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Differences in Coughing and Other Responses to Intrabronchial Infection with *Bordetella pertussis* among Strains of Rats

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Four strains of rats were each infected intrabronchially with approximately 10^8 CFU of *Bordetella pertussis* 18-323 encased in fine agarose beads. After 8 days, Sprague-Dawley rats developed the highest incidence of coughing paroxysms, as monitored with voice-activated tape recorders; Brown Norway, Lewis, and Hooded Lister rats coughed significantly less frequently. Marked leukocytosis, with counts up to four times the normal levels, and retardation of normal weight gain occurred in all four rat strains. Both coughing and leukocytosis were greater in animals that were infected at 4 weeks of age than in those infected at 6 weeks of age. Total serum immunoglobulin E (IgE) rose in all four rat strains 9- to 244-fold by day 8 after infection and returned to near preinfection levels at 6 weeks. Sprague-Dawley and Lewis rats, which had the lowest basal levels of total IgE in serum, showed the greatest degrees of elevation. All four rat strains had IgG to *B. pertussis* whole-cell sonicate and to filamentous hemagglutinin in 6-week-postinfection sera. However, the strains differed in production of IgG to pertussis toxin, with Sprague-Dawley rats having the highest titers and Hooded Lister and Lewis rats being nonresponders. These studies highlight the importance of rat strain as a variable in the coughing-rat model of pertussis and validate the choice of the Sprague-Dawley rats in previous studies.

In unimmunized humans, particularly infants, *Bordetella pertussis* causes the distressing respiratory symptoms of whooping cough. The uncomplicated disease is generally not fatal, although respiratory complications and central nervous system disturbances can occur and are due mainly to the pressure effects and anoxia resulting from severe coughing paroxysms (2, 20, 29). Marked leukocytosis is also a characteristic clinical sign. Despite the detailed molecular and biological characterization of pertussis toxin (PT) and other virulence factors of *B. pertussis*, their role in causing the paroxysmal cough is unknown (21). Such studies have long been hampered by the lack of a coughing-animal model of this human disease. The mouse, which has been much used for experimental infection with *B. pertussis*, does not cough when infected via the respiratory route. The rabbit and various monkey species may also be infected experimentally with *B. pertussis*, but only some primates have been reported to develop paroxysmal coughing (26).

In a very early but apparently overlooked paper, Hornibrook and Ashburn (12) first reported that young rats coughed after respiratory-tract infection with *B. pertussis*. Nearly 50 years later, Woods et al. (33) infected Sprague-Dawley (SD) rats intrabronchially with *B. pertussis* encased in microscopic agar beads and found that the animals "coughed with whooping sounds similar to humans" and that the infection was nonlethal. In our previous studies (6, 22, 30), these findings were confirmed and the coughing-rat model was further developed. Low-gelling-temperature agarose was used instead of agar to make the microscopic beads containing *B. pertussis*. A fentanyl-fluanisone (Hypnorm)–midazolam hydrochloride (Hypnovel) injection mixture was used instead of ether as the anesthetic for the intrabronchial instillation of the infecting dose, although ether was still used postoperatively since it enhanced the coughing response. Paroxysmal coughing was monitored with sound-activated tape recorders with microphones above the rat cages in sound-insulated booths; coughing started at about 5 days after infection and could be detected for up to 21 days. In addition to strain 18-323, other phase I strains of *B. pertussis* induced paroxysmal coughing, whereas phase IV *B. pertussis* and *Bordetella parapertussis* did not (22). Transposon mutants of *B. pertussis* lacking PT did not induce coughing or leukocytosis, whereas a mutant lacking the heat-labile dermonecrotizing toxin was indistinguishable from wild-type *B. pertussis*. Convalescent-phase sera from animals previously infected with wild-type *B. pertussis* contained immunoglobulin G (IgG) specific for PT and filamentous hemagglutinin (FHA), and prior immunization with whole-cell pertussis vaccine prevented both coughing and leukocytosis after infection (6, 22).

In the present study, the effect of using different strains of rat in the coughing-rat model of pertussis was investigated. SD rats were compared with animals of the Brown Norway (BN), Hooded Lister (HL), and Lewis strains. These other strains were chosen not only because of their commercial availability as high-health-status animals but also to represent high- and low-IgE responders since PT is a well-known IgE adjuvant and this immunoglobulin may have some role in pathogenesis or immunity in pertussis.

MATERIALS AND METHODS

Animals. Outbred female SD and HL rats and inbred BN (Brown Norway/Ssn) and Lewis rats were purchased at 3 and 5 weeks of age from Harlan Olac Ltd. (Shaws Farm, Blackthorn, Bicester, United Kingdom). The animals were barrier reared, and each group was certified as being free from a range of viral, protozoal, and bacterial pathogens, including *Bordetella* and *Pasteurella*. They were allowed to acclimatize for 1 week before use.

Bacterial suspension and bead preparations. The organisms were grown and maintained as described previously (6). Briefly, *B. pertussis* 18-323, phase I (ATCC 9797), was grown on Bordet-Gengou agar (Difco Laboratories) containing 20% (vol/vol) defibrinated horse blood (E & O Products, Bonnybridge, Stirlingshire, United Kingdom). Growth from plates which had been heavily inoculated and incubated overnight at 37°C was suspended in a solution containing 1% (wt/vol) Casamino Acids (CAA) (Difco), and the suspension was standardized by visual comparison with the fifth international reference preparation of opacity developed by the World Health Organization (24). This opacity

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standard, of 10 opacity units, is equivalent to approximately 2×10^9 CFU of *B. pertussis* per ml. Dilutions of this suspension were plated out onto Bordet-Gengou agar to obtain viable counts.

In our previous studies, the microscopic beads of bacteria in agarose were prepared by mixing the *B. pertussis* suspension with molten, low-gelling-temperature agarose and homogenizing the mixture in warm liquid paraffin (6, 22, 30). The resultant paraffin-gel-bead mixture required extensive washing by centrifugation to remove all traces of oil. To avoid this time-consuming stage, an oil-free method which greatly reduced the preparation time was devised. Additionally, to provide optimal environmental conditions for the bacteria, 2,6-*O*-dimethyl-β-cyclodextrin (MeCD) was added to the agarose mixture. MeCD, kindly provided by H. Ikeda (Teijin Ltd., Tokyo, Japan) is frequently used to supplement *B. pertussis* culture media and enhances growth and virulence factor production (13).

Low-gelling-temperature agarose type VII (4% [wt/vol]; Sigma) was dissolved in CAA solution containing 0.2% (wt/vol) MeCD, sterilized by autoclaving, and transferred to a water bath at 38°C for equilibration. Likewise, the bacterial suspension previously standardized to approximately 4×10^9 CFU/ml was warmed to 38°C. Molten agarose (4 ml) was mixed with an equal volume of bacterial suspension and then drawn up into a 10-ml syringe. The mixture was solidified on ice, expelled into 8 ml of CAA containing 0.1% (wt/vol) MeCD, and homogenized with a Silverson mixer-emulsifier at low speed for 15 s. The final bacterium-agarose bead slurry contained approximately 10^9 CFU/ml in 1% (wt/vol) agarose in CAA and 0.1% (wt/vol) MeCD.

Infection procedure. Rats were infected intrabronchially as described previously (6, 22). Each animal was given 0.1 ml of *B. pertussis* bead slurry containing approximately 10^8 CFU. Three independent experiments were performed, each including four 4-week-old and four 6-week-old rats of each strain, for a total of 96 infected rats, plus 50 untreated controls.

Postinfection observations. The animals were weighed on the day of infection and at intervals thereafter, and on day 8 they were monitored for coughing and leukocyte count as described previously (6). One cassette tape (45 min) was used for recording each group overnight with a sound-activated tape recorder, and coughing data, expressed as number of paroxysms per rat per tape, were calculated by dividing the total number of paroxysms heard on the tapes by the number of rats in the group. On the basis that the coughing paroxysms were independent random events, the Poisson distribution was assumed to apply, and confidence limits were assigned from a standard table (4) for count data. For antibody measurements, the animals were bled from the tail vein before and at intervals after infection and the sera were stored at −20°C.

Anti-rat IgE antiserum production and purification. A goat was injected intramuscularly with 1 mg of purified rat IgE myeloma IR162 (purified IR162 and IR2 were gifts to the late Ellen Jarrett, University of Glasgow, from Herve Bazin, University of Louvain, Louvain, Belgium) and emulsified in an equal volume of Freund's complete adjuvant (Sigma). During the subsequent 14 months, six boosters, consisting of 200 to 300 μg of IR162 emulsified with Freund's incomplete adjuvant (Sigma), were given at approximately 2-month intervals, and the animal was bled 2 weeks after the last dose. The serum was frozen at −20°C and, before purification was begun, was tested in Ouchterlony immunodiffusion against the IgE myelomas IR162 and IR2 and against rat IgG. There were clear precipitin reactions to all three.

To remove the anti-rat IgG content, the goat serum was passed through an immunosorbent column of rat IgG (Sigma) coupled to cyanogen bromide (CNBr)-activated Sepharose (CNBr-Sepharose; (Pharmacia) (50 mg of rat IgG per 8 g of Sepharose) according to the manufacturer's instructions. This absorption procedure was done three times, until there was no reaction with IgG visible in immunodiffusion. To remove antibodies other than that to IgE present in the antiserum, the serum was passed twice through a CNBr-Sepharose column to which had been coupled normal rat gamma globulin (120 mg of protein coupled to 10 g of Sepharose). Finally, the partially purified goat anti-rat IgE was affinity purified on an immunosorbent column prepared with 6 mg of rat IgE myeloma IR2 coupled to 4 g of CNBr-Sepharose, and the bound fraction was eluted with 0.1 M glycine-HCl buffer (pH 3), followed by dialysis against phosphate-buffered saline. A different IgE myeloma was used on the affinity column to eliminate anti-idiotypic antibodies raised after immunization, thereby ensuring that the resultant sample contained only IgE class-specific antibodies. Biotinylation of anti-IgE antibodies was done by the method of Harlow and Lane (7). The biotinylated antibodies were aliquoted and stored at −20°C.

ELISA for total serum IgE. Nunc immunosorbent plates (Life Technologies, Paisley, United Kingdom) were coated with nonbiotinylated goat anti-rat IgE diluted 1:10,000 in 0.1 M $NaHCO_3$, pH 9.0, overnight at 4°C in a moist box; 100-μl volumes of reagents were used unless otherwise stated. The wells were washed three times with wash buffer (WB) containing 0.15 M NaCl, 0.001 M KH_2PO_4, 0.008 M Na_2HPO_4, 0.002 M KCl, and 0.05% (wt/vol) Tween 20 (Sigma). Bovine serum albumin (2% [wt/vol]; Sigma) in WB was used for blocking at 37°C for 1 h, followed by three washes in WB. Serial dilutions of samples and different concentrations of IR2 (100 to 0.01 ng/ml), both prepared in WB with 1% (wt/vol) bovine serum albumin, were added to the wells and after 1 h at 37°C washed off, and biotinylated goat anti-rat IgE diluted 1:5,000 in WB was added to each well. After 1 h and a further washing, streptavidin-peroxidase (Sigma) diluted 1:1,000 in WB was added for 40 min at 37°C and, after a washing, finally developed with 0.1 M *O*-phenylenediamine substrate (tablet form; Sigma) at 34 mg/ml in 0.1 M citrate–0.2 M phosphate buffer, pH 5, containing 20 μl of H_2O_2 (Sigma) per 100 ml of buffer. The reaction was allowed to proceed in the dark for 20 min and was then stopped by adding 50 μl of 12.5% (vol/vol) H_2SO_4. Absorbance was read at 492 nm in a Titertek Multiscan MC enzyme-linked immunosorbent assay (ELISA) reader (ICN/Flow, Thame, United Kingdom). A standard curve was constructed from the readings obtained for IR2, and the IgE content of the samples was determined from their interpolated absorbancies. Each test included 10-fold dilutions of a normal rat (SD) serum pool which had total IgE content of around 10 ng/ml.

Antigen-specific IgG ELISA. ELISAs with *B. pertussis* whole-cell sonicate, FHA, and PT were performed as described previously (6). Mean absorbance readings at 492 nm were plotted against $\log_{10}$ dilutions of the samples, and the end point at an A_{492} of 0.5 was taken as the titer of the serum. Each test included 10-fold dilutions of normal rat serum and a pooled reference serum from rats hyperimmunized with *B. pertussis* (6). The reference serum had been assigned ELISA titers of antibodies to *B. pertussis* whole-cell sonicate, FHA, and PT antigens of 20,000, 80,000, and 500, respectively, and enabled allowance to be made for day-to-day variation between tests. The normal rat serum and background levels had A_{492} values ranging from 0.1 to 0.3.

Antigen-specific IgE ELISA. To determine the conditions under which antigen-specific IgE antibody might be measurable, egg albumin (EA) (Sigma) was first used as coating antigen to take advantage of an available IgE anti-EA reference preparation. This sample was a pool of high-titer IgE anti-EA rat sera as measured by the radioallergosorbent test (RAST), in which it had a titer of 6,000 (5). By ELISA (with EA coated overnight at 10 μg/ml, biotinylated anti-IgE used at 1:1,000, and all other steps done as in the total serum IgE assay), the reference preparation had a titer of 10,000, and normal rat serum and background levels had A_{492} values around 0.2. For measurement of IgE to the bacterial antigens, the method for the antigen-specific IgG ELISA was performed except that biotinylated anti-IgE was used at 1:1,000, in place of labelled anti-IgG, and all subsequent steps were done as in the total serum IgE ELISA.

Removal of IgG antibodies from rat convalescent-phase sera. A rabbit was immunized intramuscularly with 1 mg of rat IgG (Sigma) emulsified in an equal volume of Freund's complete adjuvant (Sigma) and thereafter immunized at monthly intervals with 100 μg of IgG in Freund's incomplete adjuvant (Sigma). Two weeks after the third injection, the animal was bled. After dialysis against 0.1 M Tris buffer, pH 8.0, the serum was passed through an immunosorbent column of rat IgG (as described above), and the bound antibodies were eluted with 0.1 M glycine-HCl buffer, pH 3, followed by dialysis against phosphate-buffered saline. The affinity-purified rabbit anti-rat IgG preparation (10 mg) was coupled to 5 g of CNBr-Sepharose as described above. Eight representative individual rat serum samples obtained on day 21 after *B. pertussis* infection (about 3 ml each) were passed through the anti-rat IgG column, and the first peaks from these fractionations were collected and concentrated back to 3 ml by dialysis against polyethylene glycol (15,000 to 20,000 Da; Sigma). EA-RAST reference serum (3 ml) was treated in the same way.

Statistics. Minitab statistical software (Macintosh version, release 8; Clecom Microsoft Specialists, Birmingham, United Kingdom) was used to obtain mean values and the standard errors of the means, median values and their 95% confidence limits, to calculate significance values by the Mann-Whitney U test, and to perform analysis of variance.

RESULTS

Coughing. Previous studies had shown that the peak of the leukocyte response to *B. pertussis* infection in SD rats occurred at around day 8 and coughing occurred from day 5 onwards, with a peak around days 8 to 14 (6, 30). In this study, only the SD animals coughed to any appreciable extent during the period of observation, and those that were 4 weeks old at the time of infection were more prolific coughers than animals infected at 6 weeks of age. Figure 1 summarizes the quantitative observations on paroxysmal coughing at day 8 after infection from three independent experiments involving a total of 96 infected rats of the four strains. The 4-week-old HL rats gave a few paroxysmal coughs but markedly fewer than the SD rats, while BN and Lewis animals gave too few coughs to be of any use in the coughing-rat model of pertussis. No paroxysmal coughing was heard in tapes from a total of 50 control, uninfected animals of the four strains.

Mortality and weight gain. Before day 8 after infection, there was an overall 18% mortality (17 of 96) in the infected animals (Table 1). There were no deaths within 24 h of the operation, and the median day of death was day 5 for the 17 animals that died (9 of which were sacrificed due to ill health, i.e., when there was a change in their appearance or behavior such as a ruffled coat and hunched stance). Fourteen of these

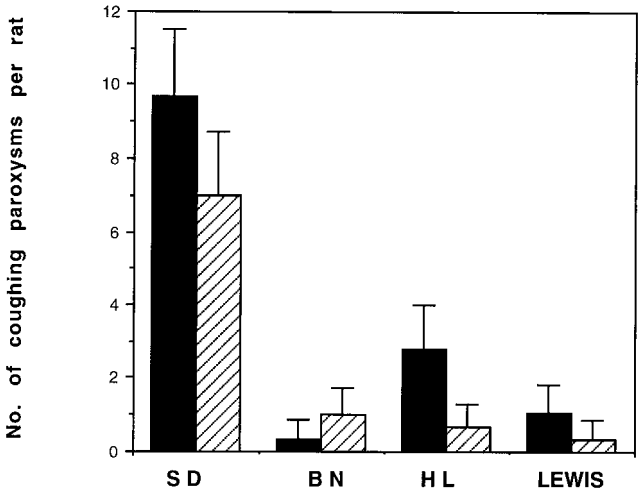

FIG. 1. Coughing in four strains of rat on day 8 after intrabronchial infection with *B. pertussis* 18-323. Solid bars represent 4-week-old animals, and hatched bars represent 6-week-old animals. Error bars are upper 95% confidence limits.

were 4 weeks of age when infected, while only three deaths occurred among animals that were 6 weeks old when infected. Thus, there were more deaths among the younger animals, but the difference in proportion of deaths was not significant by the χ^2 test ($\chi^2 = 1.53$). No deaths occurred among control, untreated rats.

The rats were weighed individually on the day of infection and on days 1, 3, and 7 thereafter. The day 0 mean weights, in grams, for each strain at 4 weeks of age were as follows: SD, 115; BN, 85; HL, 114; and Lewis, 105. The day 0 mean weights, in grams, for each strain at 6 weeks of age were as follows: SD, 168; BN, 121; HL, 156; and Lewis, 150. Figure 2 shows that control animals had weight gains of between 10 and 20% during the period of observation, the younger animals having the greater increases. In contrast, infected animals of all four strains had lost weight at 24 h but subsequently gained weight at approximately the same rate as the controls but not catching up with them during the period of observation. Exceptions were the Lewis rats of both ages, which failed to gain any weight during the 7 days postinfection or even to regain their preinfection weights. The SD, BN, and HL rats were similar in their weight changes after infection, despite the marked differences in their coughing responses.

Leukocytosis. Leukocytosis is one of the characteristic features of severe pertussis in the human infant, and leukocyte counts were therefore investigated in the infected rats. The geometric mean leukocyte counts and their 95% confidence limits for the 16 groups of rats are detailed in Table 1. All four strains of rat had developed leukocytosis at day 8 after infection with *B. pertussis*, and animals that were 4 weeks old when infected had higher levels of leukocytosis than animals infected at 6 weeks of age.

The highest mean level of leukocytosis occurred in 4-week-old infected Lewis rats and was 6.5-fold over that in the uninfected controls. In 4-week-old SD rats the figure was 5.5-fold. In contrast, 4-week-old BN and HL animals showed leukocytosis levels only 2- and 2.8-fold, respectively, higher than that in the uninfected controls. Analysis of variance of the data summarized in Table 1 confirmed that there were highly significant differences ($P < 0.001$) between the strains and ages of rat and between infected and control animals. For the four interaction terms generated by the three independent variables (strain, age, and infection), there was highly significant interaction between strain and infection and between age and infection but not between age and strain or among the three variables.

IgG responses. Detailed studies were performed by ELISA of the IgG responses to *B. pertussis* antigens in sera taken on days 8, 21, and 42 after infection. Since there were no significant differences between titers in 4- and 6-week-old animals, the results for the two age groups were pooled (Table 2). A positive serum was one having a titer of 10 ($\log_{10} = 1$) or higher. Because many of the sera had titers below the limit of detection, it was not possible to summarize group results as the means. Instead, the median values with the range and the 95% confidence limits were used.

On day 8 after infection there were no detectable IgG responses to any of the three *B. pertussis* antigens in sera from SD or Lewis rats. However, a low response to FHA was detected in BN and HL animals, with the former also producing a modest response to antigens in *B. pertussis* sonicate. By day 21 after infection, there was an increase in all four strains in the number of positive responders to the sonicate, while all the infected rats showed titers of antibodies to FHA. The latter titers were in the order BN > SD > HL > Lewis. The day 21 ELISA titers of antibodies to PT were much lower and less

TABLE 1. Survival and blood leukocyte count in rats of different strains and ages on day 8 after infection with ca. 10^8 *B. pertussis* 18-323 organisms, compared with uninfected controls

Rat strain	Age at infection (wk)	Rats infected with *B. pertussis*		Control rats	
		No. of rats surviving/ no. tested	No. of leukocytes[a]/mm^3 (10^3) (95% CL[b])	No. of rats surviving/ no. tested	No. of leukocytes/mm^3 (10^3) (95% CL)
SD	4	8/12	57.5 (36.8, 89.9)	5/5	10.5 (9.05, 12.1)
	6	12/12	21.5 (15.2, 30.5)	7/7	8.8 (6.5, 11.9)
BN	4	10/12	25.0 (17.7, 35.5)	7/7	12.1 (9.9, 14.8)
	6	11/12	13.1 (9.9, 17.3)	4/4	9.1 (5.1, 16.2)
HL	4	8/12	22.1 (14.2, 34.4)	8/8	7.8 (7.0, 8.7)
	6	11/12	12.2 (8.1, 18.4)	9/9	7.9 (7.3, 8.7)
Lewis	4	8/12	62.0 (48.0, 80.0)	5/5	9.4 (7.8, 11.3)
	6	11/12	41.0 (31.4, 53.4)	5/5	9.8 (7.6, 12.7)

[a] Geometric mean values.
[b] CL, confidence limit.

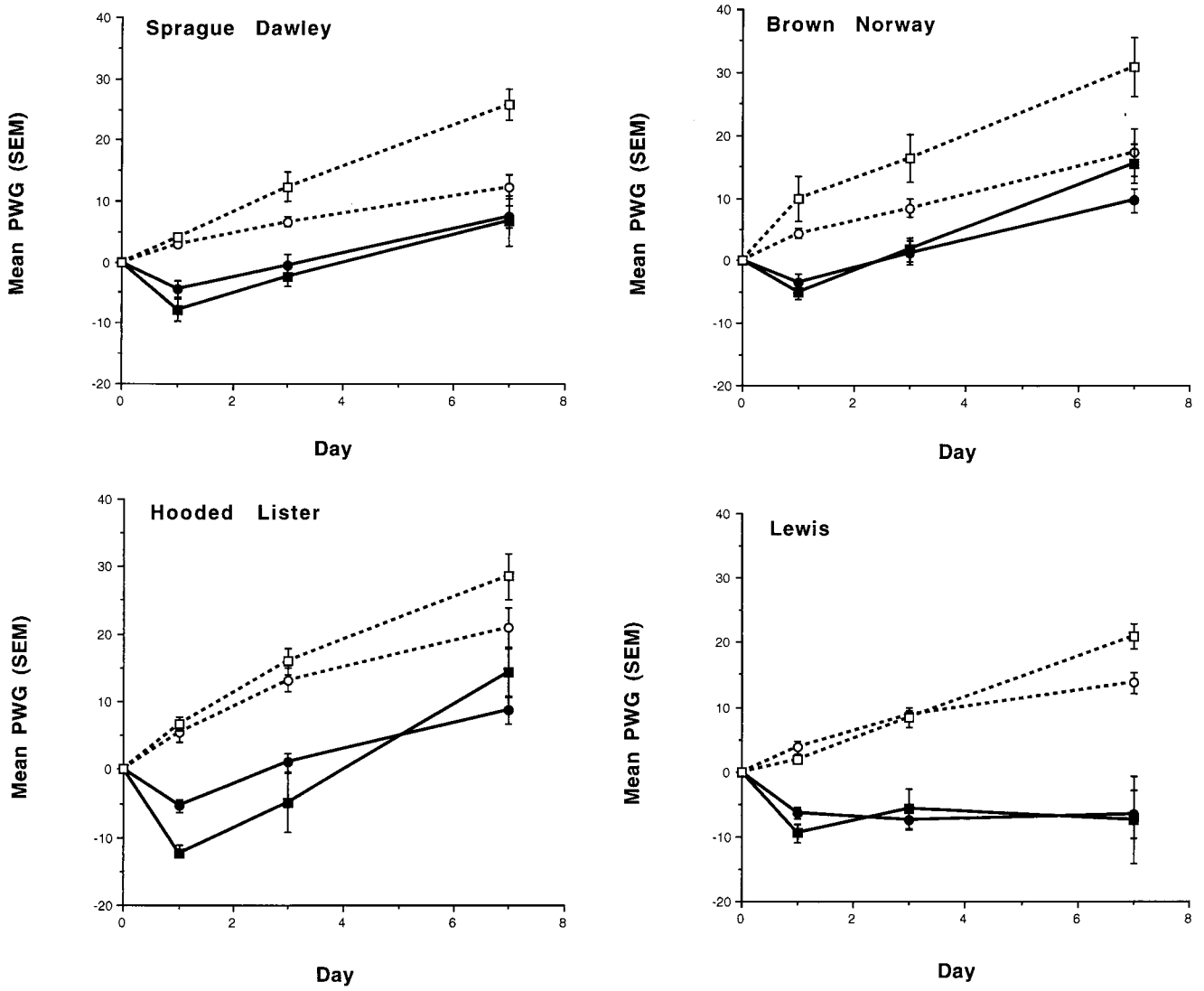

FIG. 2. Percent weight gain (PWG) of infected and control rats, calculated relative to day 0. Results are the means and standard errors of the means (SEM) of three experiments for each age group (12 rats per experimental group and 4 to 8 rats per control group). The graphs show PWG for each strain of rat: ■, 4 weeks old, infected; □, 4 weeks old, control; ●, 6 weeks old, infected; ○, 6 weeks old, control.

consistent than those to FHA: only 10 of 15 SD rats, the best-responding strain, had detectable levels of anti-PT IgG. The median titer of 95 ($\log_{10} = 1.9$) was drawn from the wide range of <10 to 400,000 in individual animals. Only 3 of 15 BN animals contained detectable levels of anti-PT, while none of the HL or Lewis rats responded at all.

By day 42, the antisonicate titer was higher than on day 21 in BN rats, whereas among the other three strains the proportion of responders declined, although the ranges increased slightly. The anti-FHA responses, in contrast, were higher on day 42 than on day 21, with every single animal in each of the four strains having a high titer. The order of median anti-FHA titers in the day 42 sera was SD = BN > Lewis > HL. Unlike the responses to FHA, the IgG responses to PT had fallen by day 42 in SD animals and similarly in BN rats, while there was still no detectable response to this antigen in Lewis or HL rats. Sera from untreated control animals all gave titers of <10 in ELISAs specific to each of the three antigens (results not shown).

Total serum IgE levels. Pre- and postinfection levels of total serum IgE are presented in Fig. 3. As there were no significant differences between the levels obtained in the 4- and 6-week-old animals, the results were pooled for convenience. A marked increment in total serum IgE on day 8 after infection was apparent in all four rat strains. Mean levels in Lewis rats increased from 0.5 to 122 ng/ml (244-fold), while in SD rats there was a 100-fold increase, from a mean basal serum IgE level on day 0 of 1.5 ng/ml to a mean level of 150 ng/ml. A 26-fold increment was seen in HL rats, in which the level increased from 7 to 185 ng/ml, while the serum IgE level in the BN strain increased 9-fold, from a basal value of 250 ng/ml to a mean value of 2,240 ng/ml. By day 21, total serum IgE levels had dropped by various degrees in most of the groups with the exception of the Lewis rats, in which the elevation was maintained, while the day 42 levels had returned nearer to preinfection readings in Lewis, SD, and BN rats (1.3, 5.6, and 375 ng/ml, respectively), with an elevation still apparent in HL rats (42 ng/ml). Levels of total serum IgE in untreated controls

TABLE 2. Summary of $\log_{10}$ IgG ELISA titers in sera of rats of different strains infected with *B. pertussis* and tested with coating antigens

Strain and day	ELISA titer with coating antigen[a]					
	BP		FHA		PT	
	Median and range (95% CL)	No. of sera positive/ no. tested	Median and range (95% CL)	No. of sera positive/ no. tested	Median and range (95% CL)	No. of sera positive/ no. tested
SD						
8	<1	0/15	<1	0/15	<1	0/15
21	<1 <1–2.1 (NC)	4/15	3.8, 2.7–5.1 (3.1–4.3)	15/15	1.9, <1–5.7 (<1–4.9)	10/15
42	<1, <1–2.6 (NC)	1/7	5.0, 4.6–7.3 (4.9–7.0)	7/7	<1, <1–3.4 (<1–3.0)	2/7
BN						
8	<1, <1–1.3 (NC)	2/15	<1, <1–4.2 (<1–1.4)	6/15	<1	0/15
21	1.1, <1–2.3 (<1–1.9)	9/15	4.6, 3.5–5.6 (4.4–5.0)	15/15	<1, <1–5.1 (NC)	3/15
42	2.6, <1–3.8 (<1–3.0)	5/7	5.0, 3.9–5.9 (4.6–5.0)	7/7	<1, <1–3.7 (<1–3.4)	4/7
HL						
8	<1	0/14	<1, <1–2.9 (<1–1.1)	2/14	<1	0/14
21	1.7, <1–2.6 (<1–2.1)	9/13	3.5, 2.4–4.5 (2.6–4.2)	13/13	<1	0/13
42	<1, <1–4.2 (<1–3.1)	4/7	3.6, 4.3–4.9 (4.4–4.7)	7/7	<1	0/7
Lewis						
8	<1	0/14	<1	0/14	<1	0/14
21	<1, <1–1.3 (<1–1.2)	2/8	3.0, 2.8–4.1 (1.9–3.5)	8/8	<1	0/8
42	<1, <1–1.3 (<1–1.2)	1/5	4.8, 3.6–5.3 (NC)	5/5	<1	0/5

[a] BP, *B. pertussis* whole-cell sonicate; CL, confidence limit; NC, noncalculable.

were measured on each of the above-mentioned days and did not differ significantly from preinfection levels (results not shown).

Antigen-specific IgE ELISA. Antigen-specific IgE ELISAs were performed on the same sera as used in the above-described experiments. All experimental samples had undetectable (<10) titers of antibodies against the three antigens tested, i.e., *B. pertussis* whole-cell sonicate, FHA, and PT. To overcome any possible interference from IgG antibodies, these were removed from eight representative sera, taken on day 21 after infection, by passage through an affinity column of anti-rat IgG and then reassayed on both IgG and IgE antigen-specific ELISAs. Before absorption, the group of eight sera (two per rat strain) had detectable IgG titers of antibodies to each of the three bacterial antigens, e.g., a median IgG anti-FHA titer of 50,000 within a range of 80 to 220,000, and after passage through the anti-rat IgG column all read <10 in IgG ELISAs specific to the three antigens, indicating the effective removal of IgG antibodies. The samples were reassayed on *B. pertussis* whole-cell sonicate, FHA, and PT IgE ELISAs and again read <10. The EA-RAST reference preparation which contained IgG anti-EA (ELISA titer, 20,000) in addition to IgE anti-EA (ELISA titer, 10,000) was also passed through the anti-IgG column, after which it read <10 in EA IgG ELISA and 5,000, indicating a slight reduction, in EA IgE ELISA. Thus, although attempts to detect IgE to specific *B. pertussis* antigens were made, none was found.

DISCUSSION

The main objective of this work was to compare the coughing responses of four different strains of rat after intrabronchial infection with *B. pertussis* encased in agarose beads. We previously showed that SD rats coughed following infection and that this response was accompanied by leukocytosis and retardation of weight gain (6, 30). We report here that, of all strains compared, SD animals are indeed the only rats to cough consistently, regardless of age, although the other symptoms of infection, namely, leukocytosis and retardation of weight gain, were demonstrated in all four rat strains.

Age-related susceptibility to infection was apparent, with appreciable mortality, during the first week postinfection in all rats infected at 4 weeks of age. Retardation of weight gain was particularly evident in Lewis rats infected at either 4 or 6 weeks of age. The degree of weight gain retardation was paralleled by susceptibility to leukocytosis, which was most pronounced in Lewis and SD rats in both age groups. In comparison, the leukocyte count in HL rats was significantly altered when the rats were infected at 4 weeks of age and in BN rats less so, but neither showed leukocytosis when infected at 6 weeks of age.

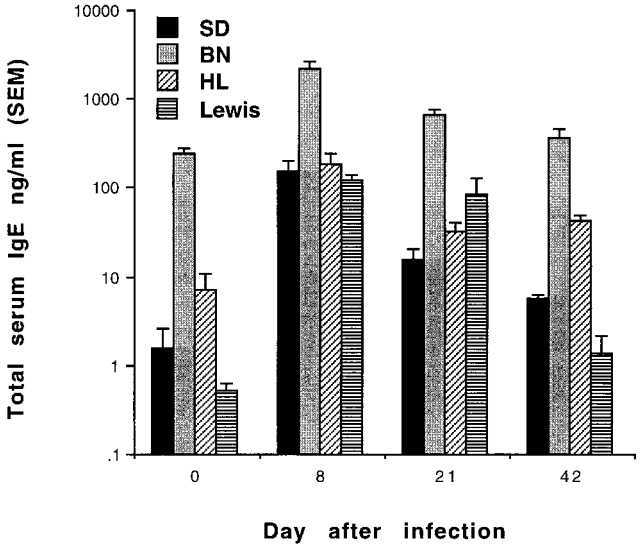

FIG. 3. Mean total serum IgE levels before and after *B. pertussis* infection in four strains of rat. Means and standard errors of the means (SEM) were obtained from two experiments on days 0, 8, and 21 and from one experiment on day 42.

Without better knowledge of the time course of bacterial persistence in the lungs, we cannot be certain that the cause of deterioration in young animals was the multiplication of pertussis organisms or even their persistence. In the original study by Woods et al. (33) and in a preliminary investigation of our own (unpublished data), *B. pertussis* was cleared fairly rapidly from rat lungs and could not be recovered from lung homogenates by day 10, although in the former study, the bacteria reappeared at day 21. Since the deaths occurred in the younger rats and during the first week, it is possible that the infecting dose was directly toxic for that age group.

It is well established that rat strains vary in their abilities to produce IgE, from the poorly producing Lewis rats to the very highly producing BN rats, and our pattern of basal serum IgE measurements obtained on day 0 confirm these earlier distinctions (1). An elevation in total serum IgE was evident by day 8 after infection in all strains, and the degree of increment was inversely proportional to the basal serum IgE levels of the strain, with the greatest increases occurring in the low-level-IgE-producing Lewis and SD rats. The levels gradually declined, returning close to normal in Lewis, SD, and BN rats, but an elevation was still apparent in HL rats 6 weeks after infection. There are some inconsistencies we cannot explain, such as why the HL rat day 42 level was higher than the day 21 level, but these measurements were repeated, with the same result.

The mechanisms of regulation of IgE production have yet to be fully characterized. A series of in vitro experiments with helminth-infected rodent lymphocytes highlighted soluble factors with modulatory effects specific for IgE (14). The fact that this mechanism existed in vivo was demonstrated by the induction of IgE-potentiating factor in the sera of *B. pertussis*-vaccinated rats (11). The T-cell subset population T_h2 and its cytokine secretions, which include interleukin-4, have been shown to increase IgE production in vitro by enhancing B-cell-isotype switching to IgE (3). In a mouse model, simultaneous immunization with PT and antigen caused a rise in interleukin-4 and serum IgE (17). The dramatic rise in total serum IgE observed in all four strains here demonstrates the powerful IgE-enhancing effect of *B. pertussis* live organisms in the lungs and presumably reflects some of the IgE-specific enhancement mechanisms described above.

One of the characteristic features of pertussis is leukocytosis, which is evident in humans, mice, and rats. PT is responsible for this leukocytosis-promoting activity by causing leukocytes, particularly lymphocytes, to digress from their normal migratory route to lymph nodes and to persist in the blood (16, 18). This supports the hypothesis that the IgE adjuvant action of PT may be due to the depletion of lymphocytes (which would include suppressor populations) in spleen and lymph nodes, allowing IgE production to proceed unrestrained (27). Our comparison of rat strains following infection has given some conflicting results, namely, that leukocytosis occurred in the absence of an anti-PT response (Lewis and HL) and that increments in total IgE have occurred in the absence of significant leukocytosis (BN and HL). The rise in IgE without concomitant leukocytosis occurred in the groups more resistant to infection, as seen from their weight gains, and it is possible that the bacteria were eliminated from these animals before leukocyte levels were affected. However, even if PT is not being actively secreted, some lingering traces may have an effect on IgE levels, which are known to be sensitive to minute amounts of this toxin (19). It has been suggested that the symptoms of pertussis may not be due directly to live organisms but to the long-lasting effects of released toxins (25). That PT had eventually provided an antigenic stimulus in BN rats is borne out by the detectable IgG responses in some of these rats at 3 and 6 weeks after infection.

TABLE 3. Summary of responses[a] to *B. pertussis* infection in 6-week-old rats of different strains

Rat strain	Coughing (day 8)	Leukocytosis (day 8)	Wt gain (day 7)[b]	Anti-PT IgG (day 21)	Total IgE (day 8)	IgE elevation (day 8)
SD	+++	++	+	++	++	+++
BN	±	±	+	±	+++	+
HL	+	±	+	−	++	++
Lewis	±	+++	−	−	++	+++

[a] Except as indicated, responses are expressed on a scale of − to +++, where − is the lowest response and +++ is the highest response.
[b] +, positive for weight gain; −, negative for weight gain.

The IgG antibacterial responses to infection were measured by ELISA for up to 6 weeks after infection and generally developed with time (Table 2). The response to FHA was particularly strong and persistent, with some exceptionally high titers recorded for the SD rats at day 42. The tendency for the anti-*B. pertussis* whole-cell sonicate titers to be lower than those of antibodies to FHA, which was reported in our previous studies (6, 22), presumably reflects the heterogeneous mixture of antigens present in this preparation. With regard to the anti-PT responses, the wide variation in IgG responses in SD rats was also noted previously (6, 22). There was a conspicuous absence of an IgG response to PT in Lewis and HL rats. This suggests that some rat strains are unable to respond, or are poor responders, to PT, but this should be confirmed with larger numbers of rats of different strains. As PT is regarded as a major virulence factor in *B. pertussis*, the results highlight the interesting point that the greatest number of responders to PT (at day 21) were of the rat strain (SD) heard to cough most consistently. These strain differences presumably reflect genetic differences which may also occur in humans and contribute to variations in disease severity and vaccination responses in infants. However, the role of PT in cough production is uncertain. *B. parapertussis* and *B. pertussis* mutant strains lacking PT did not produce coughing in the rat model (22), whereas some human parapertussis patients have been reported to show coughing paroxysms similar to those in pertussis patients (10, 32).

A summary of some of the key responses to *B. pertussis* infection in 6-week-old rats is presented in Table 3. This study has highlighted that the ability to cough varies with the strain of rat, as does responsiveness to PT, both as an antigen and as a toxin, and that the normally concomitant symptoms of pertussis, namely, leukocytosis, retardation of weight gain, and a rise in total serum IgE, can occur independently.

B. pertussis-specific IgE antibodies were not detected in any of the four strains of rats during the 6-week period after infection. This was not due to interference from high levels of specific IgG antibodies already present, because their removal from representative sera did not alter the results of the specific IgE ELISA. There was, however, a reduction in the EA-specific IgE titer of the RAST reference preparation from 10,000 to 5,000, which implies that there was some contribution to the measurement of IgE titer by the IgG antibodies present. A rise in total IgE has been reported to occur in convalescent-phase human sera following whooping cough (28), and a low incidence of IgE antibodies to *B. pertussis* whole cells has been detected (31). IgE antibodies with specificity for *B. pertussis* whole cells and PT have been reported to be present in children after immunization (8, 9). Investigations with experimen-

tal animals often use pertussis vaccine as adjuvant when the subsequent IgE response to the accompanying antigen is measured. However, in two studies, Pauwels et al. (23), using RAST, detected IgE antibodies to various pertussis vaccines in BN rats, and Lindsay et al. (15), using purified components, induced low titers of mouse anti-FHA and anti-PT IgE measurable by passive cutaneous anaphylaxis. In the present study, IgE antibodies specific to *B. pertussis* whole-cell sonicate, FHA, or PT did not appear to contribute to the raised levels of circulating IgE in the serum of *B. pertussis*-infected rats.

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