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Effect of Passive Immunization with Purified Specific or Cross-Reacting Immunoglobulin G Antibodies against *Treponema pallidum* on the Course of Infection in Guinea Pigs†

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Whole immune serum or highly purified immunoglobulin G (IgG) antibodies to *Treponema pallidum* exhaustively adsorbed with three strains of nonpathogenic treponemes (TPI-IgG) were used for passive immunization of inbred strain 2 guinea pigs before and after intradermal challenge with 3.4×10^7 virulent *T. pallidum* Nichols organisms. Before challenge, control animals received a similarly purified IgG fraction containing either a cocktail of antibodies against three nonpathogenic treponemes (NPTI-IgG) or IgG prepared from normal guinea pig serum (NGPS-IgG). The purified fractions contained both IgG1 and IgG2 isotypes. The antibody levels (detected by fluorescent treponemal antibody test and enzyme-linked immunosorbent assay) and molecular specificities (immunoblot) of sera obtained from recipient animals before infection reflected those of the purified fractions used for immunization. Three protocols of passive immunization were used. Whole immune serum containing specific and cross-reacting antibodies afforded better protection than TPI-IgG even though asymptomatic animals were not fully protected. A single intradermal injection (0.1 ml) of TPI-IgG or NPTI-IgG into one hind leg 22 h before infection at the same site provided relatively higher protection than multiple intravenous injections (total, 15 ml) of the respective individual preparations. Since purified NGPS-IgG injected in the same animals, into the opposite hind leg, failed to protect against the challenging infection, it is reasonable to assume that specific and cross-reacting antitreponemal antibodies of the IgG1 subclass, which in guinea pigs are homocytotropic, play a relevant role in local protection.

Natural and experimental infections with *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) evoke complex humoral and cellular responses, but the extent to which these immunological forces contribute to the process of resistance has not been fully delineated. Adoptive transfer experiments indicated that the cellular arm of the immune response plays an important role in the acquisition of resistance against *T. pallidum* (for references, see reference 29). However, some animals infused with relatively large numbers of treponemal immune T lymphocytes (9, 29) developed asymptomatic infections, suggesting that additional factors, possibly of a humoral nature, are required to effect full protection. It has been demonstrated that immune serum enhances the phagocytosis of treponemes by macrophages (1, 16) and inhibits in vitro the attachment of the pathogen to mammalian cells (10) and to extracellular matrix components (11). These properties, if effective in vivo, can hardly be considered irrelevant to the process of host defense against a microbial pathogen. Notwithstanding, attempts to demonstrate a humoral mechanism of resistance by passive transfer of immune serum in rabbits (7, 12, 21–24, 26) or guinea pigs (19, 20) by using whole immune serum (7, 12, 21, 23, 24, 26) or its immunoglobulin G (IgG) fraction (20, 22) have been encouraging but not totally successful. In the hamster model, however, pooled sera collected 10 weeks or more after infection with *T. pallidum* subsp. *endemicum* (4) or *T. pallidum* subsp. *pertenue* (5) provided full protection to normal syngeneic animals against a challenge infection with the homologous strains of treponemes. In the latter report (5), it has been demonstrated that the immunoglobulin fraction IgG2 exerts better treponemicidal activity in vitro than the IgG1 fraction does. The paradoxical situation is that while the immune sera of hamsters are fully effective in adoptive transfer experiments, they are unable to eliminate the pathogen from the donors of immune sera.

Currently available data indicate that during the course of infection, there is an extensive production of cross-reacting treponemal antibodies (30–33), and only a few antibodies are specific for *T. pallidum* (31, 32). Attempts to confer protection by immunization with nonpathogenic treponemes or their antigenic fractions (for references, see reference 17) provided no protection to subsequent challenge with *T. pallidum*.

The possibility exists, therefore, that the predominant production of cross-reacting antibodies interferes with rather than contributes to the process of resistance. This could be one of the pathogenic mechanisms utilized by *T. pallidum* to prolong its persistence in the host. Moreover, to our knowledge, protection afforded by purified specific antibodies to *T. pallidum* and a comparison of this protection with that provided by a similarly purified cocktail of antibodies to nonpathogenic treponemes have so far not been attempted.

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† This article is dedicated to the memory of Thomas J. Fitzgerald of the Department of Medical Microbiology and Immunology, University of Minnesota, Duluth.

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TABLE 1. Protocol of passive immunization

Group (n = 6)	Antibodies	Before infection			After infection		
		Route	No. of injections	Injection vol (ml)	No. of injections	Injection vol (ml)	Total vol (ml)
I	TPI-IgG	i.v.	3	2.5	3	2.5	15
II	NPTI-IgG	i.v.	3	2.5	3	2.5	15
III	NGPS-IgG	i.v.	3	2.5	3	2.5	15
IV	R[a]-TPI-IgG	i.d.	1	0.1			
	L[a]-NGPS-IgG	i.d.	1	0.1			
V	R[a]-NPTI-IgG	i.d.	1	0.1			
	L[a]-NGPS-IgG	i.d.	1	0.1			
VI	Whole immune serum	i.v. and i.p.	3	2.5	7	2.5	25
VII	Whole NGPS	i.v. and i.p.	3	2.5	7	2.5	25

[a] R, right hind leg; L, left hind leg.

These are the subjects of the present article, explored in the guinea pig model for syphilis.

MATERIALS AND METHODS

Animals. Young (300- to 450-g), male, inbred strain 2 guinea pigs bred at the Wadsworth Center for Laboratories and Research facilities were originally obtained from the National Institutes of Health, Bethesda, Md. (27). All animals were individually housed at a controlled temperature of 18 to 20°C and given antibiotic-free food and water ad libitum.

New York State Flemish Giant rabbits (28), with nonreactive Venereal Disease Research Laboratory serology, were used for propagation of *T. pallidum* and as recipients in the in vitro-in vivo neutralization test (8) or infectivity test (29). At the end of the experiments, all animals were sacrificed by intravenous (i.v.) injection of Somlethol (J. A. Webster, Inc., North Billerica, Mass.).

The animal procedures were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center for Laboratories and Research.

Microorganisms. The following treponemes were used: *T. pallidum* Nichols (TP), *T. phagedenis* biotype Reiter (TR), *T. refringens* Noguchi (TN), and *T. vincentii* N-9 (TV). (The abbreviations here designate the antigens, not the organisms.) A *T. pallidum* suspension was obtained from rabbit orchitic tissue (9 to 12 days postinfection) by extraction in phosphate-buffered saline (PBS) (10 ml per testis). After an adjustment to the desired concentration, this preparation was used for passage in rabbits or for infection of guinea pigs. Percoll-purified (13) *T. pallidum* was used as a source of antigen for in vitro assays. The nonpathogenic treponemes were cultured for 4 to 5 days at 37°C in pyruvate-yeast-glucose medium (25) containing 10% inactivated normal rabbit serum (NRS). The cultures were centrifuged, and the treponemes were washed exhaustively in PBS, counted by dark-field microscopy, and kept at −60°C until use.

Preparation of immune serum. (i) TPI. *T. pallidum*-immune serum (TPI) was obtained from 40 guinea pigs infected intradermally (i.d.) in the hind leg or scrotal region with 8×10^7 freshly extracted *T. pallidum* organisms. All animals developed treponeme-containing typical lesions within 2 to 3 weeks. Resistance was established 5 months postinfection by i.d. challenge (5×10^7 organisms) of representative immune and control animals. Sera were obtained from animals at 6 months or more postinfection. The animals were bled under anesthesia (Ketaset; Bristol Laboratories, Syracuse, N.Y.) at regular intervals. Final bleeding provided 20 to 35 ml of blood per animal. Serum samples were separated, pooled, and kept at −60°C until use. The final pool to be used for fractionation consisted of 900 ml of immune serum.

(ii) NPTI. To obtain serum immune to nonpathogenic treponemes (NPTI), three groups of 10 guinea pigs each were immunized with TR, TN, or TV. Sonicated antigens were incorporated in complete Freund's adjuvant for the first injection and in incomplete adjuvant for the next five injections given i.d. biweekly (10^{10} treponemes per injection) at multiple sites. The animals were bled at various intervals before the final bleeding. Sera were pooled within a group, and the enzyme-linked immunosorbent assay (ELISA) antibody titers were adjusted to a similar titer within each group to prepare a final pool (500 ml) containing a cocktail of antibodies to nonpathogenic treponemes for further IgG purification.

Preparation of IgG fractions. IgG fractions from TPI, NPTI, and normal guinea pig serum (NGPS) (i.e., TPI-IgG, NPTI-IgG, and NGPS-IgG) were prepared by precipitation with saturated ammonium sulfate, followed by ion-exchange chromatography on DEAE-Trisacryl M (LKB, Bromma, Sweden) (3) and further purification by concanavalin A-Sepharose (Pharmacia, Uppsala, Sweden) (2). The purified TPI-IgG fraction was then sequentially adsorbed with CNBr-activated Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) coupled (8 mg of protein per g of gel) to either NRS (two times) or solubilized TR (five times), TN (two times), or TV (three times) (the numbers in parentheses indicate repeats of adsorption) until a negative reaction was achieved by ELISA with the homologous antigen (32). The NPTI-IgG fraction was adsorbed only with CNBr-activated Sepharose coupled to NRS, and the purified NGPS-IgG fraction was used without further manipulation. The protein concentrations of the IgG fractions were determined (15) and adjusted to contain 7 to 8.3 mg/ml.

Immunologic methods. Treponemal antibodies were quantitated by the fluorescent treponemal antibody test (FTA) adapted for guinea pigs (27) and by ELISA using solubilized TP and sonicated TR, TN, and TV as antigens as described previously (32). Molecular analyses of the various antisera preparations were performed by Western blot (immunoblot) (32).

In vitro-in vivo neutralization test. Treponemicidal activity in the IgG preparations was examined by the neutralization

TABLE 2. Characteristics of antibodies used for host protection against *T. pallidum*

Immunoglobulin fraction	Protein concn (mg/ml)[a]	Titer determined by:					
		FTA	ELISA of:				
			TP	TR	TN	TV	NRS
TPI-IgG	7.0	800	3,200	100	<25	<25	<25
NPTI-IgG	8.3	ND	12,800	25,600	25,600	25,600	<25
NGPS-IgG	7.4	ND	400	3,200	50	50	ND

[a] Protein concentration determined by the Lowry method.

assay described by Bishop and Miller (8), with slight modifications as reported previously (30).

Passive immunization and challenge infection. Three different protocols of immunization were used (Table 1). (i) Within three groups consisting of six syngeneic animals each, each animal received three i.v. injections (2.5 ml per injection) of either TPI-IgG (group I), NPTI-IgG (group II), or NGPS-IgG (group III) within 48 h before infection and three additional injections on days 3, 7, and 10 postinfection. (ii) A single i.d. injection (0.1 ml) of either TPI-IgG (group IV) or NPTI-IgG (group V) into the right hind leg and one of NGPS-IgG into the left hind leg were administered 22 h before infection. (iii) Two groups received a total of 10 injections (2.5 ml per injection) of either whole immune serum (group VI) or NGPS (group VII), the same sera as those used for preparation of the purified IgG fractions. Three i.v. injections were given within 48 h before infection, and seven additional intraperitoneal (i.p.) injections were given every 3 days postinfection. One hour before infection, all animals were bled to determine the antibody titers and molecular specificities of the recipient sera. All 42 experimental animals and 10 age- and sex-matched controls were infected i.d. with 0.1 ml (3.4×10^7) of freshly extracted *T. pallidum* organisms.

Statistical evaluation. Incubation periods, maximum diameters of the lesions, and duration of the lesions in passively immune animals were compared with those characteristics in unprotected controls by the Mann-Whitney U test or Student's *t* test.

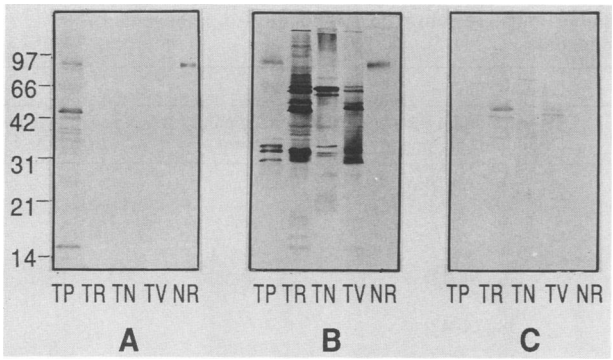

FIG. 1. Western blot of IgG fractions used for passive immunization. (A) TPI-IgG after adsorption with NRS, TR, TN, and TV; (B) NPTI-IgG after adsorption with NRS; (C) NGPS-IgG examined against various treponemes and NRS. NR, normal rabbit serum. The TPI-IgG fraction reacted with approximately 9 to 10 *T. pallidum* peptides (15 to 97 kDa). The NPTI-IgG fraction responded strongly against multiple TR, TN, and TV peptides. The reaction against TP in panel B represents antibodies to common antigens. The weak reaction of the NGPS-IgG represents natural antibody activity.

RESULTS

Properties of the immunoglobulin fractions. Purified IgG fractions examined by immunoelectrophoresis against rabbit anti-whole guinea pig serum showed a single sharp precipitation line covering the mobilities of IgG1 and IgG2 regions (results not shown). The FTA and ELISA results of the preparations used for passive transfer are presented in Table 2. Sequential adsorption of the TPI-IgG fraction with NRS and with TR, TN, and TV removed all cross-reactivities except a minor reaction in the ELISA with TR (1:100), which could be eliminated only by adsorption with CNBr-treated TP. Adsorption of NPTI-IgG with NRS removed reactivity against this antigen, yielding a preparation with high and identical titers of antibodies reacting with TR, TN, and TV, and, to a relatively lower degree, with TP (reaction against common antigens). The antibody activity present in the NGPS-IgG fraction represents the level of natural treponemal antibodies (14).

Molecular analysis of the adsorbed fractions confirmed the FTA and ELISA results (Fig. 1). The TPI-IgG fraction contained antibodies to multiple TP peptides, with the most intense reactivities being against peptides of 17 and 47 kDa. The NPTI-IgG fraction showed strong reactivities against TR, TN, and TV and also cross-reacted with TP. The reaction with the NRS is considered nonspecific. The NGPS-IgG fraction showed a weak reaction with all nonpathogenic treponemes (natural antibodies).

Molecular analysis of the sera of recipients bled just before infection showed, in recipients of TPI-IgG (Fig. 2A), antibodies active against *T. pallidum* peptides of 15, 17, 33, 39, 45, and 47 kDa. The reaction against TR must be due to natural antibodies present in the sera of the recipient. The mean FTA titer in this group was 1:112. Recipients of NPTI-IgG showed a very strong reaction against nonpatho-

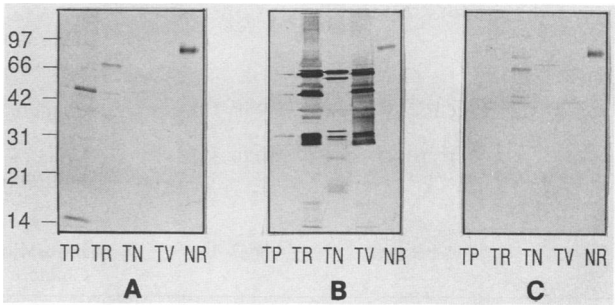

FIG. 2. Western blot. Antibody specificities in sera of guinea pigs passively immunized with TPI-IgG (A), NPTI-IgG (B), and NGPS-IgG (C) obtained 1 h before challenge infection with *T. pallidum*.

TABLE 3. Course of *T. pallidum* infection in inbred strain 2 guinea pigs receiving (i.v.) antibodies to pathogenic or nonpathogenic treponemes

Group	Animal injected with:	Lesion development				
		Day of onset (group mean range)	Lesion[a]	Days of duration (group mean range)[b]	Avg maximum size (mm)	No. of *T. pallidum* organisms[c]
I	TPI-IgG	12 (7–16)	Induration (n = 4)	49.8 (40–59)	7	17
			Chancre (n = 2)		10	17
II	NPTI-IgG	10 (10)	Induration (n = 2)	36.8 (24–49)	9	24
			Chancre (n = 4)		9.5	24
III	NGPS-IgG	9 (7–10)	Induration (n = 1)	44 (38–51)	8	18
			Chancre (n = 5)		10	18

[a] Induration, atypical, nonprogressive, nonulcerative lesion; chancre, typical, progressive ulcerative lesion.
[b] These values apply to both indurations and chancres within each group.
[c] Dark-field examination of skin punch biopsies obtained from two representative animals in each group. Number of organisms in 50 fields (magnification, ×1,200).

genic treponemes and cross-reactivity with TP (Fig. 2B), and recipients of NGPS-IgG showed, as expected, a weak activity against nonpathogenic treponemes (Fig. 2C).

Examination of the three preparations (each injected at six sites on the clipped backs of two normal rabbits) by the in vivo-in vitro neutralization test provided the following results. In the presence of a complement-positive control, immune guinea pig serum neutralized (100%) the virulence of *T. pallidum*, whereas a pool of nonimmune guinea pig serum in similar conditions did not (6%). Both sera in the absence of complement either failed to neutralize or, as in the case of immune guinea pig serum, showed a relatively low level of neutralizing activity (20%). Analysis of the total number of sites (six sites per specimen per rabbit) of inoculation with *T. pallidum*-IgG fraction mixtures showed in the presence of complement similar degrees of neutralization, i.e., 91% (11 of 12 sites) for TPI-IgG and 83% (10 of 12 sites) for NPTI-IgG. In the absence of complement, there was still treponemicidal activity, i.e., 66% for TPI-IgG and 50% for NPTI-IgG. Control NGPS-IgG showed 33% (4 of 12 sites) neutralizing activity in the presence of complement and 0% (0 of 12 sites) in the absence of complement.

Resistance to challenge infection with *T. pallidum*. After challenge with *T. pallidum*, a small erythematous reaction developed at the site of injection, lasting more than 24 h. Injection with TPI-IgG (group I) afforded only partial protection against *T. pallidum*; four of six animals developed indurated papules and two developed ulcerative lesions between 7 and 16 days of infection (Table 3). Much less protection was afforded by the NPTI-IgG fraction; two animals developed atypical lesions, and four developed typical lesions. No protection at all was demonstrated by the NGPS-IgG; one animal developed atypical lesions and five developed typical ulcerative lesions. No significant differences were noted among the three groups in the onset, duration, or maximum size of typical or atypical lesions or in the number of treponemes found in imprints from 3-mm punch biopsies taken from cutaneous lesions from two representative animals in each group.

A single injection of similar preparations given i.d. provided a relatively higher degree of protection than that provided by multiple i.v. injections (Table 4). Thus, in animals injected with the TPI-IgG fraction into the right hind leg, one was negative, four developed an atypical lesion, and

TABLE 4. Course of *T. pallidum* infection in inbred strain 2 guinea pigs receiving (i.d.) antibodies to pathogenic or nonpathogenic treponemes

Group	Animal injected with:	Lesion development				
		Day of onset (group mean range)	Lesion[a]	Days of duration (group mean range)[b]	Avg maximum size (mm)	No. of *T. pallidum* organisms[c]
IV	R^d-TPI-IgG	13 (10–15)	None (n = 1)	31.4 (24–43)		
			Induration (n = 4)		8	7
			Chancre (n = 1)		8	7
	L^d-NGPS-IgG	9 (7–10)	Chancre (n = 6)	36 (24–43)	9	15
V	R^d-NPTI-IgG	10 (9–12)	None (n = 1)	38 (24–42)		
			Induration (n = 3)		8	8
			Chancre (n = 2)		10	8
	L^d-NGPS-IgG	9 (7–11)	Induration (n = 1)	42 (24–68)	8	16
			Chancre (n = 5)		9	16

[a] For description, see Table 3, footnote *a*.
[b] These values apply to both indurations and chancres.
[c] Dark-field examination of skin punch biopsies from two representative animals in each group. Number of organisms in 50 fields. When >10 organisms were found, 50 fields were examined, when <10 organisms were seen, 100 fields were examined.
[d] R, right hind leg; L, left hind leg.

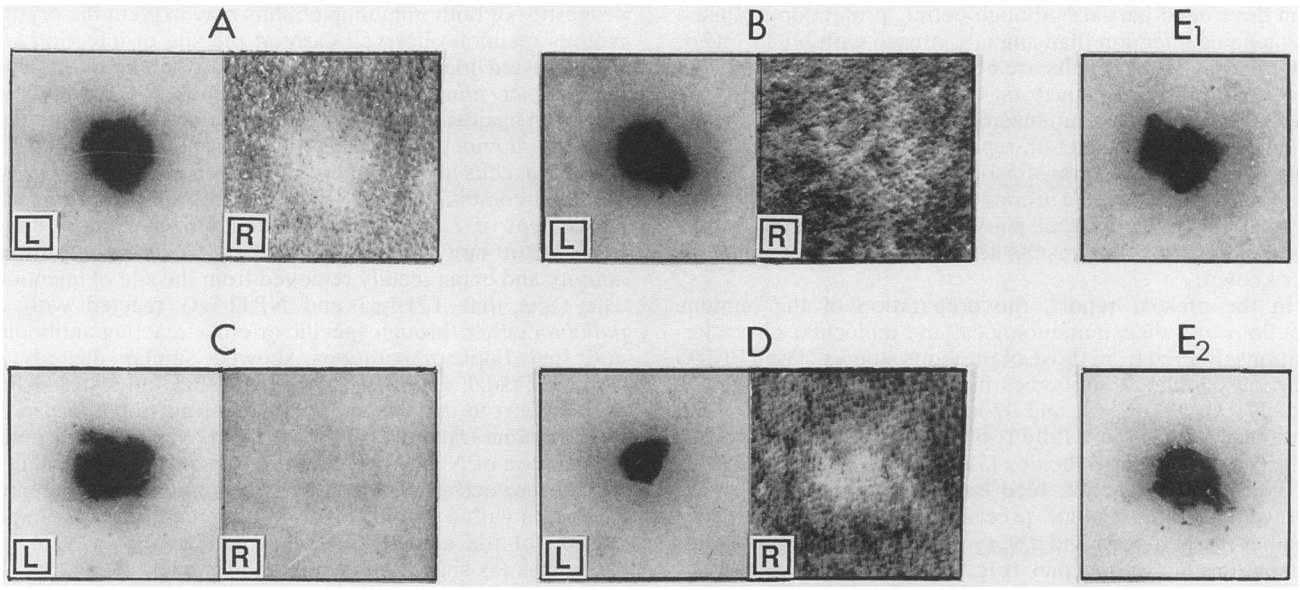

FIG. 3. Lesions resulting from injection i.d. with IgG fractions 22 h before challenge at the same sites with 3.4×10^7 (0.1 ml) of virulent *T. pallidum* organisms. (A and B) Lesions of two representative animals injected in the right (R) hind leg with TPI-IgG and in the left (L) hind leg with NGPS-IgG; (C and D) lesions of two animals injected in the right (R) hind leg with NPTI-IgG and in the left (L) hind leg with NGPS-IgG; (E_1 and E_2) lesions of unprotected control guinea pigs. The lesions from all animals were photographed at the same time, 10 to 11 days after challenge.

one developed an ulcerative lesion, whereas all six animals whose left sides were injected with NGPS-IgG developed ulcerative lesions. To a lower degree, the NPTI-IgG fraction also afforded some protection when given i.d. in a single injection. One animal was negative, three developed atypical lesions, and two developed chancre. In the animals whose left sides were injected with NGPS-IgG, one developed an atypical lesion and five developed ulcerative lesions. Although statistically insignificant ($P > 0.05$), there were differences within each group of animals in the onset, duration of lesions, and number of treponemes in the lesions which developed on both hind legs. These differences are shown in Fig. 3 by two representative animals of each group. At the time when ulcerative lesions were fully developed on the unprotected left side, there was no or slight induration on the right side that was protected either by TPI-IgG (Fig. 3A and B) or NPTI-IgG (Fig. 3C and D). Cutaneous lesions developed in unmanipulated normal guinea pigs are shown in Fig. 3E_1 and E_2.

Much better protection was afforded by passive immunization with whole TPI serum (Table 5) given i.v. and i.p. in a total volume larger than that used in protocol (i) described in Materials and Methods. Only three of six animals developed small indurated papules during a significantly ($P < 0.01$) prolonged incubation time compared with controls injected with NGPS (mean, 30.5 versus 10.2 days, respectively). These papular lesions, which developed after injection with immune serum was discontinued, contained substantially lower numbers of treponemes than control animals. In the three animals apparently protected by passive immunization, however, treponemes disseminated into the lymph nodes, as demonstrated by infectivity test.

DISCUSSION

In the present report, syngeneic recipients of injections of highly purified guinea pig IgG antibodies specific for *T. pallidum* (TPI-IgG) by two different protocols of immuniza-

TABLE 5. Course of *T. pallidum* infection in inbred strain 2 guinea pigs receiving (i.v. and i.p.) *T. pallidum*-immune whole serum

Group	Animal injected with:	Lesion development				
		Day of onset (group mean range)	Lesion[a]	Days of duration (group mean range)[b]	Avg maximum size (mm)	No. of *T. pallidum* organisms[c]
VI	TPI	30.5 (27–34)	None (n = 3)	27 (14–40)		
			Induration (n = 3)		6	5
VII	NGPS	10.2 (8–21)	Induration (n = 1)	25.2 (15–28)	8	20
			Chancre (n = 5)		8	20
VIII	Nothing (unmanipulated)	10 (7–12)	Induration (n = 2)	50.8 (38–77)	9	25
			Chancre (n = 8)		9	25

[a] For description, see Table 3, footnote *a*.
[b] These values apply to both indurations and chancres.
[c] For details, see Table 3, footnote *b*.

tion developed partial, although better, protection against a challenging infection than animals infused with NPTI-IgG or NGPS-IgG. These results are not significantly different from those of other investigators who used *T. pallidum* and various protocols of immunization (7, 12, 19–24, 26). On the other hand, Azadegan et al. reported full protection in naive hamsters by passive transfer of immune serum against the homologous pathogenic treponeme *T. pallidum* subsp. *endemicum* (4) or *T. pallidum* subsp. *pertenue* (5). Whether the apparently conflicting results are pathogen or host related is not known.

In the present report, the preparation of the immune fractions and their immunological and molecular characterizations differed from those of previous studies. The TPI-IgG fraction contained antibodies in quantities and specificities (15, 17, 33, 39, 45, 47, and 97 kDa; Fig. 1) similar to those previously shown in a fully resistant host after the removal of cross-reacting specificities (31, 32). Moreover, recipients of the TPI-IgG fraction bled immediately before infection showed by immunoblot precisely the same specificities against the pathogen and a very mild or no reaction against nonpathogenic treponemes (Fig. 2A). Thus, the inability of TPI-IgG to provide full protection cannot be attributed either to the lack of specific antibodies or to an interference affected by cross-reacting antibodies.

To answer the question whether the purification procedures influenced the activity of the TPI-IgG, passive transfer experiments were done with a crude unadsorbed IgG fraction. The results (not shown) were almost identical to those obtained with whole immune serum (Table 5). We may conclude, therefore, that the exhaustive adsorption most likely eliminated the antibodies to fibronectin or extracellular matrix components known to block attachment of the pathogen to host cells (10, 11), contributing to the lesser protective activity of the TPI-IgG fraction. Indeed, the combined action of specific, cross-reacting and nonspecific antibodies present in the whole immune serum apparently afforded better protection than either TPI-IgG or NPTI-IgG alone. Only three of six animals developed indurated papules after injections with whole immune serum were discontinued, and three animals did not develop lesions, whereas all controls injected with NRS developed either a papular lesion (one animal) or chancres (five animals). In the asymptomatic animals, however, the protection was by no means complete. An infectivity test in rabbits performed with lymph nodes removed from two asymptomatic guinea pigs indicated systemic dissemination of the pathogen.

More interesting were the results obtained by i.d. injection of a single dose of TPI-IgG and NPTI-IgG, 22 h before challenge at the same sites with *T. pallidum*. Both IgG fractions containing specific or cross-reacting antibodies afforded substantial local protection when compared with the results of NGPS-IgG injected in the same animal on the opposite hind leg. This finding is remarkable in view of the relatively small amount of antibodies injected (0.1 ml), 150 times lower than that used in protocol (i). The guinea pig IgG fractions contained both IgG1 and IgG2. Although in guinea pigs the IgG1 is in a much lower concentration (20%) than IgG2 (ca. 80%), the former is known to increase after immunization (18) and possibly after infection. Both immunoglobulins have different biological properties. IgG1 is a homocytotropic antibody, attaches through the Fc fragment to mast cells and basophils (6), and is responsible for the immediate type of hypersensitivity reaction. On the other hand, IgG2 does not bind to cells, fixes complement, and is responsible for the Arthus-type reaction (18). The biological properties of both immunoglobulins may explain the erythematous reaction observed early at the site of infection and which lasted longer than 24 h. We do not know to what degree each immunoglobulin contributed to the protection observed against the challenging infection. It is quite possible that homocytotropic IgG1 antitreponemal antibodies bound to cells displayed locally a substantial number of antibody-combining sites (Fab) capable of preventing the attachment of *T. pallidum* and immobilized it more effectively than larger quantities of IgG2 lacking cytotropic activity and being readily removed from the site of injection. The facts that TPI-IgG and NPTI-IgG reacted with *T. pallidum* either through specific or cross-reacting antibodies and that both preparations showed similar degrees of treponemicidal activities by the neutralization test add further support to that assumption. The protection afforded by both immune fractions can be considered specific inasmuch as injection of NGPS-IgG containing normal IgG1 and IgG2 did not protect at all. Moreover, by immunizing syngeneic recipients with a single dose of an immune fraction, amplification of the actual level of protection by antiallotypic antibodies (as suggested in the rabbit model [21, 24, 26]) or even antiidiotypic antibodies (as suggested for an inbred strain of guinea pigs [19, 20]) can be totally excluded for all practical purposes.

In summary, on the basis of present and previous results on adoptive transfer experiments (29), we may conclude that antibodies produced during the course of syphilitic infection, while unable to afford total protection, undeniably constitute an adjunct effector mechanism of host defense against the pathogen. The availability of the guinea pig model offers the additional possibility of exploring comparatively the relative protection afforded by both the IgG1 and IgG2 antitreponemal antibodies, which have different biological properties.

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