

Differences in susceptibility to infection with *Treponema pallidum* (Nichols) between five strains of guinea pig

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SUMMARY Groups of 10 young male guinea pigs of inbred strains 2 and 13 and outbred strains Hartley A, Hartley B, and one deficient in the fourth component of complement (C4D) were infected intradermally with 80×10^6 *Treponema pallidum* (Nichols). The course of infection and production of antitreponemal antibody were examined. Strain C4D guinea pigs were the most susceptible to infection (100%); inbred strains 2 and 13 and outbred strain Hartley B showed 80-90% symptomatic infection; and the Hartley A strain was the least susceptible to infection (10%). Strain 13 animals responded with the highest antitreponemal antibody activity, and the Hartley A strain with the lowest. The results suggest that genetic factors or complement, or both, may influence the degree of susceptibility to infection with *T pallidum* in guinea pigs.

Introduction

Neither clinical investigations in man nor experimental studies in the rabbit have provided complete information on the nature of syphilis. The guinea pig, because it is less susceptible to infection with *Treponema pallidum* than the rabbit, has been neglected as an experimental model. Yet studies with this animal could be important for exploration of the immunopathological mechanisms of the disease.^{1,2}

The median infectious dose (ID_{50}) for outbred guinea pigs is about 0.1×10^6 organisms,¹ and similar results have been obtained with inbred strains 2 and 13.³ Pierce *et al* showed that when young male Hartley guinea pigs were infected with proper concentrations of *T pallidum* (Nichols) they responded with dark field positive lesions, production of treponemal IgG antibody, and histopathological changes in the lymph nodes and spleen.¹ The animals did not produce treponemal IgM¹ or cardiolipin antibodies.^{1,2}

In anticipation of using this animal model for studies of the genetic factors influencing susceptibility to infection and the course of the disease and of the role of complement in syphilis, we examined five

species of guinea pigs differing in genetic make up and complement activity. These strains were tested for their susceptibility to infection with *T pallidum*, and the course of infection was monitored. We report here that the strains differed appreciably in their susceptibility to infection with *T pallidum* (Nichols).

Materials and methods

ANIMALS

Inbred and outbred strains of guinea pigs were used in the study. Animals of inbred strains 2 and 13 were bought by our centre's animal production facility (Griffin Laboratory) from the National Institutes of Health (NIH) in the 1970s and have since been reared as a line bred colony. Outbred Hartley guinea pigs (Hartley A) have been reared in our institution as a closed colony since 1917. The colony was started by Caesarean section and bred at random. Brothers and sisters were mated during 1954-68, after which random breeding was resumed. Another Hartley strain (Hartley B), (bought from Buckberg Animal Farm, Tompkins Cove, New York, USA) is the strain used in previous studies.^{1,2} A strain deficient in the fourth component of complement (C4D), which was discovered and established at NIH,⁴ was obtained by Griffin Laboratory in 1975 and has since been bred at random.

All animals were male, weighed 300-400 g, and were housed individually in air conditioned quarters

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TABLE I *Development of lesions and antitreponemal antibody in guinea pigs infected with T pallidum (Nichols)*

Strain	Lesions			Antibody on day 17:		Maximum antibody titre:			
	No positive (n = 10)	Appeared on day:	Duration (days)	No positive (n = 10)*	Titres (range)	Single animal	At day:	Group mean	At day:
Hartley A	1	7	5	6	1/5-1/40	1/80	30	1/35	60
Inbred 13	8	7	11-30	9	1/5-1/80	1/320	60	1/220	90
Inbred 2	9	7-9	6-11	10	1/5-1/40	1/320	90	1/205	120
Hartley B	9	7-14	11-18	8	1/5-1/40	1/160	90	1/80	90
C4D	10	7-14	21-60	7	1/5-1/40	1/320	60	1/160	60

*Dilution 1:5.

C4D = Deficient in C4.

(18-22°C) and fed food free of antibiotics and water ad libitum.

INFECTION

T pallidum (Nichols) was obtained from the testes of infected Nys Flemish Giant (FG) rabbits killed with euthenasia agent T-61 (American Hoechst) at the peak of orchitis (10 days). The organisms were extracted into phosphate buffered saline (PBS) pH 7.2 and counted. All 50 guinea pigs (groups of 10 animals of each strain) were depilated in the pubic region and infected intradermally under general anaesthesia (Ketaset; Bristol Laboratories, Syracuse, New York, USA) with 80×10^6 organisms. During inoculation (about two hours for the 50 animals) the treponemal suspension was kept at room temperature and stirred. Single animals were selected alternately from the five groups for infection. The animals were examined daily for lesions, and blood was collected to measure antibody at regular intervals from day 17 of infection.

FLUORESCENT TREPONEMAL ANTIBODY TEST

Heat inactivated serum samples were initially diluted (1:5) in a sorbent, and further dilutions were made in PBS. Serum samples were applied to commercially prepared Treposlides (Beckman Instruments) and incubated in a humidified chamber at 37°C for 30 minutes. After washing in PBS, appropriately diluted fluorescein isothiocyanate conjugated rabbit antiserum to guinea pig IgG was applied to the slides,¹ and they were reincubated for 30 minutes at 37°C. After another washing the slides were dried, mounted, and examined under a Nikkon Optiphot microscope equipped with interference filters, xenon illumination, and 10 × ocular and 40 × dry objective lenses. The end point titre was the last dilution to give 1+ reactivity.

CARDIOLIPIN ANTIBODIES

Selected serum samples from all five strains were examined for cardiolipin antibodies by the Venereal Disease Research Laboratory (VDRL) test.⁵

Results

Table I summarises the course of infection and antibody response. For six days no change was seen at the site of infection, excluding the possibility of a contaminated inoculum. On day 7 an erythematous area of about 5 mm in diameter was seen in most animals. In time it developed (except in the Hartley A strain) to a definite induration, which exceeded 15 mm in diameter in some animals of the C4D strain. Table II shows the size and course of lesions in six C4D animals.

Central necrosis occurred in almost all lesions, especially during the regression phase, which varied in different animals. Figures 1 and 2 show lesions in animals of four strains. The duration of the lesions varied: it was longest (21 to 60 days) in the C4D animals and shortest (five days) in the single Hartley A animal. The percentage of symptomatic infection was also highest (100%) in the C4D guinea pigs and lowest (10%) in the Hartley A. Dermal lesions were examined by dark field microscopy in at least three animals of each group except Hartley A. All lesions contained motile *T pallidum* micro-organisms, which appeared to be slightly more slender than those from rabbit orchitic fluid. During the course of infection the animals did not have raised temperatures, and their increase in body weight was similar to that of control non-infected animals of corresponding age

TABLE II *Size and course of lesions in six* C4D (deficient in C4) guinea pigs*

Animal No	Appeared on day:	Maximum		Disappeared on day:
		Size (mm)	On day:	
41	7	about 15	23	47
42	14	about 15	37	50
45	9	about 12	37	40
46	7	about 10	30	60
49	7	about 15	37	50
50	14	about 8	37	60

*Lesions of four of 10 animals were used for dark field examination and are not included here.

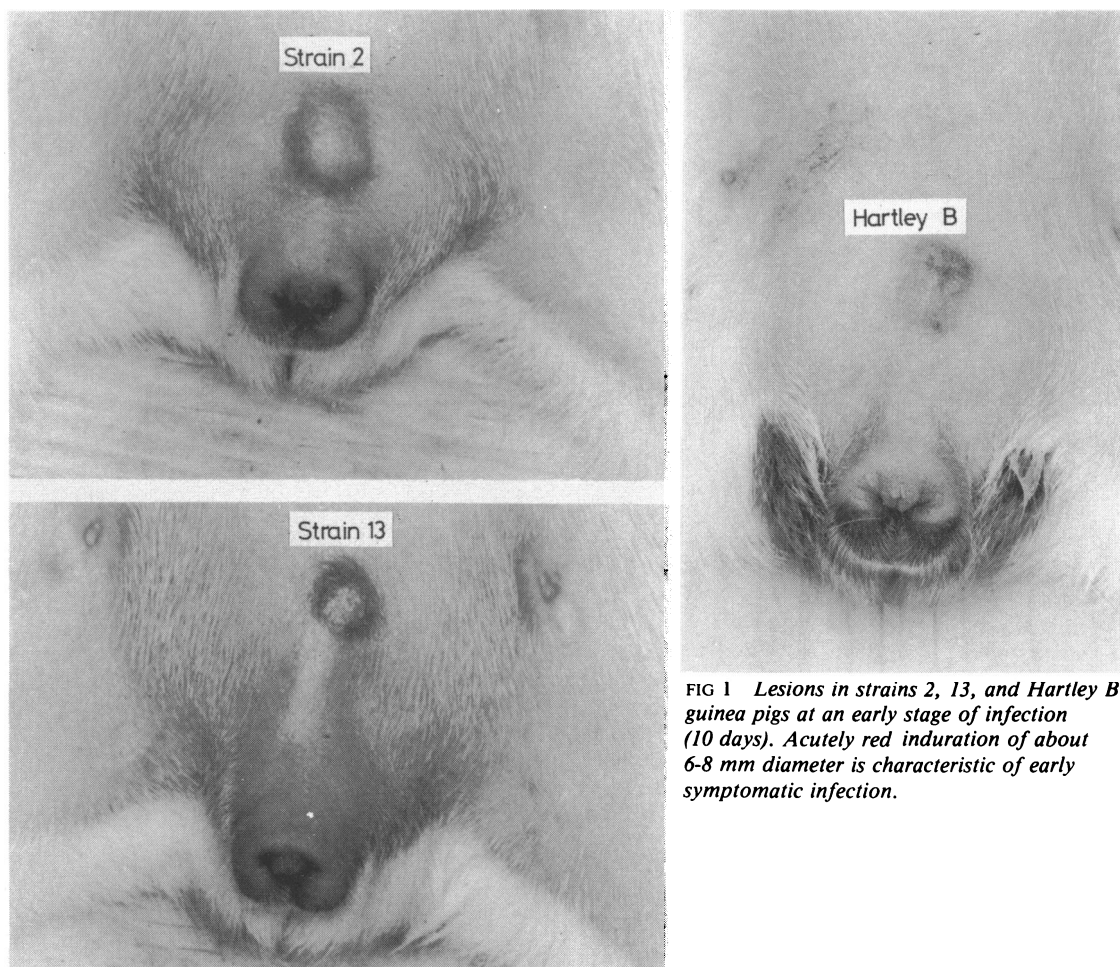


FIG 1 Lesions in strains 2, 13, and Hartley B guinea pigs at an early stage of infection (10 days). Acutely red induration of about 6-8 mm diameter is characteristic of early symptomatic infection.

groups. The regional lymph nodes were not enlarged on palpation.

Blood samples were taken from all animals on day 17 after infection and tested for antitreponemal antibodies (table I). Four animals from each group with detectable antibodies were selected for quantitative serological studies (tables I and III). The maximum titre in individual animals was reached by day 30 in the Hartley A strain and by day 90 in strains 2 and Hartley B. The highest group mean titres were found in the inbred strains 13 and 2, and the lowest in Hartley A. Antibody persisted for long periods in representative animals of each strain examined for seven months. In some instances (animals 5, 18, 22, and 43) the antibody fluctuated, suggesting a continuous but variable antigenic stimulus. Serum

samples examined for cardiolipin antibodies gave negative results, which confirmed our earlier results with outbred guinea pigs.^{1 2}

Discussion

During the past few years research on experimental syphilis has been leaning more towards molecular description of *T pallidum* and recombinant DNA. Though newer technology may offer more excitement and is theoretically very promising, we may have to wait a long time for the practical results of these approaches. Meanwhile syphilis maintains an unhealthy fluctuation of incidence and remains high on the list of notifiable venereal diseases (third in the

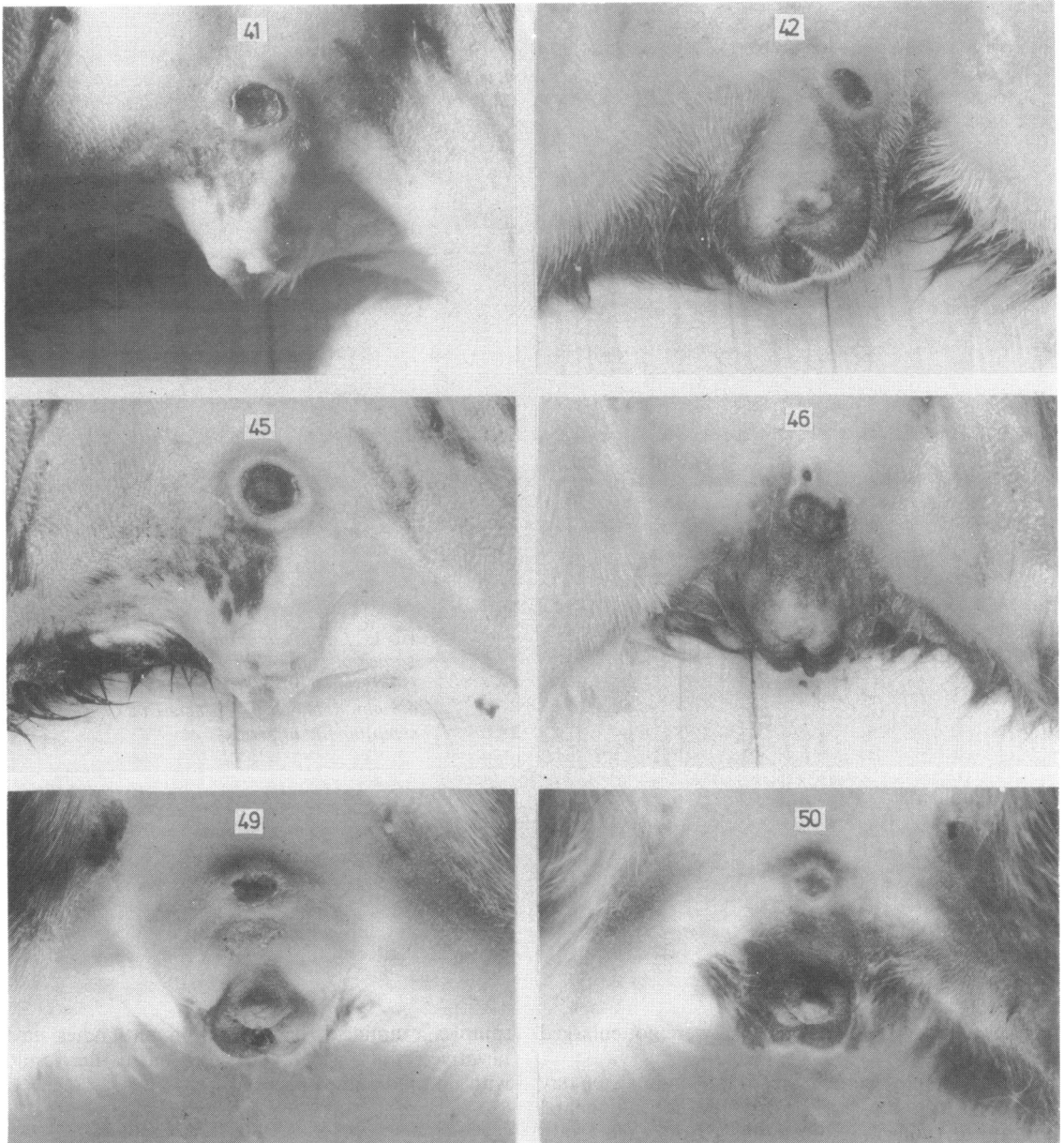


FIG 2 Lesions in six C4D (deficient in C4) guinea pigs at a later stage of infection. Individual differences were seen in the development of lesions and in the healing process (see table III). Most of these photographs were taken when the lesions were at maximum size.

United States of America). All efforts should be made to learn more about this disease.

The rabbit has not yet been exhausted as a model for syphilis, but it has serious limitations. One is the difficulty of dissecting the specific from the non-

specific responses because the rabbit produces both cardiolipin antibodies and antibodies to various non-pathogenic treponemes.⁶ Another is that in rabbits the *T pallidum* micro-organisms hide in an envelope of rabbit proteins, thus presenting a difficult target

TABLE III Kinetics of antitreponemal antibody production in guinea pigs infected with *T pallidum*

Guinea pig		Titre on day:						
Strain	No	17	30	60	90	120	150	210
Hartley A	22	1/40	1/80	1/80	1/80	1/40	1/20	1/40
	25	1/10	1/10	1/20	1/20	1/20	1/20	Died
	26	1/10	1/20	1/20	1/20	1/20	1/10	1/10
	28	1/5	1/20	1/20	1/20	1/20	1/10	1/10
Inbred 13	31	1/40	1/160	1/160	1/80	1/160	1/160	1/40
	34	1/20	1/160	1/160	1/160	1/80	Died	
	38	1/10	1/160	1/160	1/320	1/160	1/40	1/20
	40	1/80	1/160	1/320	1/320	1/320	1/80	1/20
Inbred 2	11	1/10	1/20	1/20	1/40	1/20	1/20	Died
	12	1/20	1/40	1/40	1/80	1/160	1/40	1/20
	18	1/40	1/80	1/160	1/160	1/320	1/160	1/160
	20	1/40	1/80	1/160	1/320	1/320	1/80	Died
Hartley B	3	1/20	1/40	1/40	1/40	1/40	1/40	1/40
	5	1/20	1/80	1/80	1/160	1/160	1/40	1/160
	7	1/5	1/40	1/40	1/80	1/80	1/80	1/80
	10	1/40	1/40	1/40	1/40	1/20	1/10	1/10
C4D	41	1/20	1/160	1/320	1/160	1/80	1/80	Died
	43	1/40	1/80	1/80	1/80	1/40	1/40	1/80
	46	1/20	1/160	1/160	1/80	1/40	1/20	1/40
	47	1/10	1/80	1/80	1/160	1/160	1/80	1/40

for the host immune mechanism. The guinea pig, on the other hand, produces only antitreponemal IgG antibodies,¹ which are not affected by absorption with intact *T phagedenis* biotype Reiter (Pierce CS, Wicher K, unpublished observation), and it mounts a specific T cell response to *T pallidum*.⁷ This animal thus lends itself to studies of immune responses, as well as of genetic factors of the host's susceptibility to disease.

Our results with Hartley B guinea pigs confirmed the percentage (87.5%) of symptomatic infections previously reported in these animals.¹ In contrast, the outbred Hartley A animals, which have been reared in our laboratory as a closed colony since 1917, showed a pronounced resistance to infection. The susceptibility of the inbred strains 2 and 13 was similar to that of Hartley B. The C4D animals were the most susceptible and produced the largest lesions, which lasted for two months. Whether the heightened susceptibility of this strain to syphilitic infection is due to the lack of C4 alone or to other genetic characteristics as well has still to be clarified.

Complement activity (total haemolytic activity) (192 to 215 U/ml) was identical in age matched non-infected guinea pigs of strains 2 and Hartley A. Serum samples from non-infected C4D guinea pigs had no detectable haemolytic activity (<10 U/ml). The lack of susceptibility to infection in the Hartley A animals may therefore not be attributable to their high complement activity. In the C4D animals, however, the severity and duration of infection may be related to complement. This strain has defects in a number of immunological functions, such as diminished opsonic and bactericidal activity, which

require sequential activation of early components in the classic pathway.⁸

As previously described, the humoral response in all five strains was restricted to production of anti-treponemal antibodies, with a total lack of anti-cardiolipin antibodies.^{1,2} It is generally assumed that cardiolipin antibodies in an infected host reflect tissue damage and are therefore not produced in animal species with little or no susceptibility to infection.^{9,10} Our results do not support this assumption, however, as neither resistant (Hartley A) nor highly susceptible (C4D) animals produced anti-cardiolipin antibodies. For reasons at present unknown, lipids do not seem to be immunogenic for the guinea pig. Several protocols of immunisation used previously by Pierce with susceptible Hartley B guinea pigs failed to produce antibodies to lipids.¹ By using immune complexes consisting of rabbit cardiolipin antibodies and VDRL antigen we were recently able to obtain a very low titre of antibodies reacting with VDRL antigen, but this was achieved in only one of 10 Hartley A guinea pigs after 10 intradermal injections. A rabbit used as a control had a higher anticardiolipin titre after three injections (Wicher V, Wicher K, unpublished observation). The resistance of guinea pigs to the production of cardiolipin antibodies is a welcome characteristic, as in experimental infection with *T pallidum* the animals mount an immune response to the causative organism only.

Our previous studies and that reported here provide supportive evidence that the guinea pig is a useful model for the investigation of syphilis. This animal offers the opportunity to study, not only specific immune responses, but also genetic factors

and the role of complement in the host's susceptibility to infection.

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