Published in final edited form as:

Pediatr Infect Dis J. 2008 November ; 27(11): 1004–1008. doi:10.1097/INF.0b013e31817d53c5.

Antibody Responses After Inactivated Influenza Vaccine in Young Children

Peter F. Wright, MD*, Edith Sannella, MT*, Jian R. Shi, MS, MPH*, Yuwei Zhu, MD, MS†, Mine R. Ikizler, MLI*, and Kathryn M. Edwards, MD*

*Department of Pediatrics, Vanderbilt School of Medicine, Nashville, TN.

†Department of Biostatistics, Vanderbilt School of Medicine, Nashville, TN.

Abstract

Background—The frequency and duration of antibody responses after trivalent inactivated influenza vaccine (TIV) in young children are not well defined and assume greater importance with the expanded recommendations for vaccine use in children aged 6 months–5 years.

Methods—Forty-three children aged 6–23 months were vaccinated with TIV in the fall of 2002. At enrollment the majority of children were seronegative to one or more of the vaccine antigens and had no previously documented influenza. Postvaccination sera were collected in the subsequent fall and winter seasons. Acute antibody responses to TIV were determined using standardized hemagglutination inhibition (HAI) and neutralization assays. In calculating the duration of responses, sequential sera were analyzed to the last available sera, to the point at which antibody became undetectable, or to intercurrent influenza infection.

Results—Forty-three subjects contributed 121 sera that were analyzed for HAI responses to TIV. Four-fold HAI rises after 2 doses of TIV in naive individuals were seen in 13 (72%) to H3N2, 22 (92%) to H1N1, and 15 (60%) to influenza B. Fewer 4-fold rises were seen in those with preexisting antibody. The results of microneutralization assays to H3N2 correlated well with HAI results. The time for antibody to decay to one-half of the postvaccination titer ($t_{1/2}$) was approximately 126 days for H1N1 and 258 days for H3N2.

Conclusions—Although not all children responded with 4-fold rises in antibody or achieved the putative protective titer of 1:32, the half-life of antibody suggested that children immunized in the fall should have immune responses sustained throughout the ensuing influenza season.

Keywords

influenza; vaccine; antibody

Studies have shown that influenza infection significantly impacts young children by causing excess hospitalizations with rates in children <2 years of age similar to those of persons >65 years of age.^{1–3} Evaluation of outpatient visits and use of antibiotics during the influenza season also clearly demonstrates the under-recognized burden of influenza, particularly in the children aged <2 years.³ The Advisory Committee on Immunization Practices (ACIP) has responded to these data by broadening the recommendations for vaccination of infants and children to include all aged 6 months–5 years.⁴

With these expanded influenza vaccine recommendations in children, it is important to evaluate the frequency and duration of immune responses to trivalent inactivated influenza vaccines (TIV) in children. Several recent papers have addressed this question in school age and younger children and support the currently recommended 2-dose regimen.^{5–8} Duration of immunity is also relevant to the timing of administration of vaccine before the influenza season and to a basic understanding of the immunologic memory induced by these vaccines—particularly when TIV is a child's primary exposure to influenza. In response to ACIP recommendations encouraging the use of influenza immunization in children, virtually all the eligible children aged 6–24 months in the Vanderbilt Vaccine Clinic (VVC), a prospectively followed experimental study population previously described,⁹ were given TIV in the fall of 2002.

There was relatively little influenza in the winter of 2002–2003 either nationally or in the VVC with a good match of all strains with vaccine content.¹⁰ In the VVC in which active viral surveillance of respiratory illness was done, there was a single H3N2 isolate, 2 H1N1 isolates, and 10 influenza B isolates of 135 samples screened between January and April of 2003. As a result of the limited local circulation of influenza A, we were provided an opportunity to examine the rise and duration of influenza A antibodies in young children after TIV. The observed decline in titer will need to be factored into our understanding of the nature of induction of immunity after TIV vaccination.

METHODS

In the fall of 2002 TIV was offered to all children being followed in the VVC who were between the ages of 6 and 23 months. Two doses of 0.25 mL of vaccine containing 7.5 μ g of each of A/Moscow/10/99-like (H3N2), A/New Caledonia/20/99-like (H1N1), and B/Sichuan/379/99-like viruses manufactured by Wyeth Vaccines were offered. This was the first year TIV had been given in this age group in the VVC.

The VVC children given TIV in the fall of 2002 had sera obtained at frequent intervals to determine the seroprevalence of antibody to common respiratory pathogens under a Vanderbilt University Institutional Review Board-approved protocol. They were provided primary care in the VVC and cultured for viral pathogens with each episode of respiratory illness as previously described.⁹

HAI antibodies were determined using CDC protocols¹¹ with representative antigens from A/Panama/H3N2, A/Wyoming/H3N2, an emerging H3N2 variant in 2002–2003, A/New Caledonia/H1N1, B/Hong Kong, the vaccine strain in the B/Victoria lineage, and B/Sichuan, in the cocirculating B/Yamagata lineage.¹¹ Receptor destroying enzyme and reference antisera were kindly supplied by the CDC. Sera that had been continuously frozen at -20°C were thawed, treated with receptor destroying enzyme, and run with a starting dilution of 1:8 with negative titers being assigned a value of 1:4 for calculation of geometric mean titers (GMT). A microneutralization assay¹² was run according to the current CDC protocol (personal communication, Jacqueline Katz, March 8, 2005). We participated in running a blinded panel of sera circulated to standardize the neutralization assay for the H3N2 virus as described in a report of a WHO meeting.¹³ The results from the Vanderbilt laboratory were in every instance within 2-fold of the CDC results. For a negative control for both assays, we selected 8–10 sera from children 6–18 months of age drawn in August–November in the early 1980s.

Data analysis of acute responses was performed after cells with <4 subjects were censored from analysis. Acute responses to a single dose were determined for the 19 subjects who had sera drawn before the first dose and before receiving the second dose. The responses to 2

doses were assessed in 31 subjects who had sera drawn before the first and after the second dose. The assays that gave a negative result at 1:8 were considered 1:4 for calculation of the acute titers and decay curves.

In calculation of antibody duration, sequential sera after the second dose in seronegative children were censored after their antibody became undetectable or when they experienced natural infection. Those with detectable preexisting antibody titers to a strain were not analyzed because their high antibody titer was most likely because natural exposure and would be expected to be of longer duration.¹⁴ From the available data, best fit curves of the HAI values were drawn.

RESULTS

Forty-three children received either 1 or 2 doses of TIV. The mean interval between injections was 47 days with a range of 28–110 days. The 43 vaccinated children had 1 or more subsequent sera drawn as part of the routine surveillance effort in the vaccine clinic and had sera after the 2001–2002 influenza season that predated their next influenza immunization, Table 1. A total of 121 sera were tested. Safety was not specifically examined in this study but no telephone or visit complaints related to the injections were noted in a review of the office charts.

As the children followed in the VVC were routinely cultured for respiratory illnesses in the presence of fever, otitis media, or respiratory symptoms, isolation of influenza virus had been sought before immunization and during the subsequent winter after immunization. Four vaccinees had had A/Panama-like H3N2 influenza strains and 1 child had B/Yamanashi isolated in the winter before vaccination. Additional children were seropositive to one or more of the vaccine strains, as reflected in Table 2.

The acute serologic responses to 1 and 2 doses are shown in Table 2. A consistent immune response in the naive children to H1N1 was achieved with a single dose of A/New Caledonia vaccine, H3N2 A/Panama required 2 doses of vaccine, and B/Hong Kong had a marginal response (60% having a 4-fold rise but with a GMT of only 24) even after 2 doses. The immunogenicity of the strains in TIV in naive subjects after 2 doses of vaccine can thus be ranked with the best responses to the H1N1 strain (79% achieving a 1:32 protective titer), intermediate to H3N2 (67% achieving a protective titer), and the lowest to influenza B (52% with a protective titer). The response to the emerging variant strain A/Wyoming was less than to the A/Panama contained in the TIV vaccine (GMT 15 versus 40). The responses to 2 influenza B variants, B/Hong Kong, and B/Sichuan, were similarly low (Table 2).

The geometric mean titer per vaccine in the 8 subjects who had previously experienced natural A/Panama infections was 1:271—considerably higher than the 1:40 to A/Panama after 2 doses of TIV. However, the percent responding to the TIV vaccine strains was consistently less in the preimmune children.

Neutralization to the H3N2 strain gave results in this and our other unpublished data sets from adults that very closely paralleled the HAI results (Fig. 1, <http://links.lww.com/A533>). The measure of TIV-induced immunity to A/Panama was very similar by HAI, Table 2 and by neutralization, Table 3.

The antibody duration in naive children reflected the previously noted decay in titers after inactivated vaccine.^{14–16} We excluded from consideration all sera that followed a rise in titer or isolation of influenza during the influenza season. In the winter after vaccination, 4 of the vaccinated children had influenza isolated—2 of A/New Caledonia-like/H1N1 and 2

of B/Hong Kong-like virus. Figure 2A, B show the decay in titer in the previously naive children for A/Panama/H3N2 and A/ New Caledonia/H1N1.

DISCUSSION

The broadening recommendations for influenza vaccine require that humoral immunogenicity in targeted age groups be examined as this is the presumed surrogate of protection for TIV. This examination is particularly relevant for TIV as the alternative live, attenuated vaccine appears to have broader and enhanced immunity in children that may depend on stimulation of other components of the immune response.

The specific settings in which TIV vaccine efficacy has been critically examined against placebo are limited and involve small trials indicating protection against otitis media,^{17,18} retrospective studies in the setting of history of vaccine administration in the office practice setting,¹⁹ a casecontrol study in an office practice setting²⁰ and one larger comparative trial of TIV and live vaccine compared with placebo in the age group from 2 to 15 years.²¹ Several recent reviews have summarized the efficacy data upon which the recommendations for TIV immunization of young children are based.^{22–24} They reach differing conclusions including that there may be no efficacy of TIV in those younger than 2 years of age.²² Other epidemiologically based studies have suggested community-wide or even country-wide protection that extends beyond the age of a vaccinated pediatric population.^{25,26} The very high efficacy of live, attenuated vaccines was shown in prelicensure trials.²⁷ A recent paper demonstrated the greater efficacy of live, attenuated vaccine in children in comparison with TIV but did not have an unvaccinated control group.¹⁶

This study confirms several observations. Children older than 6 months of age have a moderately good response to the influenza A components delivered in 2 doses of split product influenza vaccine. The responses to the influenza A components of the vaccine in naive children are lower than that seen with natural influenza A infection, and surprisingly the frequency of responses is lower in those with preexisting antibody. Thus, there is no evidence of priming by prior infection or reason to think that responses in subsequent years will be enhanced over primary vaccination. This was also suggested by the diminished responses in adults to sequential yearly immunization or from previous data in adults by successive yearly vaccinations.²⁸ Again we saw that the HAI response to the influenza B vaccine component, and to natural influenza B infection, in young children is often limited.^{29,30}

The previously observed strain-specific response to TIV was again suggested by the limited response to the variant A/Wyoming/H3N2 strain in those without pre antibody titer.^{31,32} The response to the variant B/Sichuan was comparable but low to the B/Hong Kong in the vaccine. Previously, the response to TIV in children has been shown to be quite strain specific.

A neutralization assay did not change the observations made with the HAI assay of the young child's response to influenza A/Panama/H3N2. The very high correlation of the 2 assays coupled with the variability of the neutralization assay suggests that the latter is not a major innovation in interpretation of the response to annual vaccination.

The antibody response in young children after TIV is not sustained. The data reported here are from a retrospectively analyzed sample of convenience but the data is very consistent with previous studies. The pediatric trials of the A/New Jersey (swine) vaccine in 1976 presented an early opportunity to examine the height and duration of the immune response to TIV.³³ Although 11/14 subjects developed had titers 1:40 (GMT 59) after 2 doses of A/New Jersey vaccine, the titers rapidly declined 4–7 months later so that only 2/14 subjects

had persistent titers 1:40 (GMT 13). Another report from that era extended the observations to children 6 months to 10 years of age who had sera available up to 1 year after immunization.³⁴ Immediately after 2 doses of TIV, 85% had titers of 1:40, by 2–5 months only 47% had such titers, but 6–9 months later only 13% had titers 1:40. Thus, the GMT declined with an approximate $t_{1/2}$ of 4 months. Johnson et al¹⁵ directly compared the response to live, intranasally administered vaccine and TIV in children seronegative to influenza A/H3N2. By 6 months antibody titers had fallen approximately 3-fold in the TIV group. There was virtually no fall in antibody titer 1 year later in live vaccine recipients. At that time, 59 children including the above vaccine recipients were challenged with homologous live vaccine.³⁵ Virus shedding was of low titer and short duration in the live vaccine recipients—similar to that in those with previous natural infection. Virus shedding in TIV recipients did not significantly differ from that seen in those who were influenza naive and had never been vaccinated.

Although the data suggest a longer $t_{1/2}$ with H3N2 strains that may reflect the relatively small number of samples and is an observation that requires confirmation in other more prospective studies to validate. Although antibody decay is seen, in aggregate, this study is supportive of the recommendation that young children more than 6 months of age be immunized and that a protective antibody response will be predicted to occur in many that will last through the influenza season after vaccination. However, further studies of TIV in infants need to be done to define correlates of protection, to understand its effectiveness in clinical use and height and breadth of induced immunity in both those naive to influenza and those with preexisting antibody after repeated use of TIV becomes routine.

Acknowledgments

Supported by the Vanderbilt Vaccine and Treatment Evaluation Unit NO1-AI-25462 and the Respiratory Pathogens Research Unit NO1-AI-65298. The studies were conducted on the Vanderbilt Clinical Research Center supported by grant M01 RR-00095 from the National Center for Research Resources, National Institutes of Health.

REFERENCES

1. Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med*. 2000; 342:232–239. [PubMed: 10648764]
2. Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med*. 2000; 342:225–231. [PubMed: 10648763]
3. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med*. 2006; 355:31–40. [PubMed: 16822994]
4. CDC. Prevention and Control of Influenza. *MMWR Morb Mortal Wkly Rep*. 2006; 55 Early Release.
5. Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. *Pediatrics*. 2005; 115:1039–1047. [PubMed: 15805382]
6. Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. *Pediatrics*. 2006; 118:e579–e585. [PubMed: 16950949]
7. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naïve 5–8-year-old children. *J Infect Dis*. 2006; 194:1032–1039. [PubMed: 16991077]
8. Walter EB, Neuzil KM, Zhu Y, et al. Influenza vaccine immunogenicity in 6–23-month-old children: are identical antigens necessary for priming? *Pediatrics*. 2006; 118:e570–e578. [PubMed: 16950948]

9. Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. *J Infect Dis*. 2002; 185:147–152. [PubMed: 11807687]
10. CDC. Influenza Activity United States and Worldwide, 2002–03 Season, and Composition of the 2003–04 Influenza Vaccine. Jun 6. 2003 Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5325a1.htm>
11. Kendal, AP.; Pereira, MS.; Skehel, JJ. Hemagglutination inhibition.. In: Kendal, A.; Pereira, M.; Skehel, J., editors. *Concepts and Procedures for Laboratory-Based Influenza Surveillance*. Centers for Disease Control and Prevention and Pan American Health Organization; Atlanta, GA: 1982. p. B17-B35.
12. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol*. 1999; 37:937–943. [PubMed: 10074505]
13. Report of an informal scientific meeting. Dresden assays for neutralizing antibody to influenza viruses. *Weekly Epid Record*. 2003; 78:285–296.
14. Wright PF, Ross KB, Thompson J, Karzon DT. Influenza A infections in young children. Primary natural infection and protective efficacy of live-vaccine-induced or naturally acquired immunity. *N Engl J Med*. 1977; 296:829–834. [PubMed: 846492]
15. Johnson PR Jr, Feldman S, Thompson JM, Mahoney JD, Wright PF. Comparison of long-term systemic and secretory antibody responses in children given live, attenuated, or inactivated influenza A vaccine. *J Med Virol*. 1985; 17:325–335. [PubMed: 4078559]
16. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med*. 2007; 356:685–696. [PubMed: 17301299]
17. Clements DA, Langdon L, Bland C, Walter E. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. *Arch Pediatr Adolesc Med*. 1995; 149:1113–1117. [PubMed: 7550814]
18. Heikkinen T, Ruuskanen O, Waris M, Ziegler T, Arola M, Halonen P. Influenza vaccination in the prevention of acute otitis media in children. *Am J Dis Child*. 1991; 145:445–448. [PubMed: 1849344]
19. Allison MA, Daley MF, Crane LA, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003–2004 season. *J Pediatr*. 2006; 149:755–762. [PubMed: 17137887]
20. Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003–2004. *Pediatrics*. 2007; 119:e587–e595. [PubMed: 17332179]
21. Neuzil KM, Dupont WD, Wright PF, Edwards KM. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. *Pediatr Infect Dis J*. 2001; 20:733–740. [PubMed: 11734733]
22. Jefferson T, Smith S, Demicheli V, Harnden A, Rivetti A, Di Pietrantonj C. Assessment of the efficacy and effectiveness of influenza vaccines in healthy children: systematic review. *Lancet*. 2005; 365:773–780. [PubMed: 15733718]
23. Ruben FL. Inactivated influenza virus vaccines in children. *Clin Infect Dis*. 2004; 38:678–688. [PubMed: 14986252]
24. Wright PF. The use of inactivated influenza vaccine in childhood. *Semin Pediatr Infect Dis*. 2006; 17:200–205. [PubMed: 17055371]
25. Monto AS, Davenport FM, Napier JA, Francis T Jr. Modification of an outbreak of influenza in Tecumseh, Michigan by vaccination of school-children. *J Infect Dis*. 1970; 122:16–25. [PubMed: 5433709]
26. Reichert TA, Sugaya N, Fedson DS, Glezen WP, Simonsen L, Tashiro M. The Japanese experience with vaccinating schoolchildren against influenza. *N Engl J Med*. 2001; 344:889–896. [PubMed: 11259722]
27. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. *N Engl J Med*. 1998; 338:1405–1412. [PubMed: 9580647]

28. Edwards KM, Dupont WD, Westrich MK, Plummer WD Jr, Palmer PS, Wright PF. A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. *J Infect Dis.* 1994; 169:68–76. [PubMed: 8277200]
29. Wright PF, Bryant JD, Karzon DT. Comparison of influenza B/Hong Kong virus infections among infants, children, and young adults. *J Infect Dis.* 1980; 141:430–435. [PubMed: 7373080]
30. Wright PF, Sell SH, Thompson J, Karzon DT. Clinical reactions and serologic response following inactivated monovalent influenza type B vaccine in young children and infants. *J Pediatr.* 1976; 88:31–35. [PubMed: 1245939]
31. Belshe RB, Gruber WC. Safety, efficacy and effectiveness of cold-adapted, live, attenuated, trivalent, intranasal influenza vaccine in adults and children. *Philos Trans R Soc Lond B Biol Sci.* 2001; 356:1947–1951. [PubMed: 11779396]
32. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr.* 2000; 136:168–175. [PubMed: 10657821]
33. Noble GR, Kaye HS, O'Brien RJ, et al. Persistence of influenza A/New Jersey/76 (Hsw1N1) antibody one year after vaccination. *Dev Biol Stand.* 1977; 39:253–260. [PubMed: 604107]
34. Lerman SJ, Wright PF, Patil KD. Antibody decline in children following A/New Jersey/76 influenza virus immunization. *J Pediatr.* 1980; 96:271–274. [PubMed: 6985957]
35. Johnson PR, Feldman S, Thompson JM, Mahoney JD, Wright PF. Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. *J Infect Dis.* 1986; 154:121–127. [PubMed: 3711685]

FIGURE 2.

A, Duration of antibody in influenza naive individuals to A/Panama/H3N2 after receipt of trivalent inactivated vaccine. B, Duration of antibody in influenza naive individuals to A/New Caledonia/H1N1 after receipt of trivalent inactivated vaccine.

TABLE 1**Demographics of Children Followed After Influenza Immunization**

Characteristics of Group	Number Studied
Age at vaccination	
<1 yr	13
1-2 yr	30
Gender	
Male	22
Female	21
Race	
Black	4
White	31
Other	8
No. vaccine doses	
1	43 *
2	31
No. sera available	
2	20
3	13
4	10

* 19 had sera predating dose 2 what could be used to calculate response to a single dose.

TABLE 2

HAI Responses to Trivalent Inactivated Influenza Vaccine in Children

Vaccine Strains/Serostatus	Postvaccination 1 Dose				Postvaccination 2 Doses			
	N	4-fold Rise in Titer	32 in Titer	GMT	N	4-fold Rise in Titer	32 in Titer	GMT
A/Panama/H3N2								
<8 in titer	11	6 (55)	4 (36)	17	18	13 (72)	12 (67)	40
8 in titer	8	2 (25)	6 (75)	362	13	7 (54)	12 (92)	271
A/New Caledonia/H1N1								
< 8 in titer	16	15 (94)	13 (81)	67	24	22 (92)	19 (79)	72
8 in titer	3	*	*	*	7	2 (29)	4 (57)	52
B/Hong Kong								
< 8 in titers	19	6 (32)	3 (16)	8	25	15 (60)	13 (52)	24
8 in titers	0	*	*	*	6	2 (33)	2 (33)	25
A/Wyoming/H3N2 [†]								
<8 in titer [‡]	13	1 (8)	1 (8)	8.5	25	7 (28)	7 (28)	15
>8 in titer	6	2 (33)	5 (83)	288	6	2 (33)	5 (83)	455
B/Sichuan [†]								
< 8 in titers	9	0	0	5	15	11 (73)	9 (60)	28
8 in titers	10	0	5 (50)	42	16	4 (25)	10 (63)	56

* Where data on less than 4 subjects in a cell values are not shown.

[†] Variant strains not in vaccine.

[‡] For A/Wyoming there were a number of subjects with titers of 1:8. That is assumed to be nonspecific as they had no evidence of the previously circulating A/Panama/H3N2.

TABLE 3

Neutralization Responses to Trivalent Inactivated A/Panama/ H3N2 Influenza Vaccine in Children

Vaccine Response	<u>Vaccine Strains/Serostatus A/Panama/H3N2</u>	
	<10 in Titer	10 in Titer
Postvaccination 1 dose		
N	9	10
4-fold rise in titer	2 (22)	1 (10)
40 in titer	4 (44)	10 (100)
GMT	20	80
Postvaccination 2 doses		
N	17	14
4-fold rise in titer	11 (65)	4 (31)
40 in titer	11 (65)	11(79)
GMT	40	160