J. Hyg., Camb. (1980), **84**, 433
Printed in Great Britain

The response of institutionalized Down's syndrome subjects to enterovirus infections

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(*Received* 10 *October* 1979)

SUMMARY

In a study comparing the responses of institutionalized Down's syndrome (DS) and non-Down's (ND) inmates to enterovirus infections, the frequency of wild enteric viruses and the excretion patterns of oral polio vaccine (OPV) viruses were similar in both groups. Antibody titres developed to poliovirus types 2 and 3 following vaccination were similar in DS and ND vaccinees, but the response to type I virus was significantly less in DS vaccinees. As judged by the development of poliovirus antibody in non-vaccinees, the spread of virus from OPV-immunized to unimmunized subjects in the institution was not noticeably different in DS and ND subjects.

An unexpected finding was that the excretion patterns of all three serotypes of poliovirus were strikingly similar for each individual, although the patterns varied considerably from individual to individual. The similarity of excretion occurred despite wide differences within an individual in the titres of neutralizing serum antibodies to the three serotypes. It is suggested that the rate at which a given individual eliminates enteroviruses may be largely determined by factors, the activities of which are not reflected in serum antibody titres.

INTRODUCTION

Institutionalized Down's syndrome (DS) subjects tend to be more susceptible to certain diseases than inmates with mental retardation of non-Down's (ND) aetiology. This is particularly true of respiratory infections (Carter, Mikkelsen & Nielsen, 1964) and leukaemia (Kessler & Lilienfeld, 1969).

Perhaps the most thoroughly investigated manifestation of the differing responses of DS and ND individuals to infection concerns the hepatitis B virus (HBV), where the frequency of chronic HBV carriers is much higher in institutionalized DS inmates than in their ND counterparts (Blumberg *et al.* 1970). Other features of this association are a greater susceptibility of DS inmates to HBV infection, regardless of whether the outcome is chronic or not, and an apparently impaired capacity to maintain high antibody titres to the surface antigen of the virus

0022–1724/80/0093–1979 $01.00

(HBsAg) in those subjects eventually eliminating the virus (Boughton *et al.* 1976).

The present study was carried out to determine whether institutionalized DS subjects differ from ND inmates in their response to the enteroviruses, which, like HBV, are usually transient inhabitants of humans. DS and ND inmates were compared from three viewpoints. First a survey was carried out in an institution to determine the frequency of 'wild' enteric viruses and to provide data for possible interference effects. This was followed by the administration of oral poliovaccine (OPV) to a sample of the population and subsequent monitoring of faecal excretion patterns and antibody development. Finally some measure of the communicability of the OPV from vaccinees to non-vaccinees within the institution was determined by monitoring non-immunized inmates for the development of poliovirus antibodies during the study.

MATERIALS AND METHODS

The institution

This was an institution in Sydney caring for about 200 inmates, about half of whom were DS. Conditions of hygiene were good and DS and ND subjects were housed, fed and generally cared for together. A more detailed description is provided elsewhere (Boughton *et al.* 1976).

Study groups

Enteric virus survey group

To compare the frequency of wild enteric viruses in DS and ND inmates, 28 DS inmates, aged 4–18 years, and 28 ND inmates aged 4–27 years, were sampled. These 56 subjects possessed the lowest poliovirus neutralizing antibody titres of 96 young inmates screened. DS and ND groups were reasonably well matched for sex, age and pre-study poliovirus antibody status.

Immunized subset

From the enteric virus survey group, a subset of nine DS and nine ND subjects were selected for OPV administration. These possessed the lowest antibody titres of all the inmates screened, and DS and ND groups were matched as closely as possible for age (range 4–17 years both groups), sex (DS 6 males, ND 4 males), pre-study antibody titres to poliovirus types 1–3 and history of previous poliovirus immunization. Relatively more of the DS group (4) than ND (1) had a negative history of poliovirus immunization, an unavoidable reflection of vaccination policy. However, even the unimmunized inmates possessed poliovirus antibody, presumably from natural exposure to wild or vaccine-derived strains (see Table 2). On the first day of the study, the 18 subjects in this group were fed one dose (0·2 ml) of trivalent OPV (Connaught Laboratories).

In another study (Hawkes *et al.* 1980) sera taken previously from the inmates had been screened for HBsAg by radioimmunoassay (Ausria II, Abbott Laboratories). Three of the DS group appeared to be chronic carriers; no ND subjects were in this category.

Specimen collection

Sera for poliovirus antibody determinations were collected from all 56 subjects shortly before, and 10 weeks after the commencement of the study (August 1978).

Faeces were collected from all 56 subjects on the day before immunization and also from the OPV immunized subset on days, 4, 7, 11, 14, 21, 28, 42, 56 and 70 thereafter, with occasional unavoidable omissions. Specimens were stored at −20 °C for later testing in batches.

Laboratory testing

Sera

Neutralizing antibody titres to poliovirus types 1, 2 and 3 in sera were determined in microtitre tray cultures of HEp 2 cells by the method of Murphy *et al.* (1972). To facilitate comparison between the results of DS and ND subjects, pre- and post-immunization sera from all 56 subjects were titrated, in duplicate, under identical conditions in one run, with appropriate controls.

Faeces

An accurately weighed 20% suspension of each faecal sample in phosphate buffered saline (PBS) was vigorously shaken for 30 min, centrifuged at 1000 ***g*** for 20 min and then at 10000 ***g*** for 1 h. The supernatant was stored at −20 °C and used for monitoring poliovirus excretion patterns in the OPV-immunized subset. For wild enteric virus detection in the pre-immunization sample of all 56 subjects, 4 ml of the 10000 ***g*** supernatant was further centrifuged for two hours at 100000 ***g*** and the deposit resuspended in 6 drops of PBS. Half of this was used for electron-microscopic examination and the remainder used in cell culture for virus isolation.

Virus detection in the enteric virus survey was accomplished by standard techniques involving electron microscopy (negative staining) and inoculation of the specimen into primary monkey kidney, human diploid fibroblast (MRC 5 strain) and HEp 2 cell cultures.

The faecal suspensions of the eighteen OPV vaccinees were monitored for poliovirus excretion by inoculation of 0·2 ml samples of the 10000 ***g*** supernatants into tube cultures of HEp 2 cells. If one or more poliovirus serotypes were present, quantitation and typing was carried out by the 'deletion pool' method of McCollough *et al.* (1969). Briefly this entails the reacting of doubling dilutions of the positive 10000 ***g*** faecal extracts with all possible combinations of diluted monotypic poliovirus antisera and inoculation of quadruplicate microtitre HEp 2 cultures with each mixture for detection of residual (un-neutralized) virus. To ensure comparability, DS and ND titrations were always done simultaneously, the testing was done in five large batches, and two separate titration series of a standard poliovirus type 1 stock were performed on each occasion. The system was validated with artificial mixtures of high titred poliovirus stocks before use in the study.

Analysis of results

To compare the prevalence of wild enteric viruses in the 28 DS and 28 ND subjects, and to compare the spread of vaccine virus to unvaccinated DS and ND subjects as judged by antibody response, the chi-square test was used.

For each vaccinee, three measurements were defined for statistical analysis of OPV excretion patterns. These were: the maximum amount of virus present in any one sample (peak height, (PH)); the day on which this occurred (Peak day (PD)); and the last day, relative to the day of immunization, on which a titratable amount of virus (i.e. $\geqslant 10^{2\cdot3}$ TCID 50/g of faeces) could be detected (length of excretion (LE)). To compare DS and ND vaccinees for these measurements, and for comparison of antibody status and response in DS and ND vaccinees, the F test was used. If this showed significance, the Student's t test was implemented. All 18 vaccinees were included in the analysis of antibody status and response, but one ND vaccinee was excluded from analysis of excretion measurements because of the unavailability of specimens between day 10 and day 70.

RESULTS

Enteric virus survey

No virions of any type were detected in the 56 pre-immunization faecal samples by electron microscopy. However, a cytopathic agent, ECHO 15 virus, was detected in 7 out of the 56 (12·5%) subjects (5/28 DS; 2/28 ND). The prevalence in DS and ND subjects was not significantly different ($P > 0{\cdot}2$). Four of the seven positive subjects were members of the OPV-immunized subset (3 DS, 1 ND) and the duration of ECHO 15 virus excretion was determined. Two subjects (1 DS, 1 ND) had no detectable virus when sampled 4 days after the first specimen, one (DS) became negative for virus after 10 days and the other (DS) after 28 days. Unlike the more transient enteric virus positives, these latter two DS subjects had no counterparts with prolonged enteric virus excretion in the ND immunized group. The possibility of bias arising because of the interfering effects of ECHO 15 virus infection on OPV persistence and antibody development in these two inmates had to be allowed for in analysis of those results. This was accomplished by comparing the three excretion measurements (PH, PD, LE) and antibody titres of these two subjects with the mean values of the seven 'matched' members of the DS immunized group. The only measurements found to vary by more than two standard deviations from the mean occurred in the 28-day carrier of ECHO 15 (DS 1), where poliovirus types 2 and 3 were excreted significantly longer than in the seven matched subjects. Because of this, comparison of the DS and ND groups for this measurement was carried out both with and without DS 1.

OPV excretion patterns in immunized subset

All 18 members of the immunized subset excreted all three polio types following OPV ingestion. The general pattern for all three serotypes was a rise in virus titre to a peak between 1 and 2 weeks, followed by a gradual decline, the last day of viral detectability ranging between day 21 and day 56. Occasionally such decline was

Table 1. *Poliovirus excretion (mean values) of nine Down's and eight Non-Down's OPV immunized subjects*

Inmate group	Polio Type 1			Polio Type 2			Polio Type 3		
	PH	PD	LE	PH	PD	LE	PH	PD	LE
Down's	4·1 (0·61)	9·9 (2·9)	39·4 (13·5)	3·7 (0·39)	12·0 (6·7)	33·0 (16·1)	3·9 (0·45)	10·8 (2·3)	39·4 (13·5)
Non-Down's	4·2 (0·58)	7·9 (2·9)	28·7 (10·5)	4·0 (0·63)	7·4 (2·7)	29·6 (8·9)	3·9 (0·37)	7·4 (3·2)	28·4 (6·6)
Significance, Down's *v* Non-Down's	NS	NS	NS	NS	NS	NS	NS	NS	NS

PH = Peak virus excretion in $\log_{10}$ TCID50 per gram of faeces. PD = Day of peak excretion in days after OPV ingestion. LE = Length of virus excretion in days after OPV ingestion. NS = Not significant (Student's *t* tests).

Figures in parentheses refer to standard deviations.

broken by a transitory increase in titre. There was considerable variability between individuals in their patterns of excretion, but inspection of the excretion curves and analysis of the three excretion measurements (Table 1) revealed no significant differences between the DS and ND groups, with or without the inclusion of DS 1 in the analysis.

Further, there was no obvious correlation between the pattern of excretion and age, sex, history of previous poliovirus immunization or pre-vaccination antibody titre to any of the three serotypes. The three DS subjects with chronic HBsAg did not show prolonged excretion of enteroviruses, or any gross abnormality in the other excretion measurements.

Although some individuals differed greatly from others in excretion patterns, there was a strong tendency for all three poliovirus serotypes to be excreted in a strikingly similar pattern by any given individual. This occurred in all 18 subjects. The similarity of excretion patterns for all three serotypes in a given individual occurred despite often widely differing titres of pre-vaccination antibody to those serotypes in such subjects. There was also no obvious relation between duration of OPV virus excretion and such factors as age, sex or previous polio immunization. Examples of representative excretion patterns are given in Fig. 1.

Antibody responses in immunized subset

Pre-immunization antibody titres to all three serotypes were not significantly different in the DS and ND groups. Following vaccination, the DS group developed significantly lower polio type 1 geometric mean antibody titres (GMT) than did the ND group (Table 2). This finding was a reflexion of the fact that all nine ND subjects underwent fourfold or greater elevations of antibody titres to poliovirus type 1,

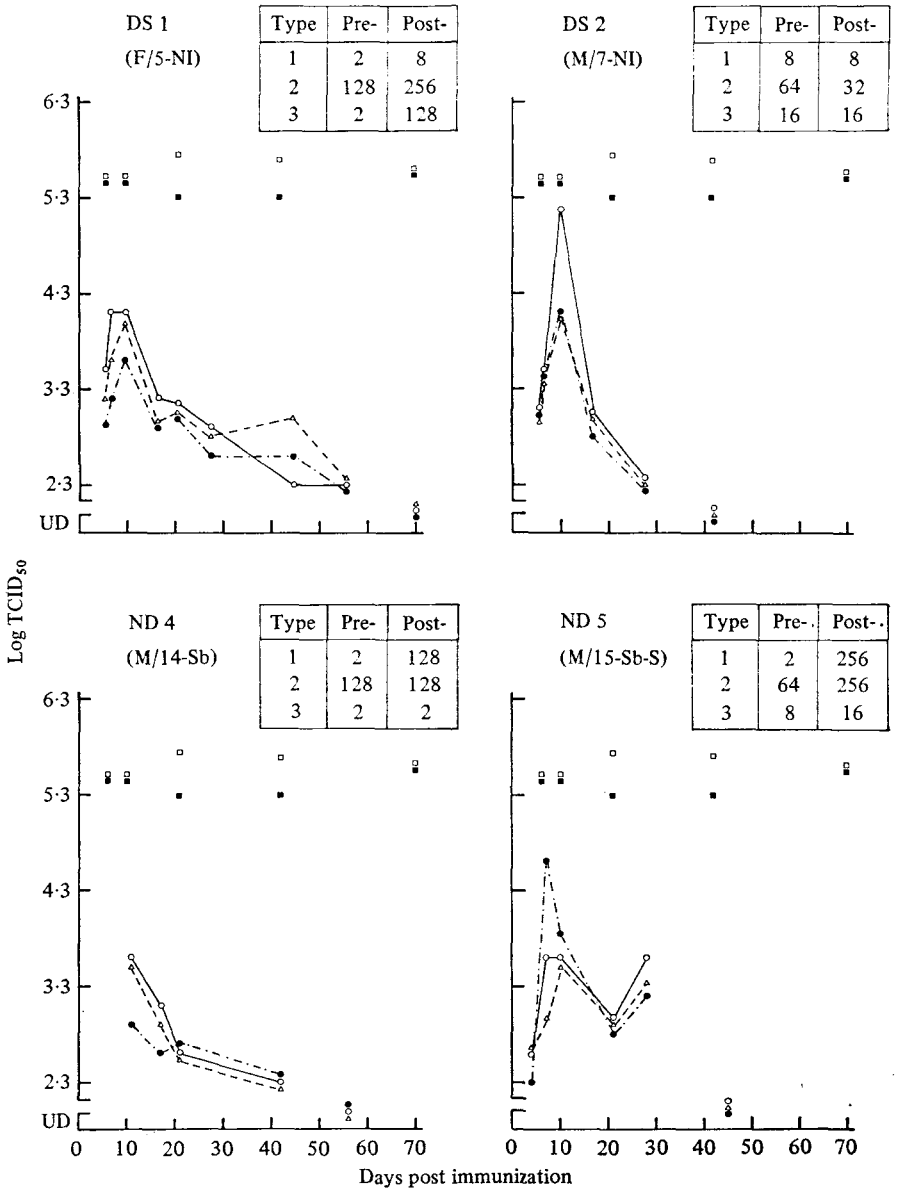

Fig. 1. Representative faecal excretion patterns of poliovirus types 1 (○—○), 2 (●–·–●) and 3 (△—△) in Down's syndrome (DS) and non-Down's (ND) inmates. UD indicates virus undetectable (less than $10^{2\cdot3}$ TCID 50 per g faeces). □, ■, Titres (TCID 50/0·25 ml) of the standard poliovirus type 1 stock titrated in duplicate when specimens titrated (see Materials and Methods). Figures in boxes refer to the inmates' neutralizing antibody titres, prior (pre-) and subsequent (post-) to immunization. Data adjacent to boxes refer to inmate sex (M = male; F = female), age in years and poliovaccination history (NI = not immunized; Sb = oral polio vaccine; S = inactivated poliovaccine).

https://doi.org/10.1017/S0022172400026978 Published online by Cambridge University Press

Table 2. *Poliovirus neutralizing antibody status and responses in nine DS and nine ND OPV-immunized subjects*

Inmate group	Type 1		Type 1		Type 3	
	Pre-*	Post-*	Pre-	Post-	Pre-	Post-
Down's						
GMT	4	7	14	51	3	9
SD of mean	(11·3)	(12·4)	(19·0)	(21·0)	(15·9)	(96·8)
Range of titres	2–8	2–16	2–128	8–256	2–8	2–128
Non-Down's						
GMT	3	64	44	101	5	13
SD of mean	(10·0)	(61·3)	(117·0)	(53·9)	(15·9)	(13·5)
Range of titres	2–8	8–256	8–128	8–256	2–16	2–32
Significance (Down's *v.* Non-Down's	NS	$P < 0{\cdot}01$	NS	NS	NS	NS

GMT = Geometric mean antibody titre. NS = Not significant.
*Pre- and Post- refer to sera taken just before and 10 weeks after ingestion of OPV.

whereas only three of the nine DS subjects did so. For the other serotypes the numbers showing significant antibody development were more nearly equal (Type 2; DS 4, ND 2: Type 3; DS 3, ND 3).

Antibody responses of non-vaccinees.

As a crude measure of relative sensitivity of DS and ND groups to naturally acquired poliovirus infection, the proportion of each group acquiring infection from the immunized subset during the study was determined by identifying those unvaccinated subjects undergoing fourfold or greater development in antibody titres during the study. It was retrospectively realized that this would provide an underestimate of infection, since, in the immunized subset itself, where all vaccinees were demonstrably infected, only 24 out of a possible 54 (i.e. 3 serotypes × 18 subjects) antibody rises occurred. Only five instances of antibody development were seen in the 38 unimmunized subjects; two of these occurred in DS subjects (type 1) and three in ND subjects, an incidence of 5/114 or 4·4% of possible rises.

DISCUSSION

The study was designed to determine whether institutionalized DS subjects differed from ND inmates in their response to enteroviral infection in a way similar to that seen with HBV infection. By analogy, this would involve the DS subjects having a lower threshold of infection, a greater propensity to chronic carriage and lower antibody titres following enteroviral infection (Boughton *et al.* 1976). With respect to the first two features, this study provided no evidence of any difference between DS and ND subjects. The point-prevalence survey of the type which originally revealed the abnormally high carriage rate of HBV in DS subjects (Blumberg *et al.* 1970) did not yield a similar finding for enteroviruses here.

Furthermore the admittedly limited and insensitive studies designed to compare communicability of OPV to DS and ND non-vaccinees did not indicate that DS subjects had a lower threshold of infection to these viruses. From the standpoint of chronic carriage, although the DS subjects tended to excrete all three serotypes of poliovirus for somewhat longer periods of time than did the ND group, the difference was not significant and all 18 OPV-immunized subjects stopped excreting within the 70-day period of the study. There was no tendency observed for the three chronic HBsAg carriers to excrete polio viruses for longer periods than other inmates.

The pre-vaccination antibody status of the DS and ND groups was quite similar, a finding consistent with the recent survey of McKay *et al.* (1978). Similarly, the antibody responses to poliovirus types 2 and 3 revealed no differences between the DS and ND subjects. However the type 1 response was significantly poorer in the DS subjects, both in the lower mean titre achieved and in the proportion of the group developing a fourfold or greater elevation of antibody titres following immunization. This is a confusing finding since there is no readily apparent reason for DS subjects to differ from ND subjects in response to one component only of the trivalent vaccine. More extensive studies are required to substantiate this finding. If such a study did confirm the result, poliovirus type 1 would be the only antigen of ten tested, other than HBV-associated ones, to which DS subjects in this institution differed in response from their ND counterparts (Hawkes, Boughton & Schroeter, 1978; Hawkes *et al.* 1980).

As expected, there was considerable variability in the excretion patterns of the eighteen OPV vaccinees. However, every individual exhibited a striking similarity in the excretion pattern of all three serotypes (figure 1). This unexpected phenomenon may have been undetected in previous OPV-monitoring studies because such have not usually attempted to quantitatively assess the excretion patterns of all three serotypes in individual subjects. It is generally accepted that duration of poliovirus excretion is likely to be a reflexion of previous poliovirus infection and vaccination history, and to be related to serum antibody titres. Such was not the case in the current study.

Knowledge of the factors responsible for termination of any viral infection is incomplete. With respect to enteroviruses, titres of local antibody in the gut and even interferon may be involved, but these were not monitored in the present study. Cross-reacting antibody exerting a similar effect on all three serotypes is an unlikely explanation of the similarity of excretion patterns, as is the possibility of a mechanism involving phenotypic mixing. It is possible that the ability to terminate enteroviral infections is largely an inherent one, with little antigenic specificity involved. Further studies to confirm this aspect of the work and extend it to non-polio enteroviruses are required.

We wish to thank Mr T. Vale of the Microbiology Department, Prince Henry Hospital, Sydney, for typing of the ECHO 15 isolates. The work was carried out with the financial assistance of the Australian National Health and Medical Research Foundation (grant no. 74/357).

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