virus excretion. In human poliovirus infection, shorter duration of vaccine virus excretion in antibody-negative adults as compared to children has been observed (22), and there is some indication that the duration of fecal excretion in human adenovirus infection may be related to age (10). In the present case, the phase of low-titered, often intermittent, infectious virus excretion which precedes consistently negative cultures occurs on days 11 to 18 in newly hatched chicks and on days 9 to 11 in birds infected at 21 days of age or later, coinciding in time with the development of serum antibody response. In the newly hatched infected chick, antibody is not detectable on day 14 (7) and is first observed at 21

days (*unpublished data*) whereas, as shown in the present study, antibody response in birds infected at 35 days of age develops between day 7 and day 14 postinfection. During this phase of elimination of detectable infectious virus from the feces of newly hatched chicks, the ceca were found to be the site of highest virus concentration in the intestinal tract and, in some cases, the only site where virus was detectable (*unpublished data*). The ceca are also recognized as the site of the earliest concentrated accumulation of lymphoid tissue in the chick intestine (4). The longer elimination phase in chicks less than 21 days of age may reflect a delay in local antibody response in the ceca similar to the delay in serum antibody response observed in newly hatched chicks.

The increasing resistance with age of the mouse to coxsackievirus and herpesvirus infection has been correlated with age-associated changes in interferon response (11), abundance and distribution of cell receptor sites (18), and macrophage activity (13). Among the arenoviruses, the establishment of persistent infection appears to be related to the integrity and functioning of the thymic-dependent immune response (1). Studies in progress with the strain 93-chick system utilizing bursectomized and thymectomized birds may elucidate the role of these factors in an infection which is typically subclinical and self-limited.

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