Epidemiol. Infect. (2005), **133**, 737–748. © 2005 Cambridge University Press
doi:10.1017/S0950268805003833 Printed in the United Kingdom

Age-specific long-term course of IgG antibodies to pertussis toxin after symptomatic infection with *Bordetella pertussis*

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(*Accepted 22 December 2004*)

SUMMARY

To investigate the possible dependence on age of the rate of decline of IgG antibodies to pertussis toxin (IgG-PT) after natural infection with *Bordetella pertussis* we measured IgG-PT in follow-up sera of 121 patients (age 0–94 years) obtained after 123 episodes of *B. pertussis* infection. For analysis we applied a dynamic model for the inactivation of *B. pertussis* by the immune system. There were no significant differences in rise, peak and decline of IgG-PT between different age groups, although there was a tendency for a more rapid increase, a higher peak and a faster decline with increasing age. The IgG-PT cut-off of 100 U/ml for serodiagnosis of pertussis appeared valid in all age groups. A decline of IgG-PT to <10 U/ml was associated with increased risk of re-infection with *B. pertussis*.

INTRODUCTION

Neither vaccination against pertussis nor natural infection with *Bordetella pertussis* affords lifelong immunity [1–12]. However, the rate of waning of immunity and possible differences after both vaccination and natural infection are difficult to establish, because no method exists to unequivocally measure (non)-susceptibility for pertussis for each individual. A simple single correlate of protection has not been found; both humoral and cellular immunity play a role [13, 14]. However, a relationship between protection and levels of IgG antibodies against the virulence factors pertussis toxin, pertactin and fimbriae has been demonstrated [2, 13, 15–17]. In accordance with the concept of waning immunity, it has been observed that elevated levels of these IgG antibodies, whether induced by vaccination or natural infection quite rapidly decrease again [9, 13, 18, 19]. However, the rate of decline and its possible age dependency have not been established. Also the question of whether or not after infection, IgG antibodies to pertussis toxin (IgG-PT) eventually persist at some level or completely disappear, remains unanswered.

Of the mentioned virulence factors pertussis toxin is of special interest; when used as a single component in an acellular vaccine, it induces considerable protection and is the only virulence factor which is present in each of the newly developed acellular pertussis vaccines [20]. Furthermore, IgG-PT is also an important parameter for serodiagnosis of pertussis, because cross-reactivity of pertussis toxin with antibodies

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induced by heterologous proteins has never been established, and most individuals produce high levels of IgG-PT in response to infection with *B. pertussis* [19, 21, 22].

In our first study of the longitudinal course of IgG-PT after typical symptomatic infection with *B. pertussis*, practically all 57 participating pertussis patients were young children and an established model for assessing the rate of decline of IgG-PT antibodies was lacking [19]. For assessment of the possible age dependence of the rate of IgG-PT decline after natural infection, we extended our studies to a larger group of pertussis patients of various ages for whom follow-up sera were available, and to a group of elderly adult patients with pertussis for whom a follow-up serum sample had been drawn 1 year after infection with *B. pertussis* [23]. For analysis we applied an adapted version of a recently described dynamic model for the inactivation of *B. pertussis* by the immune system [24].

MATERIALS AND METHODS

Patients and sera

The first group of pertussis patients studied (A) consisted of 87 subjects (80 children and adolescents between 0 and 18 years and seven adults between 30 and 42 years), who suffered from clinically typical whooping cough (paroxysmal coughing for $\geqslant 2$ weeks) between 1989 and 1999, confirmed by positive IgG-PT serology (for criteria see below). These patients were from the paediatric practice of one of the authors (F.G.A.V.) in Gouda, The Netherlands, and, after the pertussis episode (diagnosed by F.G.A.V.), remained as a patient in that practice, often because of asthma or other respiratory problems. At the time of the pertussis episode, these patients or their parents gave informed consent to use future sera, which might be obtained for various reasons at irregular control visits or at consultations with new complaints, for measurement of IgG antibodies to pertussis toxin. The adults in this group were parents of participating children who had pertussis at the same time as their child and who had volunteered to participate in this study. Only patients for whom at least one follow-up serum sample, obtained >6 months after onset of disease, was available were included in this analysis. The Medical Ethical Committee of the Groene Hart Hospital approved the study.

The second group (B) of pertussis patients studied consisted of 34 people from a convent in the southern part of The Netherlands, 33 nuns (aged between 59 and 94 years) and one employee (aged 26 years). All of them had clinically typical pertussis during an outbreak of pertussis in this convent in 1992, which was confirmed by positive IgG-PT serology (for criteria see below) (for a detailed description of this outbreak see ref. [23]). Approximately 1 year after the outbreak one follow-up serum sample had been obtained from all patients. All patients gave informed consent.

Laboratory evaluation

In both patient groups the clinical diagnosis of pertussis had been confirmed by the finding, in sera obtained within 3 months after onset of disease, of a significant (i.e. $\geqslant$fourfold) increase of IgG-PT in paired sera to a level of at least 20 U/ml, or by the finding of a high IgG-PT concentration in a single serum sample, i.e. above a defined diagnostic cut-off of 100 U/ml as measured in an in-house IgG-PT ELISA of the National Institute of Public Health and the Environment, Bilthoven, The Netherlands [19]. Previously, it was shown that the sensitivity of these criteria for diagnosis of actual or very recent infection with *B. pertussis* was 90% in paired sera of two groups of patients with culture- or PCR-confirmed pertussis, studied several years apart (respectively $n=89$ and $n=56$) [19, 25]. The specificity of these criteria in paired sera of control patients with respiratory infection of other aetiology ($n=58$) was 96% [25]. The specificity of the $\geqslant 100$ U/ml criterion for single sera was 99% when assessed in population sera ($n=7756$), and was independent of age [19]. This ELISA, in which purified pertussis toxin is coated after pre-coating with fetuine, was developed and described at the start of the 1980s [26], and has been in use since then for serodiagnosis of pertussis for the whole country. For long-term consistency of potency expression in U/ml a serially diluted nationally standardized reference serum is applied on each ELISA plate. Routinely, two dilutions (1:100 and 1:400) of patient sera are used, and depending on the magnitude of optical densities (OD) of those dilutions, the OD of one is used to calculate the IgG-PT concentration of the patient serum sample relative to the defined potency of the reference serum. Although the minimal quantitation in this assay is 1 U/ml, the coefficient of variation being ~ 20% in the 5–500 U/ml range, is >30% in sera with values <5 U/ml [27]. Therefore, the detection level of the assay is considered to be 5 U/ml. Due to the limited number of dilutions of patient sera used in

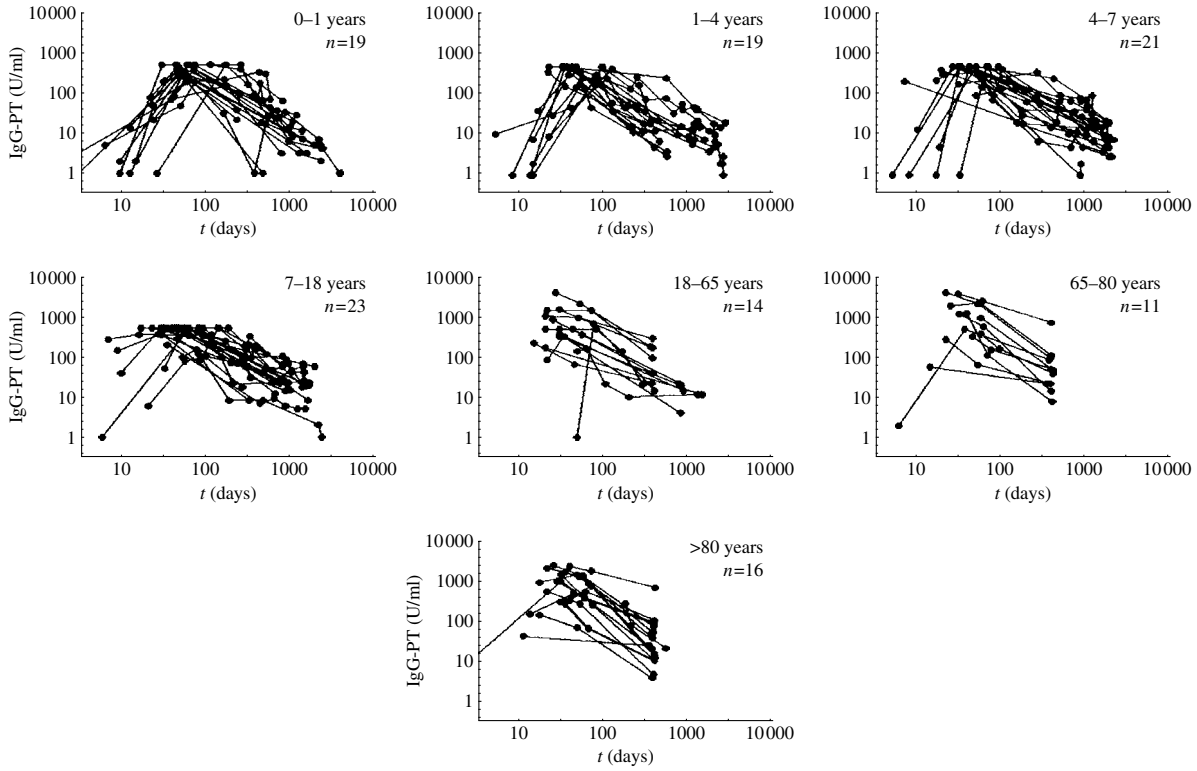

Fig. 1. Measured IgG-PT levels against time from infection [$\log_{10}$ (IgG-PT) against $\log_{10}$ (time)]. Different age groups are in separate panels; data from individual patients are connected.

the assay there is an upper limit of quantitative differentiation of 500 U/ml. However, the sera of patients from group B with IgG-PT $\geqslant$500 U/ml were subsequently retested in higher dilutions (1:800, 1:1600 and 1:3200) to obtain an exact value.

Recently, the IgG-PT ELISA used here [19, 26] has been compared to an internationally standardized IgG-PT ELISA which was recommended by the United States' Food and Drug Administration (FDA) for use in clinical trials of pertussis vaccines, and in which the 'lot 3 pertussis serum' of the FDA, with a predefined content of IgG-PT in IU/ml, is used as reference serum [27–29]. It was shown by Giammanco et al. [29] that there was a good correlation between both assays and that the relation between IU/ml in the internationally standardized ELISA and U/ml in our ELISA as calculated through linear regression of log-transformed concentrations is as follows: 5 IU/ml (detection level) = 6·5 U/ml and 125 IU/ml = 100 U/ml (diagnostic cut-off).

Data analysis

IgG-PT responses show considerable variation among individual patients, as is clear in Figure 1. Therefore we analyse these responses with a hierarchical model, allowing for variations between individual patients. For any individual patient the amount of information is limited: only a few measurements at best. For that reason we have described longitudinal responses with a model of the interaction between bacteria and the immune system. The model assumes that during infection bacteria are growing exponentially in the host. At the same time, bacteria are inactivated (killed) by antibodies (or by some action associated with the antibody response) according to a mass-action mechanism: inactivation depends on the product of the concentrations of bacteria and antibodies (or antibody-producing cells). Conversely, antibody production is controlled by the probability of antibodies (or antibody-producing cells) encountering bacteria (or being presented with antigens derived from these pathogens). Antibody removal is considered an autonomous first-order process. This is the simplest possible model for the interaction between host and pathogen, and it is well known in population biology as a Lotka–Volterra model [30]. The model can be formulated as a system of ordinary differential equations (see Appendix).

This set of equations can be solved numerically and the solution is used for fitting to the patient data. Rather than fitting the model to data from any patient at any sampling time, individual records are fitted separately, producing a set of parameters for each of the patients. We further use population distributions to describe the variation of these parameters among all the patients in an age group, thereby generalizing the set of responses from individual patients to the age-group level. Sampling from these population distributions then allows us to construct generalized immune responses typical for the whole age group, instead of individual patients in that group. All our conclusions are based on these predicted responses. The model is fitted by means of a Markov Chain Monte Carlo (MCMC) algorithm [31] that allows efficient sampling of the parameter space for such complex multilevel problems. Some details are given in the Appendix.

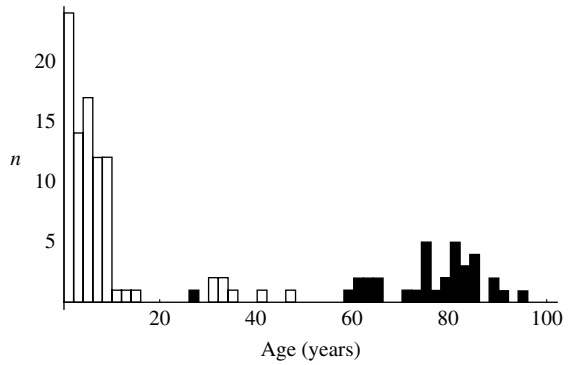

Fig. 2. Age distribution of pertussis cases from the two studies (note that these are 2-year categories, different from the age categories used for analysis). Patients <18 years are from group A (□), those >65 years from group B (■). Adults (18–65 years) are either parents of children in group A, or from group B.

RESULTS

Descriptive analysis

The age distribution of the 87 patients in group A is given in Figure 2. Pertussis-vaccination in The Netherlands at the time of the study consisted of four immunizations in the first year of life, with a nationally produced whole-cell pertussis vaccine. Fifteen of the 19 patients aged <1 year were not vaccinated at onset of pertussis or were incompletely vaccinated (fewer than three immunizations). In only one of those 15 was it documented that the patient had received immunizations against *B. pertussis* after the pertussis episode [note: at the time of the study it was normal practice to abandon (further) vaccination against *B. pertussis* in infants who already had pertussis]. In the other 68 patients five were not vaccinated. For two subjects the vaccination status was unknown (aged 30·2 and 35·6 years).

In three of the 87 patients in group A during serological follow-up after pertussis a second infection with *B. pertussis* was serologically documented, respectively 3·4, 5·8 and 6·6 years after the first (see below for details). In those three patients the follow-up of the first infection with *B. pertussis* was considered to have ended with the last serum sample obtained before onset of the second infection with *B. pertussis*. The serological follow-up of the second episode in two of these three patients was >6 months and inclusion of the follow-up of these two second episodes therefore resulted in a follow-up in group A of 89 episodes of infection with *B. pertussis* in 87 patients. From the 89 follow-up periods in group A, 403 sera were available. The distribution of those sera over time since the onset of pertussis is shown in Table 1. The mean number of sera for each follow-up period was 4·5 (range of 2–12). As shown in Figure 3 the time between onset of infection with *B. pertussis* and obtaining the last follow-up serum sample ranged from 6 months to 10·7 years, with a median of 3·1 years.

The age distribution of the 34 patients of group B is also given in Figure 2. Since the national vaccination programme in The Netherlands against pertussis started in 1952, the 33 patients of group B who were born before 1952 (i.e. all the nuns) had not been vaccinated. The one patient born after 1952 (the employee) had been completely vaccinated. From the 34 pertussis episodes of the 34 patients of group B 99 sera were available, with 64 sera in the first 6 months after onset, six sera from six patients in the second half year, and 29 sera from 29 patients in the second year (Table 1). The mean number of sera per patient/episode was 2·97 (range of 2–3). The time between onset of pertussis and obtaining the last follow-up serum sample ranged from a minimum of 11·1 months to a maximum of 17·6 months, with a median of 12·6 months.

Categorization of IgG-PT values (<5, 5–20, 20–100, 100–400 and >400 U/ml) and of time elapsed since onset of pertussis (0–6 months, 6–12 months, 1–2 years, 2–4 years, 4–6 years, 6–11 years) yielded the results shown in Table 1. All 89 episodes followed

Table 1. *Percentages of pertussis episodes with indicated category of IgG-PT serum-concentration within the indicated time category*

	Time after onset of disease					
	0–6 mo.	>6–12 mo.	>1–2 yr	>2–4 yr	>4–6 yr	>6–11 yr
Group A						
No. episodes (no. sera) …	89 (180)	39 (49)	32 (44)	48 (75)	30 (40)	8 (15)
IgG-PT concentration (U/ml)						
>400	57	5	0	0	0	0
>100 to ⩽400	43	18	19	0	0	0
>20 to ⩽100	0	51	41	42	27	0
>5 to ⩽20	0	26	28	48	46	38
⩽5	0	0	12	10	27	62
Group B						
No. episodes (no. sera) …	34 (64)	6 (6)	29 (29)			
IgG-PT concentration (U/ml)						
>400	74	0	7			
>100 to ⩽400	21	17	14			
>20 to ⩽100	5	83	48			
>5 to ⩽20	0	0	24			
⩽5	0	0	7			

Separate data for group A (89 episodes in 87 patients) and group B (34 episodes in 34 patients). Times in months (mo.) or years (yr). In case of availability of multiple sera from one episode within one time category, the serum with the highest value has been used. Apart from the numbers of episodes with at least one serum available in the indicated time category (no. episodes) also the total numbers of sera available from those episodes in that time category (no. sera) is indicated.

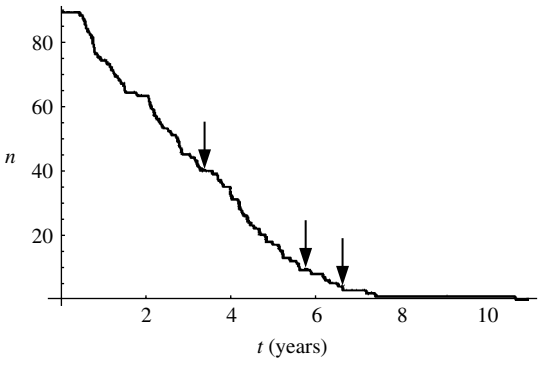

Fig. 3. Number of times patients of group A have been in the study; numbers of patients as a function of the time between onset of disease and last serum sample. The arrows denote time points of confirmed re-infections (one at each time point).

in group A were associated with an IgG-PT of >100 U/ml at some time-point in the first 6 months after pertussis, while in the second half year and in the second year after pertussis the IgG-PT in >70% of cases had fallen below the level of 100 U/ml and in some cases had already fallen below the detection level of 5 U/ml. Six to 11 years after pertussis the IgG-PT had declined to <5 U/ml in five out of eight cases (62%); in the other three IgG-PT had declined to a level between 5 and 20 U/ml (Table 1).

Of the patients of group B 95% had an IgG-PT value of >100 U/ml at some point in the first 6 months after pertussis (Table 1). In the two patients with IgG-PT <100 U/ml in the first 6 months, a more than fourfold increase of IgG-PT in paired sera to a value between 50 and 100 U/ml had been found during the pertussis episode. In the second half year and in the second year after onset of pertussis >70% of the patients of whom sera of that period were available had an IgG-PT of <100 U/ml and in one patient a value <5 U/ml was found in the second year after infection.

The distribution of IgG-PT categories in groups A and B in the first 6 months after infection (for each individual the highest value in that period is taken) and in the second year after infection (also highest value) are remarkably similar suggesting that the rate of decline over time in both groups was similar (Table 1).

Re-infections

In the three patients in group A with re-infection after 3·4, 5·8 and 6·6 years, the maximum IgG-PT titre in the first months after the first typically symptomatic infection was ⩾500, ⩾500 and 248 U/ml respectively,

the high titres had declined in sera obtained after 2·8, 2·9 and 3·1 years respectively (i.e. the last serum sample before onset of the second episode of infection), to 10, 8 and 6 U/ml respectively. The maximum IgG-PT titre reached in the first months after onset of the second episode was 492, 156 and 270 U/ml respectively. The patient with re-infection after 3·4 years (first infection at age 4·2 years) had had a very mild cough, lasting 1 week, 4 weeks before sampling of the serum in which a high IgG-PT, diagnostic of very recent infection, was found. At the same time a sibling had serologically confirmed pertussis. The patient with re-infection after 5·8 years (first infection at age 0·4 years) was discovered when blood was sampled at that time for other reasons; the parents recalled no recent coughing illness. The patient with re-infection after 6·6 years (first infection at age 2·7 years) suffered from mild coughing during 3 weeks; besides the finding of a high IgG-PT concentration diagnostic for very recent infection, this patient was also pertussis-PCR positive.

In Figure 3 the times (after first infection) of occurrence of the three re-infections have been indicated. It can be seen that the one re-infection after 3·4 years was among 40 patients with a follow up of 3·4 years or more (2·5%), the one re-infection after 5·8 years was among nine patients with a follow up of 5·8 years or more (11%) and the one re-infection after 6·6 years was among four patients with a follow up of 6·6 years or more (25%).

Analysis in dynamic model

For analysis in the dynamic model, the patients of groups A and B were taken together and divided into seven age groups: (*a*) <1 year ($n=19$), (*b*) 1–4 years ($n=19$), (*c*) 4–7 years ($n=21$), (*d*) 7–18 years ($n=23$), (*e*) 18–65 years ($n=14$; seven from group A, seven from group B), (*f*) 65–80 years ($n=11$), and (*g*) $\geqslant 80$ years ($n=16$).

Figure 1 shows the individual responses of IgG-PT to infection with *B. pertussis* in these different age groups. In age groups (*a*)–(*d*) and part of group (*e*) (i.e. the patients in this age group derived from group A) the IgG-PT concentrations are censored at an upper level of 500 U/ml (see Laboratory evaluation section above). In part of group (*e*) (those derived from group B) and in groups (*f*)–(*g*) the IgG-PT concentrations are given. The regression model allows for censoring of IgG-PT data from group A (as reported previously [24]), making groups A and B comparable. Note that in Figure 1, both, IgG-PT concentrations and time elapsed since onset of pertussis, are log-transformed, which is common for antibody concentrations but is unusual for time. However, in this manner both the rapidly increasing as well as the slowly decreasing phase of the immunoresponse can be shown in a single graph.

In Figure 4 the estimated variation in individual IgG-PT responses in the different age groups as calculated in the dynamic model is shown as the median (50th percentile) and the 5th and 95th percentiles of simulated responses for each age group. In all groups there is considerable variation. The older groups (*e*)–(*g*) tend to have a higher median peak response, also with a wide range. The time to reach peak levels seems inversely correlated with age (Table 2). Group (*b*) (1–4 years) seems to have the lowest predicted peak level (Table 3) and the longest time to reach peak levels (Table 2). However, the response range in the 1–4 years age group was wide and overlapped the corresponding ranges in the other age groups. The median time-periods in the different age groups for IgG-PT to reach the peak level, and to decline to levels of respectively <100 U/ml (the diagnostic cut-off) and <5 U/ml (detection level) are given in Table 2. These data also indicate that there might be an age-dependent tendency for older people to have a faster decline. Eventually, the median time for IgG-PT to decrease below the detection level of 5 U/ml varied by age group from 1·4 to 5·2 years, with wide inter-individual variations (Table 2).

The estimated variation in the older groups (*e*)–(*g*) is higher than in groups (*a*)–(*d*). While it cannot be excluded that this is also – in part – caused by the influence of censoring, it should also be noted that the records of older patients (of group B) consist of fewer measurements (two or three) than those of the juvenile patients (of group A). We checked the influence of censoring by recalculation after artificially censoring the data from group B, finding similar results, confirming that correction for censoring of the data from group A in the dynamic model was appropriate (data not shown).

In Figure 5 the predicted age profile of IgG-PT responses at different time-points after onset of pertussis is given (10, 20, 50, 100, 200, 365 and 730 days). At 10 and 20 days there is large variation in response in all age groups, possibly partly due to large variations in the rapidity of the increasing phase of the IgG-PT response to infection, and possibly partly due to inaccurate reports of the first day of the disease.

Table 2. *Predicted times* (*in months post onset of symptoms*), *to reach peak levels of the IgG-PT response, and* (*during the declining phase of the IgG-PT response*) *to reach 100 U/ml and times to reach 5 U/ml: $Q_{0\cdot05–0\cdot50–0\cdot95}$ = 5th, 50th* (*median*) *and 95th percentiles*

	Time to peak			Time to 100 U/ml			Time to 5 U/ml		
Age (years)	$Q_{0\cdot05}$	$Q_{0\cdot50}$	$Q_{0\cdot95}$	$Q_{0\cdot05}$	$Q_{0\cdot50}$	$Q_{0\cdot95}$	$Q_{0\cdot05}$	$Q_{0\cdot50}$	$Q_{0\cdot95}$
0–1	0·55	1·18	2·94	1·34	8·28	38·90	15·11	36·48	104·36
1–4	0·47	1·45	2·72	1·48	5·30	97·83	4·63	26·27	257·39
4–7	0·41	0·96	1·86	1·80	14·06	34·40	21·74	62·06	159·55
7–18	0·38	0·77	1·63	3·12	12·25	29·09	20·11	47·93	113·90
18–65	0·09	0·31	1·34	2·29	8·73	25·23	4·56	23·16	100·32
65–80	0·14	0·45	1·20	2·73	10·63	24·61	8·63	25·48	75·67
⩾80	0·15	0·40	1·52	2·08	6·61	19·44	3·75	17·13	68·55

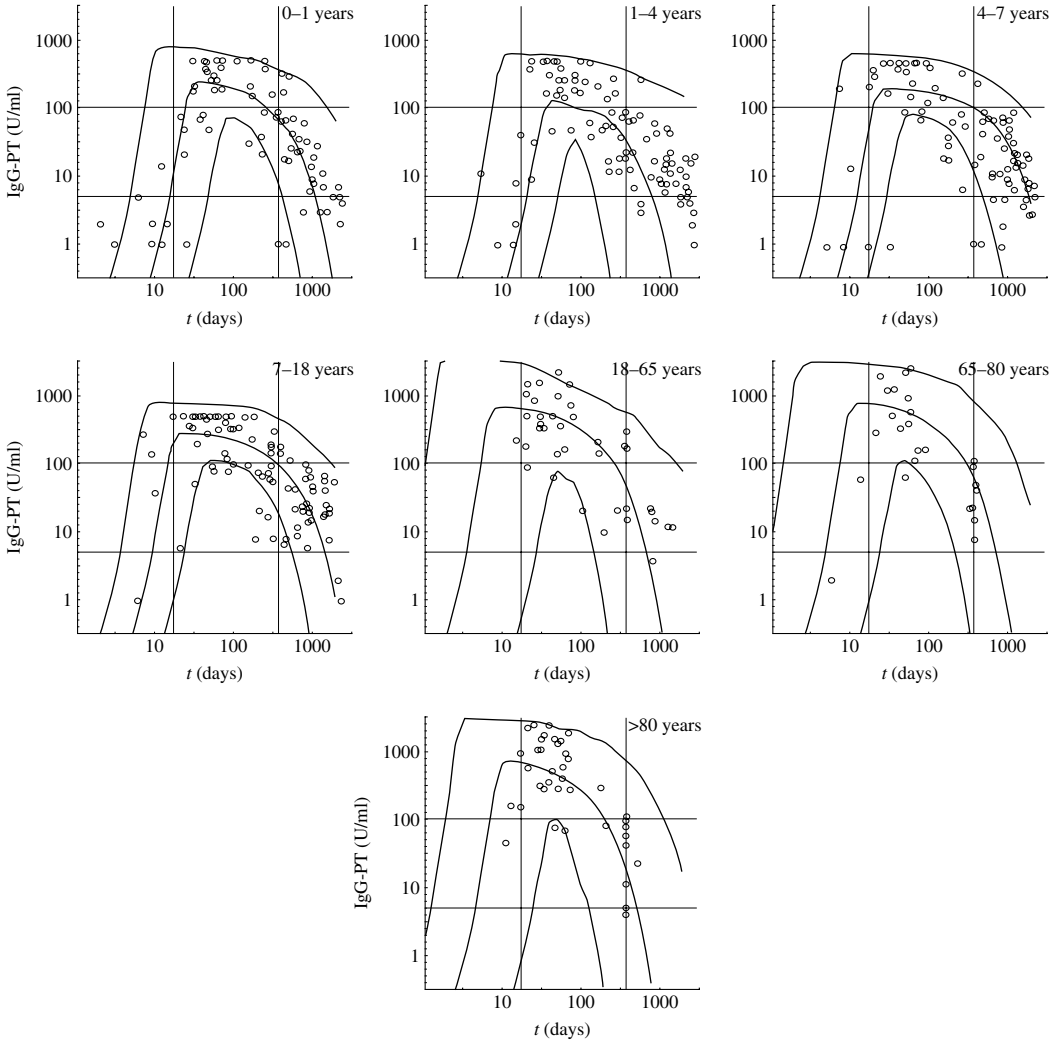

Fig. 4. Predicted IgG-PT antibody responses to infection in each of the age groups, on a $\log_{10}$ (IgG-PT) − $\log_{10}$ (time) scale to show both initial rise and final decline of the responses. Intervals show (95%) range of variation in response among individual patients. Circles indicate observed IgG-PT titres. Also indicated are 3 weeks and 1 year past onset of symptoms (vertical lines), and IgG levels (horizontal lines) of 5 U/ml (detection level), and 100 U/ml (diagnostic cut-off).

Table 3. *Predicted peak levels (U/ml) of the IgG-PT response: $Q_{0\cdot05–0\cdot50–0\cdot95}$ = 5th, 50th (median) and 95th percentiles*

Age (years)	Peak titre (U/ml)		
	$Q_{0\cdot05}$	$Q_{0\cdot50}$	$Q_{0\cdot95}$
0–1	85·9	244·3	624·7
1–4	46·2	136·2	575·1
4–7	100·3	213·0	621·6
7–18	124·3	303·0	653·7
18–65	138·7	695·2	2631·6
65–80	188·2	792·6	2384·2
>80	162·1	720·5	2503·3

However, at both time-points the IgG-PT concentrations in the three adult subgroups tended to be higher, suggesting that the IgG-PT response to infection in adults may be somewhat more rapid. At 50 and 100 days all predicted median IgG-PT levels are >100 U/ml and the variation of responses is small, without substantial differences among age groups, although peak responses in the oldest two subgroups appeared to be somewhat higher. At 200 days antibody decay has started in all age groups. In the older patients (65 to <80 and ⩾80 years) there seemed to be a tendency towards a more rapid decline of IgG-PT after 365 and 730 days than in younger patients.

The second youngest age group (1–4 years) appears to show a response, which is slightly different from the other age groups (slower increase, lower magnitude). As already stated, data in this group show markedly high dispersion, as may also be appreciated by comparing error bars (illustrating variation) in Figure 5 (see also Fig. 4 and Tables 2 and 3).

DISCUSSION

This study of 121 pertussis patients aged 0–94 years shows that elevated levels of IgG-PT induced by infection with *B. pertussis* consistently decline again to below detection levels within several years, unless this course is interrupted by re-infection with *B. pertussis*, which was observed in three cases. In each of the different age groups there was wide variation in rapidity, intensity and rate of decay of IgG-PT and between groups there was a wide overlap of the ranges of these variables. Although the pattern of IgG-PT response is not significantly age dependent, there was a tendency for the IgG-PT response after infection to be faster and stronger and the decay to be more rapid with increasing age. The pattern in the 1–4 years age group was different from all other age groups in that the IgG-PT response tended to be slower mounting and smaller in amplitude and in most cases decayed rapidly.

Our study is unique with respect to the high number of participating patients, the wide age range of the patients, the long follow-up times in part of the patients, and also the method of analysis, using a hierarchical model for induction and decay of antibodies after infection. The few studies of the course of IgG-PT after infection with *B. pertussis* that have been published all show decline over time, but assessment of possible differences in children and adults is lacking and follow-up times are relatively short. In the studies of Hodder et al. [9] and Heininger et al. [32] the course of IgG-PT over a period of 20 months after pertussis in 48 adults aged >65 years [9] and over a period of 28 months in 11 adults [32] was remarkably similar to the pattern over the same time span in adults in our study. In those studies the internationally standardized IgG-PT ELISA, recommended by the FDA was used, of which the relationship with our ELISA is known (see Materials and Methods section; [29]). For instance, the geometric mean IgG-PT titre of 11 adult pertussis patients was 242 IU/ml at 2 months after onset of infection and 45 IU/ml at 28 months after onset [32]. However, in none of those patients did IgG-PT decline below detection levels, which may be related to the limited follow-up time. Two other studies, one comprising both adults and children [2] and one comprising young children [33], also showed a significant decline of geometric mean IgG-PT titre 1 year after pertussis. The study with the longest follow- up time was from Tomoda et al. who measured IgG-PT during an outbreak of pertussis in a semi-closed adult community and again 5 years later [34]. He showed that in the large majority of 21 pertussis patients in that outbreak (37·5 ± 12·1 years) IgG-PT was >50 U/ml several weeks after onset of pertussis; 5 years later the IgG-PT in all patients had declined to <10 U/ml and in most to undetectable levels. These findings support our conclusion that in the majority of cases or perhaps in all cases of pertussis, the subsequent decay of infection-induced IgG-PT does not level off well above detection thresholds but progresses to undetectability.

The relatively short persistence, in all age categories, of infection-induced peak levels of IgG-PT supports our previous finding that a diagnostic cut-off

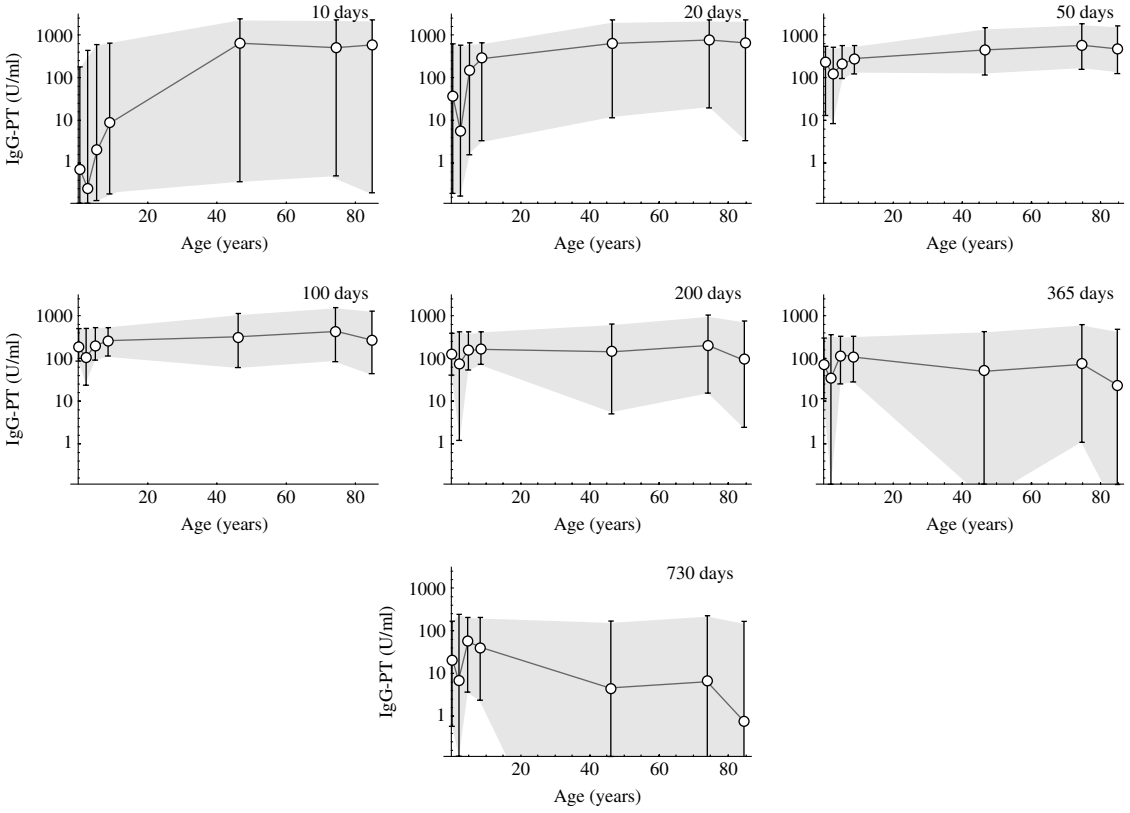

Fig. 5. Age profiles of ($\log_{10}$) IgG-PT responses: predicted antibody levels against age (category) at various times from infection. As in Figure 4 the intervals show (95%) range of variation in response among individual patients.

of 100 U/ml (equivalent to 125 IU/ml) for serodiagnosis of actual or very recent infection with *B. pertussis* is valid for all ages [19]. Although vaccination can induce IgG-PT levels >100 U/ml, interference with serodiagnosis of pertussis is minimal because IgG-PT induced by vaccination with whole-cell pertussis vaccines or acellular pertussis vaccines also declines quite rapidly. Multiple studies show a decrease after primary and booster vaccination to very low or undetectable levels within 1–8 years [13, 35–38]. For instance, Taranger et al. [39] followed 813 children after pertussis vaccination with a monovalent pertussis toxoid vaccine. There was a strong IgG-PT response to a geometric mean of 143 IU/ml at 1–2 months after vaccination and a rapid decrease to a geometric mean of 8 IU/ml at 21–32 months after vaccination.

One explanation for our finding of a relatively slow and low IgG-PT response in the 1–4 years age group, the large majority of whom had been (recently) vaccinated, might be that children who suffer from *B. pertussis* infection shortly after vaccination are a separate category, i.e. are children with a relatively poor immunoresponse to pertussis antigens. Indeed, Taranger et al. [39] showed that previously vaccinated children who within 33 months of completing vaccination developed pertussis upon exposure in their household had had a significantly lower IgG-PT response 1–2 months after the third vaccination with a monocomponent (PT) acellular vaccine (mean peak response 79 U/ml) than children who did not develop pertussis upon household exposure within that time-frame (mean peak response after vaccination 212 U/ml).

The tendency of the IgG-PT response to be more rapid and strong with increasing age may indicate the involvement of specific memory immunity through encounters with *B. pertussis* antigens earlier in life. Also Granström et al. [40] have shown that adults have a faster peak response than children after a natural infection. The intuition that such rapid and strong responses would persist longer evidently is not true. Perhaps the rapid decay of IgG-PT after a rapid and strong response may be explained by a faster eradication of the pathogen and shorter duration of antigenic stimulation of the immune system. The phenomenon as such has been noted before: Blennow & Granström [41] showed that children receiving a

booster vaccination with an acellular vaccine containing pertussis toxin and filamentous haemagglutinin showed a more rapid decay of neutralizing antibodies to pertussis toxin in the Chinese hamster ovary cells (CHO) assay than after the primary vaccination, despite the fact that after the booster vaccination the median of the neutralizing antibody titres was higher than after the primary vaccination.

The three re-infections documented in this study were in children whose IgG-PT at the time of the second infection had declined to <10 U/ml. Although the number of re-infections was small, their timing and incidence was compatible with the statement that natural infection initially induces protection against re-infection but that susceptibility to (re-)infection re-emerges when IgG-PT concentrations have fallen to <10 U/ml and that subsequently, susceptibility for disease in association with infection increases over time. In a previous paper, we have described two other patients who had infection with *B. pertussis* 12 years after the first episode. In contrast to the three re-infections 3·4, 5·8 and 6·6 years after the first in this study, the re-infections after 12 years were associated with typical symptoms, i.e. long-lasting paroxysmal cough [12].

In conclusion this study shows that the high IgG-PT concentrations induced by infection with *B. pertussis* consistently decline to low or undetectable levels within 5–6 years, this decline is associated with emergence of susceptibility for re-infection and disease. Although the pattern of decline is largely independent of age, there is a tendency for older people to have a more rapid increase, higher peak and faster decline. This study also shows that a diagnostic cut-off of 100 U/ml is not only valid in children [19], but is true for all ages.

APPENDIX

The predator–prey model of the interaction between the immune system and invading bacteria can be formulated as:

$$\begin{cases} x'(t) = ax(t) - bx(t)y(t) \\ y'(t) = -cy(t) + dx(t)y(t) \end{cases} \quad \begin{cases} x(0) = x_0 \\ y(0) = y_0, \end{cases}$$

where $x(t)$ = pathogen concentration; $y(t)$ = antibodies; a = pathogen growth rate; b = antibody-dependent pathogen inactivation; c = antibody decay rate; d = pathogen-dependent antibody production rate.

Numerical solutions to these equations (a simple analytical solution does not exist) can be fitted to the longitudinal antibody data, treating the unknown pathogen concentration as a nuisance variable.

Measurement errors were assumed lognormal: both data and model function were log-transformed (TBS: transform both sides) so that we could use a normal likelihood function. This also allows treatment of censoring as previously reported [24]. Variation between individual patients is considerable, we therefore used a hierarchical Bayesian model. For statistical analysis, parameters were transformed:

$$u = \sqrt{ac} \qquad w = \sqrt{bd}$$
$$v = \sqrt{a/c} \qquad z = \sqrt{b/d}$$

and these new parameters were log transformed, to restrict them to positive values. Parameters u and v were assumed equal in all patients, w and z vary among individuals. Parameter estimation for the hierarchical model was performed using a MCMC method, employing the Metropolis–Hastings algorithm [31].

Posterior predictive intervals for the longitudinal response to pertussis were constructed by applying the longitudinal model to a parameter sample from the MCMC output (by addition of a subject with missing data only, for convenient sampling from the population parameter distributions).

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