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NOTES

Treponema pallidum-Immobilizing Antibodies in Guinea Pig Experimental Syphilis

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***Treponema pallidum*-immobilizing (TPI) antibodies were examined in intradermally infected inbred strain 13 and adoptively immune inbred strain 2 guinea pigs. Both strains of animals produced TPI antibodies at or after 90 days of infection. TPI antibodies were not associated with the protective mechanism(s) operative after challenge in adoptively immune animals.**

Lack of a readily available inbred strain of rabbits has been a stumbling block in our understanding of the effector mechanisms operative in the process of immunity in syphilis. We have shown that *Treponema pallidum* infection in an inbred strain of guinea pigs, although milder than in rabbits, mimics critical aspects of the early stage of venereal syphilis in humans when carried out under optimal conditions (15). A number of basic parameters such as median infective dose, clinical course of infection, and humoral and cellular responses have been explored in different strains of guinea pigs (for a review, see reference 13). However, the production of *T. pallidum*-immobilizing (TPI) antibodies and their potential contributory role in recipients of chancre-immune T lymphocytes after *T. pallidum* challenge have not been examined in the inbred guinea pig model. This report presents information on the kinetics of the TPI-antibody response in inbred strain 13 and explores its role in adoptively immune inbred strain 2 guinea pigs.

Young adult male guinea pigs (300 to 400 g) of inbred strains 2 and 13 were used in the experiments. The original source of these animals and housing conditions have been previously reported (14). Manipulations such as infection, trial bleedings, and lesion biopsies were done with animals under general anesthesia by using Ketaset (Bristol Laboratories, Syracuse, N.Y.). Strain 13 animals were infected intradermally (i.d.) in the depilated pubic region with ca. 8×10^7 *T. pallidum* subsp. *pallidum* in 0.1 ml as described earlier (14). They were examined daily for lesions, and blood was collected at regular intervals for serological and immunochemical examinations. Four groups of inbred strain 2 guinea pigs were infused with 10^8 purified T cells prepared from chancre-immune (CI), *Treponema phagedenis* Reiter-immune (TRI), or *T. pallidum*-free inflammatory testicular fluid-immune (ITFI) animals or T cells from normal guinea pigs (NGP). All animals were infected i.d. with 10^8 organisms 24 h later. For the present study, we used sera from 4 of 11 adoptively immune animals that did not develop lesions for at least 6 months postinfection (for details, see reference 16). Observations of the clinical course of infection and bleedings were done as described for strain 13.

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T. pallidum immobilization test was performed as described previously (2, 11) with minor modifications. Briefly, treponemal suspensions with ≥96% active motility were combined with heat-inactivated (56°C for 30 min) test serum and unheated, undiluted guinea pig serum as a source of complement; each serum was tested in duplicate. As a complement control, inactivated guinea pig serum (56°C for 30 min) was added to the treponeme-serum mixture. Incubation was carried out for 16 h at 34°C in an atmosphere of 95% N_2 and 5% CO_2 and was followed by dark-field examination to determine the percentage of actively motile organisms on the basis of 25 randomly observed treponemes in the test (with complement) and control (without complement) tubes. If the percent difference in motility between the test and control tubes was ≥50%, the serum was considered to have immobilizing antibody and was called reactive. Differences between 21 and 49% indicated minimal but definitive immobilizing antibody and were called weakly reactive. Differences of ≤20% indicated the absence of immobilizing antibody and were called nonreactive. If discrepancies occurred between duplicate test tubes ranging from nonreactive to weakly reactive or reactive, the results were considered inconclusive. All assays were performed on coded samples.

Fluorescent treponemal antibody absorption test (FTA-ABS) was done with heat-inactivated sera appropriately diluted in commercial sorbent as previously described (14).

Immunoblotting was done by electrophoretic separation of treponemal antigen in 12% polyacrylamide gel in a discontinuous Tris-glycine system (5) in a Mini-Protean II slab cell (Bio-Rad Laboratories, Richmond, Calif.). To each well, 20 μl of antigen (1 mg/ml) was applied. The separated proteins were then electrophoretically transferred (9) onto 20-μm-pore-size nitrocellulose paper (Schleicher & Schuell, Inc., Keene, N.H.) by using a Mini Trans Blot cell with 25 mM Tris, 192 mM glycine, and 20% methanol buffer (pH 8.3) run at 100 V for 1 h. The guinea pig sera were used at a 1:20 dilution. Bound antibodies were identified with horseradish peroxidase-conjugated protein A (Zymed Laboratories, Inc., San Francisco, Calif.) with 4-chloro-1-naphthol (Bio-Rad) as indicator and H_2O_2 as substrate. Molecular weight standards

TABLE 1. Treponemal antibodies in adoptively immune and unprotected control guinea pigs of strain 2 after challenge with *T. pallidum*

Type of infused T cells	No. of animals	Bleeding day	FTA-ABS results[a]	No. of animals with immobilization test result[b]			
				NR	INC	WR	R
CI	4	30	<10	4			
		60	35	4			
		90	60	4			
		120	100			3	1
TRI	3	30	67	3			
		60	160	2	1		
		90	427			3	
		120	640			1	2
ITFI	2	30	60	2			
		60	240	2			
		90	480	1		1	
		120	640	1		1	
NGP	4	30	45	ND			
		60	120	2		2	
		90	280	2			2
		120	400	1		1	2

[a] Group mean titer.
[b] Result by *T. pallidum* immobilization test. NR, Nonreactive; INC, inconclusive results; WR, weakly reactive; R, reactive; ND, not determined.

(Bio-Rad) were run in parallel, and M_rs of polypeptides were calculated (12).

Of 20 strain 13 guinea pigs, 18 developed dark-field-positive lesions between 7 and 10 days. Twelve guinea pigs demonstrated progressive ulcerative lesions (7.5 ± 2.1 mm), one had multiple lesions, and six developed nonprogressive, indurated papules (4.8 ± 0.3 mm); the lesions lasted from 11 to 37 days. As previously reported (16), all unprotected controls of inbred strain 2 infused with cells from TRI and ITFI animals and NGP developed ulcerative lesions (7.3 ± 2.5 mm) between 12 and 19 days (14.7 ± 3.2), lasting up to 40 days (31 ± 8.2) postinfection. None of the four adoptively immune (CI) guinea pigs developed lesions during 6 months of observation.

In inbred strain 13 guinea pigs, FTA-ABS antibodies to *T. pallidum* began to appear around week 2 of infection, with titers continuing to increase 3 to 4 months later. Immobilizing antibodies appeared at or after 3 months following infection. All five individual animals examined from days 17 to 120 postinfection were weakly reactive or reactive between 90 and 120 days. The pooled sera from six additional animals confirmed the immune responses observed in individual guinea pigs. While the pattern of humoral response of inbred strain 2 animals infused with TRI, ITFI, or NGP cells was similar to that of strain 13, it differed substantially from that of CI animals. In the latter, production of FTA-ABS antibodies was substantially delayed (30 to 60 days postinfection) and the titers were three to fourfold lower than the titers from unprotected controls (Table 1). Similarly, detection of TPI antibodies was delayed and relatively weaker than that observed in seven of nine control animals. Two controls, one in the ITFI group and one in the NGP group, did not show detectable TPI antibodies during 4 months of examination.

The possibility that TPI antibodies could be associated with selective treponemal polypeptides was explored by

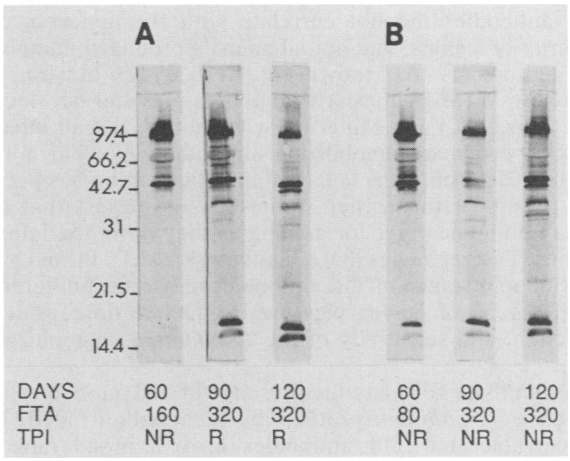

FIG. 1. Comparison of treponemal antibodies with molecular distribution of *T. pallidum* polypeptides in TPI-reactive (A) and TPI-non-reactive (B) animal sera. DAYS, Time after infection; FTA, titers; TPI, nonreactive (NR) or reactive (R) serum. Relative molecular mass of recognized polypeptides is expressed in kilodaltons.

immunoblot analysis in inbred strain 2. To this end, sera obtained 60 to 120 days postinfection from four unprotected animals were used as probes, two with nonreactive and two with reactive TPI antibodies. A representative result showing sera with similar FTA-ABS titers but different TPI reactivity is depicted in Fig. 1. The numbers and molecular masses of all polypeptides recognized by the TPI-antibody-reactive sera were also recognized by the TPI-antibody-nonreactive sera; the nonreactive sera recognized even more polypeptides than did the TPI-antibody-reactive sera.

TPI antibodies in guinea pigs have thus far been examined only by Gastinel et al. (3), Lesinski et al. (6), and A. Jakubowski (M.D. thesis, School of Medicine, Bialystok, Poland, 1964). These investigators used outbred animals and *T. pallidum* Nichols from the Fournier Institute, Paris, France. Gastinel and associates (3) observed the appearance of TPI antibodies between 150 and 185 days; the antibodies persisted for over 310 days after intratesticular infection. The reactivity ranged from 50 to 100% of immobilization. Lesinski et al. (6) infected guinea pigs by three different routes: intracardiac, intratesticular, and i.d. TPI antibodies developed in all three groups between 60 and 90 days after infection and remained in some animals for over 220 days. The most frequent appearance, strongest reactivity, and longest persistence of the immobilizing antibodies were observed in the i.d. infected animals. Jakubowski (M.D. thesis) infected outbred guinea pigs by i.d. and intracardiac routes. Of 10 animals infected i.d., the TPI antibodies were observed in 3 during month 1 of infection and in 5 by month 2. In the intracardially infected animals, only 3 of 10 produced antibodies between months 4 and 5.

In the present study, which included over 100 serum specimens obtained from guinea pig inbred strains 2 and 13 infected i.d. with approximately 10^8 *T. pallidum* and examined between 17 and 120 days after infection, the data are closer to the earlier results of Lesinski et al. (6) than to those of Gastinel et al. (3) and Jakubowski (M.D. thesis). In our experiments, the immobilizing antibodies rarely developed before 60 days after infection; they appeared at measurable levels between 90 and 120 days, at which time the FTA-ABS antibodies were at relatively high levels. The appearance of

TPI antibodies did not correlate with the appearance or severity of lesions, and not all animals produced immobilizing antibodies. As shown by Lesinski et al. (6), and Jakubowski (M.D. thesis) with guinea pigs and by Metzger and Smogor (7) and Miller (8) with rabbits, not all infected animals produce immobilizing antibodies. We did not attempt to determine the length of time that antibodies persist, but results of the earlier studies (3, 6) suggest that they remain in guinea pigs for as long as they do in the infected rabbits. Discrepancies (3, 6; Jakubowski, M.D. thesis) in the time of appearance of TPI antibodies may reflect differences in the breed of guinea pigs used, infective dose, route of infection, and sensitivity of the *T. pallidum* immobilization test.

Attempts to correlate the presence of TPI antibodies with selective *T. pallidum* proteins by immunoblot failed. It is conceivable that TPI antibodies have a broad range of reactivity associated with epitopes present on different polypeptides.

In the present study, the possibility was excluded that protection observed in animals of inbred strain 2 infused with purified CI T lymphocytes may be due to an early release of treponemicidal or treponemistatic antibodies by any contaminating B cells or as a result of helper activity of the infused T cells. Low levels of FTA-ABS antibodies appeared after 1 or 2 months of challenge, while minimal but definitive TPI antibody was detectable after 4 months. Although we examined sera from only 4 out of 11 adoptively immune guinea pigs, we believe that they are representative of the whole group. Each of these animals has been infused with exactly the same population of purified (95%) T cells, challenged at the same time, and examined under identical conditions; they developed a similar pattern of humoral response (16). Thus, we conclude from our studies that the protective mechanism(s) operative after challenge in adoptively immune animals (16) was solely T cell mediated in nature.

Although in the early literature (10) the appearance of TPI antibodies has been correlated with immunity to syphilis, Metzger and Smogor (7) and Miller (8) demonstrated unequivocally that immobilizing antibodies were not necessarily associated with the development or persistence of immunity. These findings, however, do not exclude the possibility that treponemicidal antibodies, when present, may contribute to the process of immunity. Moreover, it remains to be proven whether the treponemicidal activity present in apparently healthy individuals (1, 4) affords some degree of natural resistance against *T. pallidum*.

The present study contributes an additional parameter to the general pattern of immune responses observed in *T. pallidum*-infected guinea pigs, a model which serves well in the exploration of the immunopathologic mechanisms operative in syphilis.

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