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Natural antibodies to treponemal antigens in four strains of guinea-pigs

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SUMMARY

A total of 185 serum samples obtained from healthy male and female guinea-pigs of inbred strains 2 and 13 and outbred strains C4D and Hartley A were examined for natural antibodies to treponemal antigens by ELISA using *Treponema pallidum* (TP), *T. phagedenis* biotype Reiter (TR) and *T. vincentii* (TV) antigens and by the FTA test. The prevalence and titres of natural antibodies depended on the age and strain of guinea-pig and the treponemal antigen used. One- and 7-day-old guinea-pigs contained significantly ($P < 0{\cdot}001$) higher levels of natural antibodies than did animals 1 or 3–6 months old. The similar high levels of natural antibodies in newborn guinea-pigs and their mothers (12–30 months old) and the sharp drop observed at the age of 1 month suggested maternal transfer as the mechanism of acquisition. In young adults 3–6 months old, the age group most susceptible to TP infection, antibodies to TP and TR were at their lowest levels, but antibodies reacting to TV had already begun to rise. Natural antibodies were of the IgG1 and IgG2 but not of the IgM class. The highest levels of natural antibodies were in the C4D guinea-pigs; the lowest were in the Hartley A strain. Natural antibody activity was inhibited or adsorbed by TR antigens.

INTRODUCTION

Natural antibodies, defined as immunoglobulins reactive with antigens that were not introduced into the host by a clinically apparent or experimental infection, are found in sera from many healthy animals and man (Boyden, 1966; Wilson & Miles, 1975). The most commonly occurring natural antibodies are those reactive with erythrocyte antigens and with various microorganisms in the gastrointestinal and upper respiratory tracts, genitalia and skin. These antibodies are characterized by a greater cross-reactivity and lower titres than immune antibodies.

Production of natural antibodies seems to be one of the physiological mechanisms by which mammals acquire immunity against environmental microorganisms. Natural treponemal antibodies have been reported in man (Baker-Zander *et al.*, 1985); Hensel, Wellensiek & Bhakdi, 1985) and in rabbits (Hensel *et al.*, 1985; Wicher, Wos & Wicher, 1986). The antigenic specificities and possible immunological role of these antibodies have been investigated (Fribourg-Blanc, 1956; Hederstedt, 1976; Blanco *et al.*, 1985).

Guinea-pigs have emerged as a useful alternative model for studies of the immunopathology of syphilis. Since various strains differ in their susceptibility to *Treponema pallidum* (TP) infection (Wicher, Wicher & Gruhn, 1985), it was of interest to determine the levels of natural treponemal antibodies in four strains of this species.

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MATERIALS AND METHODS

Animals and bleedings

Guinea-pig inbred strains 2 and 13, an outbred strain deficient in the fourth complement component (C4D), and the outbred Hartley A (Albany) strain have been described elsewhere (Wicher *et al.*, 1985). Because young adult guinea-pigs are potentially more susceptible to infection with TP than old animals (unpublished observation), we examined sera from male and female animals of different ages, from 1 day to 30 months, including groups of mothers and their offspring. Each family, consisting of a mother and a litter of two to four newborns, were kept together for 1–7 days after parturition. Otherwise, the animals were housed in groups of two in air-conditioned quarters (18–22°) and fed antibiotic-free food and water *ad libitum*.

The animals were bled via cardiac puncture under sedation (Kitaset; Bristol Laboratories, Syracuse, NY), and the 1- or 7-day-old guinea-pigs were subsequently killed with euthanasia agent T-61 (American Hoechst, Sommerville, NJ). The sera were separated and kept at −20° until use.

Serological tests

Enzyme-linked immunosorbent assay (ELISA). ELISA was

carried out in Immulon I flat-bottomed microplates (Dynatech, Alexandria, VA) according to the procedure of Voller, Bidwell & Bartlett (1980). Briefly, 0·1 ml each of well-washed, sonicated TP, *T. phagedenis* biotype Reiter (TR) and *T. vincentii* (TV) (5, 3 and 3 μg/ml, respectively) in carbonate buffer was used to coat the microplates overnight at 4°. Test samples and positive and negative controls were diluted in PBS–Tween and applied in duplicate wells in a 0·1-ml volume. After 2 hr incubation, the wells were washed with PBS–Tween and incubated with 0·1 ml of alkaline phosphatase-conjugated rabbit anti-guinea-pig IgG antiserum (Miles Laboratories, Naperville, IL) at the optimal 1:1000 dilution. After 2 hr incubation the plates were washed, and 0·1 ml of *p*-nitrophenylphosphate (Sigma 104; Sigma Chemical Company, St Louis, MO) at a concentration of 1 mg/ml in 10% diethanolamine buffer was added. After an additional 30 min incubation, the reaction was stopped with 50 μl of 3 N NaOH, and the absorbance was determined at 410 nm in a Microelisa Minireader (Dynatech). An absorbance of ≥0·2, which is two SD above the mean determined for 20 non-reactive sera, was considered positive. The end-point titre therefore was the reciprocal of the last serum dilution giving an absorbance of ≥0·2.

ELISA inhibition test. The ELISA inhibition test was performed as described above but with serum diluted in commercial sorbent used for the FTA-Abs test (Clinical Sciences Inc., Whippany, NJ). This sorbent contains antigens obtained from a TR culture (Hunter, Creighton & Lewis, 1970).

Fluorescent treponemal antibody test (FTA). The FTA test was done as previously described (Wicher & Wicher, 1983). The end-point titre was the last serum dilution to give 1+ fluorescent staining.

FTA-sandwich technique. In order to determine the immunoglobulin class of the natural antibodies, the FTA-sandwich technique with monospecific rabbit antisera was applied. TP was incubated with serum diluted in PBS. The slides were then washed and incubated with monospecific rabbit antisera to IgG1, IgG2 and IgM (Miles Laboratories) at a 1:50 dilution for the two IgG and a 1:30 dilution for the IgM antisera. The slides were washed, and fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin antiserum was applied, incubated and processed as for the FTA test.

FTA inhibition test. The FTA inhibition test was performed as the FTA test but with guinea-pig sera diluted in commercial sorbent (Clinical Sciences).

Sepharose 4B immunoadsorption. Sonicated TR (8 mg/ml) was coupled onto CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's instructions, and sera were adsorbed as reported previously (Wos & Wicher, 1986).

Statistics

Results were statistically evaluated by Student's *t*-test.

RESULTS

A total of 185 sera obtained from 100 male and 85 female guinea-pigs at various ages were examined by ELISA for natural antibodies to TP, TR and TV. Most of the animals had natural anti-treponemal antibodies, but there were sharp differences in titre depending on the age and strain of guinea-pig and the antigen used (Table 1, Fig. 1). Sera of guinea-pigs 1 or 7 days old and 12–30 months old contained significantly ($P < 0{\cdot}001$) more antibodies than did sera from animals 1 or 3–6 months old. At 3–6 months strains 2 and 13 had similar levels of natural antibodies. Titres for strain 2 at 9–11 months (the only group tested at this age) and 12–30 months were not significantly different (Table 1). No differences in titre were observed between males and females within any strain. In strain 13 only 3- to 6-month-old animals were available.

Table 1. Prevalance of natural treponemal antibodies determined by ELISA in four strains of guinea-pigs at various ages

Guinea-pig strain	Age	Sex (M/F)	Titre of antibodies to (mean ± SD) TP	TR	TV
C4D	1 day	2/3	72 ± 18	1696 ± 1195	416 ± 214
	7 days	4/1	80 ± 46	1280 ± 905	800 ± 320
	30 days	3/2	18 ± 4	176 ± 131	56 ± 22
	3–6 months	17/8	5 ± 5	49 ± 48	150 ± 174
	12–30 months	4/6	86 ± 48	1580 ± 987	791 ± 742
Hartley A	1 day	3/2	7 ± 3	448 ± 175	128 ± 44
	7 days	4/1	7 ± 4	640 ± 0	88 ± 44
	30 days	2/3	< 5	40 ± 0	24 ± 15
	3–6 months	15/10	< 5	10 ± 9	37 ± 23
	12–30 months	5/5	6 ± 5	293 ± 273	124 ± 81
2	1 day	1/4	40 ± 0	904 + 557	360 ± 174
	7 days	3/2	49 ± 31	1280 ± 0	460 ± 472
	30 days	4/1	5 ± 4	145 ± 93	43 ± 27
	3–6 months	10/15	< 5	18 ± 14	61 ± 37
	9–11 months	5/5	53 + 45	737 ± 511	601 ± 487
	12–30 months	5/5	38 ± 36	842 ± 522	669 ± 416
13	3–6 months	13/12	< 5	45 ± 43	58 ± 38

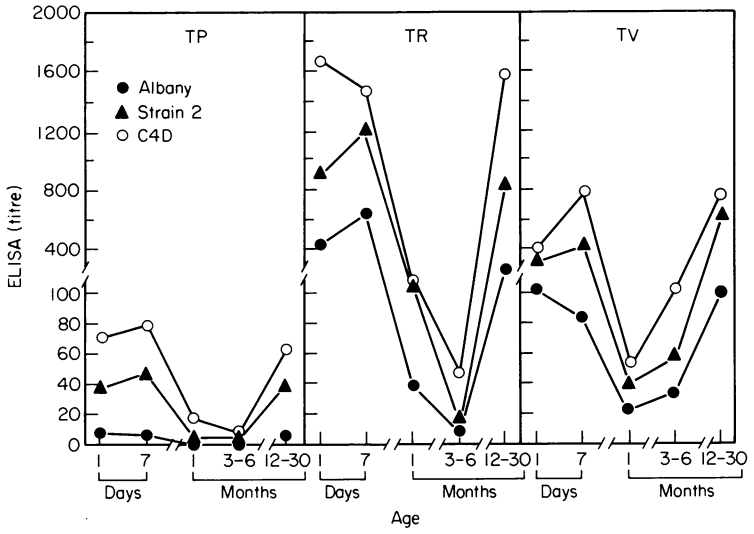

Figure 1. Mean ELISA titres of natural antibodies to TP, TR and TV in three strains of guinea-pigs. All titres dropped sharply during the first month of life and reached their lowest values (for TP and TR) at 3–6 months, when antibodies to TV were already on the rise. 'Albany' designates the Hartley A strain.

Table 2. ELISA titres of natural treponemal antibodies in representative guinea-pig families

				Titre to:		
Strain	Guinea-pig	Age	Sex	TP	TR	TV
C4D	Mother	18 months		80	2560	640
	Offspring 1	1 day	F	80	2560	320
	2	1 day	M	80	2560	640
	Mother	12 months		160	2560	1280
	Offspring 1	7 days	M	160	2560	1280
	2	7 days	M	160	1280	640
Hartley A	Mother	27 months		10	640	80
	Offspring 1	1 day	M	10	640	80
	2	1 day	M	10	640	80
	Mother	21 months		10	640	80
	Offspring 1	7 days	M	10	640	80
	2	7 days	M	10	640	80
	3	7 days	M	10	640	80
	4	7 days	F	5	640	80
2	Mother	24 months		40	1280	320
	Offspring 1	1 day	F	40	1280	320
	2	1 day	F	40	1280	320
	3	1 day	F	40	1280	160
	Mother	12 months		80	1280	80
	Offspring 1	7 days	M	80	1280	80
	2	7 days	M	80	1280	80
	3	7 days	M	40	1280	80
	4	7 days	F	40	1280	160

The highest antibody titres were consistently detected in the C4D animals and the lowest in the Hartley A (Table 1, Fig. 1). Titres to TR were usually the highest, followed by titres to TV and TP. The exception was the group 3–6 months old, where antibody to TV was already on the rise and higher than antibody to TR.

ELISA titres for representative families, consisting of a mother and litters of two to four offspring, are shown in Table 2.

Since 3–6 months is the optimal age range in our laboratory for experimental syphilis infection of guinea-pigs, we analysed additional samples from this age group by ELISA and FTA (Table 3). While practically all animals examined by ELISA

Table 3. Incidence and titres of natural treponemal antibodies in young adult (age 3–6 months) guinea-pigs by ELISA and FTA test

			ELISA		FTA	
Guinea-pig strain	No. animals	Antigen	Animals positive (%)	Titres	Animals positive (%)	Titres
C4D	25	TP	48	5–40	88	5–40
		TR	100	20–160		
		TV	100	20–640		
Hartley A	25	TP	0	<5	36	5–10
		TR	88	5–40		
		TV	100	10–80		
2	25	TP	20	5–10	64	5–20
		TR	92	5–40		
		TV	96	20–160		
13	25	TP	20	5–10	68	5–20
		TR	88	5–80		
		TV	100	20–160		

contained antibodies reacting with TV and TR, the percentage of seropositivity for TP varied by strain: 48% in C4D animals, 20% in strains 2 and 13, 0% in Hartley A. The FTA test revealed a much higher prevalence of natural antibodies reacting with TP in all four strains, but differences in seropositivity among strains were still noticeable, particularly between Hartley A 36% and C4D (88%) ($P<0{\cdot}001$). No significant difference was found between strains 2 and 13.

Natural antibodies in titres up to 160 could be readily inhibited by the commercial sorbent used to dilute the sera. Higher titres, as found in newborns and adult guinea-pigs, required additional adsorption with CNBr-Sepharose-coupled TR for total removal of the antibodies (data not shown). FTA tests of randomly selected sera of all four strains with monospecific antibodies demonstrated that the natural treponemal antibodies are IgG1 and IgG2. No IgM was detected (data not shown). We could not assay for IgA, as no reliable antiserum specific for IgA was available.

DISCUSSION

Natural treponemal antibodies detectable by ELISA and the FTA test were demonstrated in sera from healthy guinea-pigs of inbred strains 2 and 13 and outbred strains C4D and Hartley A. Although antibodies to TP and TR have been found in normal humans (Baker-Zander *et al.*, 1985; Hensel *et al.*, 1985) and rabbits (Hensel *et al.*, 1985; Wicher *et al.*, 1986), this is to our knowledge the first comprehensive study of males and females of various age groups in a single animal species, examined with antigens obtained from one pathogenic and two non-pathogenic treponemes.

In the present study, unusually high titres of natural antibodies were found for 1- and 7-day-old guinea-pigs, closely resembling the titres for the mothers. These high titres and their sharp drop after 1 month of age strongly suggest maternal transfer (via placenta or colostrum) to the newborn, whereas in adults colonization with spirochetes is the likely source of natural sensitization. The almost complete disappearance of the acquired maternal natural antibodies after 1 month (Fig. 1) is in agreement with the short half-life of IgG, which is 5–6 days in the guinea-pig (Schultze & Heremans, 1966).

All natural antibodies, regardless of their concentration in the serum, were inhibited or totally absorbed by TR antigen in the commercial sorbent or coupled to CNBr-Sepharose. This inhibition—and the fact that in most age groups the greatest seroreactivity was to TR—indicates that, whichever species of spirochetes colonize the guinea-pigs, the spirochetes exhibit a great deal of cross-reactivity with TR.

Detection of seropositivity to TP depended on the technique employed, as indicated by the higher sensitivity of the FTA test than ELISA-TP for young adults (Table 3). We may speculate that the natural antibodies have greater affinity for surface treponemal antigens in the FTA test than for the wider spectrum of cytoplasmic and surface antigens in the sonicated TP preparation used in ELISA.

Although maternal IgA treponemal antibodies might be present in the colostrum and transmitted to the 1- and 7-day-old guinea-pigs, we did not have a reliable monospecific antiserum to IgA and could not search for this class.

Results obtained with monospecific antibodies to IgM and subclasses of IgG in the FTA test confirmed our previous observations of the lack of IgM treponemal antibodies (Wicher, Wicher & Wang, 1976; Pierce, Wicher & Nakeeb, 1983; Wicher *et al.*, 1985). These results also indicated that in guinea-pigs both the immune (Wicher *et al.*, 1985) and natural treponemal antibodies are IgG1 and IgG2. This finding, together with the previous observation that immune guinea-pig serum contains a large amount of cross-reacting treponemal antibodies (Wicher & Wicher, 1985), suggests that the humoral response to syphilitic infection is for the most part an expansion of naturally presensitized clones. We do not yet know whether natural treponemal antibodies play a role in natural resistance to infection with TP. Immobilizing (Hederstedt, 1976) and treponemicidal (Blanco *et al.*, 1985) antibodies have been reported in

normal human sera, but their correlation with susceptibility to infection has not been established.

In this study, the lowest prevalence and titres of natural antibodies to TP and TR were found in the young adults, which for many years have been the age group of choice for experimental syphilis infection in our laboratory. In all five strains of guinea-pigs studied thus far, animals of this age have been the most susceptible to TP infection and have provided the most reproducible results. This empirical observation is under further investigation in our laboratory. Paradoxically, the highest prevalence and titres of natural treponemal antibodies at all ages, including the young adult group, were found amount the C4D animals, which are the most susceptible to TP infection (Wicher *et al.*, 1985). Conversely, the highly resistant Hartley A strain showed the lowest prevalence and titres of natural antibodies to nonpathogenic treponemes, and reacted weakly or not at all with TP.

Based on these results, we may assume some association between the levels of natural antibodies and the severity of syphilitic infection. Boyden (1966) speculated that natural antibodies play an important role as recognition factors in the afferent arm of the immune response. Interaction with particulate antigens renders them attractive to the phagocytic cells, and formation of immune complexes brings about the release of chemotactic substances, which in turn attracts more phagocytic cells to the site of the inflammatory reaction.

Since biological activity does not necessarily correlate with quantity, other immunological properties of the natural treponemal antibodies in the highly susceptible and resistant experimental models remain to be explored.

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REFERENCES

Baker-Zander S.A., Hook E.W., Bonin P., Handsfield H.H. & Lukchart S.A. (1985) Antigens of *Treponema pallidum* recognized by IgG and IgM antibodies during syphilis in humans. *J. infect. Dis.* **151,** 264.

Blanco D.R., Radolf J.D., Lovett M.A. & Miller J.N. (1985) Treponemicidal activity and Western blot analysis of normal human sera. *Abstracts of the Annual Meeting of the American Society for Microbiology*, **E83,** 88.

Boyden S.V. (1966) Natural antibodies and the immune response. *Adv. Immunol.* **5,** 1.

Fribourg-Blanc A. (1956) Le pouvoir treponemicide natural du serum humain. *Presse Med.* **64,** 1396.

Hederstedt B. (1976) Studies on the *Treponema pallidum* immobilizing activity in normal human serum. III. The kinetics of the immobilization reaction of normal and immune sera. *Acta Path. Microbiol. Scand. Sect. C*, **84,** 142.

Hensel U., Wellensiek H.J. & Bhakdi S. (1985) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting as a serological tool in the diagnosis of syphilitic infections. *J. clin. Microbiol.* **21,** 82.

Hunter E.F., Creighton E.T. & Lewis J.S. (1970) An improved antigen for the FTA-ABS test. *Health Lab. Sci.* **7,** 237.

Pierce C.S., Wicher K. & Nakeeb S. (1983) Experimental syphilis: guinea pig model. *Br. J. vener. Dis.* **59,** 157.

Schultze H.E. & Heremans J.F. (1966) *Molecular Biology of Human Proteins, With Special Reference to Plasma Proteins*, Vol. 1, p. 479. Elsevier, New York.

Voller A., Bidwell D. & Bartlett A. (1980) In: *Manual of Clinical Immunology* (eds N. R. Rose and H. Friedman), 2nd edn. p. 359. American Society of Microbiology, Washington.

Wicher K. & Wicher V. (1985) Immunoblotting analysis of sera from *Treponema pallidum*-infected guinea pigs. *Abstract of the Annual Meeting of the American Society for Microbiology*, **B204,** 52.

Wicher K., Wicher V. & Gruhn R.F. (1985) Differences in susceptibility to infection with *Treponema pallidum* (Nichols) between five strains of guinea pig. *Genitourin. Med.* **61,** 21.

Wicher K., Wicher, V. & Wang M.C.C. (1976) Cellular and humoral immune response of guinea pig infected with *Treponema pallidum*. *Int. Arch. Allergy appl. Immun.* **51,** 284.

Wicher K., Wos S.M. & Wicher V. (1986) Kinetics of antibody responses to polypeptides of pathogenic and nonpathogenic treponemes in experimental syphilis. *Sex Trans. Dis.* (in press).

Wicher V. & Wicher K. (1983) Studies of rabbit testes infected with *Treponema pallidum*. II. Local synthesis of antibodies. *Br. J. vener. Dis.* **59,** 359.

Wilson G.S. & Miles A.A. (1975) The natural antibodies: their nature, origin and behaviour. In: *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, 6th ed, Vol. 2, p. 1419. Williams & Wilkins, Baltimore.

Wos S.M. & Wicher K. (1986) Extensive cross-reactivity between *Treponema pallidum* and cultivable treponemes demonstrated by sequential immunoadsorption. *Int. Archs. Allergy appl. Immunol.* **79,** 282.