

## Adoptive Transfer of Immunity to *Treponema pallidum* Nichols Infection in Inbred Strain 2 and C4D Guinea Pigs

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**T lymphocytes purified from lymph nodes and spleens of chancre-immune, inbred strain 2 guinea pigs, when infused into syngeneic guinea pigs, conferred protection against challenge with *Treponema pallidum* subsp. *pallidum* Nichols. No protection was conferred by similar injections of cell suspensions from normal guinea pigs or guinea pigs immunized with *T. phagedenis* biotype Reiter or *T. pallidum*-free testis supernatants from infected rabbits. Similar results were obtained with homozygous C4D guinea pigs. After several months of infection, 2 of 11 strain 2 and 1 of 8 strain C4D recipients of *T. pallidum*-immune cells developed an erythematous reaction of short duration at the injection site; 2 of these recipients were positive for *T. pallidum*. Throughout the experimental period the humoral response to treponemal antigens was substantially lower in the adoptively immune guinea pigs than in various unprotected control groups. Passive immunity to infection with *T. pallidum*, however, seems to be dose related, since asymptomatic infection persisted for as long as 3 months after challenge in strain 2 guinea pigs transfused with  $10^8$  *T. pallidum*-immune lymphocytes, but not in C4D recipients of twice as many immune cells.**

It is known that natural and experimental syphilitic infection evokes a complex humoral and cellular response, but to what extent these two immunological responses participate in protection against syphilis is not clear.

Two observations have been taken as evidence for an important role of cell-mediated mechanisms in the acquisition of immunity: (i) the apparent failure of the humoral immune response developed during the early stage of infection to eradicate the pathogen and (ii) the fact that hypersensitivity to treponemal antigens and resistance to challenge with *Treponema pallidum* start to develop in late secondary and latent syphilis (for reviews, see references 5 and 19).

Although adoptive transfer of cellular immunity to normal hamsters has been reported for endemic syphilis (18, 20) and infection with *T. pallidum* subsp. *pertenue* (8, 21), conflicting results have been reported for venereal syphilis. Metzger and Smogor (13) reported that normal allogeneic rabbits infused with syphilis-immune lymph node cells were partially protected against subsequent infection with the pathogen. However, Baughn et al. (1) found that immune spleen cells failed to protect syngeneic normal rabbits. More recently, Pavia and Niederbuhl (15), using inbred strains of guinea pigs, reported that unfractionated or purified T and B cells from *T. pallidum*-immune (TPI) guinea pigs conferred partial protection against venereal syphilis.

In this report we present direct evidence of adoptive transfer of cellular immunity to *T. pallidum* infection in guinea pig inbred strain 2. We also present similar results obtained with the most susceptible strain, deficient in complement component C4 (C4D). While the 50% infective dose for strain 2 guinea pigs is  $10^5$ , similar to that for strain 13 and outbred Hartley guinea pigs (17), the 50% infective dose for the C4D animals is  $<10^3$  (unpublished observation). Moreover, as previously reported (32), the size, duration, and

severity of primary syphilitic lesions in C4D guinea pigs are substantially greater than those evoked by the same inoculum size in inbred strains 2 and 13. *T. pallidum* infection sensitizes the host not only against pathogenic treponemes but also against nonpathogenic treponemes (36) and rabbit proteins (34). It was considered appropriate, therefore, to include as controls T cells from guinea pigs immunized with *T. phagedenis* biotype Reiter or with *T. pallidum*-free testis fluid from infected rabbits (ITF; 35), neither of which was included in any of the previous studies.

### MATERIALS AND METHODS

**Animals.** Young (3 to 4 months old) male inbred strain 2 guinea pigs and homozygous C4D guinea pigs (350 to 500 g) were obtained from the animal facilities (Griffin Laboratory; 32) of the Wadsworth Center for Laboratories and Research. Our C4D colony has a restricted genetic polymorphism, as it was started in 1975 with one male and six females derived from the original National Institutes of Health stock (4). Adult male Nys(FG) rabbits, clinically and serologically negative for *T. paraluiscuniculi* infection, were used for passage of the virulent *T. pallidum* subsp. *pallidum* Nichols and as recipients in infectivity tests.

The guinea pigs and rabbits were housed in air-conditioned (18 to 20°C) quarters in pairs and individually, respectively. All were fed food free of antibiotics and given water ad libitum. All manipulations with the guinea pigs, such as injections, trial bleedings, and skin biopsies, were done with the animals under general anesthesia, using either Ketaset (Bristol Laboratories, Syracuse, N. Y.) or ether. The animals were sacrificed with Euthanasia T-61 (American Hoechst, Somerville, N. J.).

**Cell donors.** TPI guinea pigs were prepared by intradermal (i.d.) infection of inbred strain 2 and C4D guinea pigs with 0.2 ml of a suspension containing  $10^8$  *T. pallidum* organisms (11) obtained from infected rabbit testes at the peak of orchitis (32). Three guinea pigs of each strain challenged 5 months later did not develop lesions, whereas control animals did; thus, the experimental animals were considered

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immune. These donors and the cells obtained from them will be referred to as TPI.

*T. phagedenis* Reiter-immune (TRI) guinea pigs received nine weekly i.d. injections of  $10^{10}$  well-washed sonicated organisms prepared as previously described (36). These donors and the cells obtained from them will be referred to as TRI. *T. pallidum*-free ITF-immune (ITFI) guinea pigs received seven weekly i.d. injections of 0.5 ml ITF prepared as previously described (35). These donors and the cells obtained from them will be referred to as ITFI.

The experimental animals and an age-matched control group of normal guinea pigs (NGPs) were treated daily for 7 days with chloramphenicol (each injection, 50 mg/kg) to terminate infection and used 2 weeks later as donors of cells.

**Purification and examination of T cells.** Suspensions rich in T cells were obtained by a slight modification of the procedure described by Trizio and Cudkowicz (24), involving sequential passages of pooled spleen or lymph node cells through acid-treated sterile glass wool (20) and nylon wool columns. The purified nonadherent spleen and lymph node cells were pooled and constituted 15 to 23% of the total cells applied. Substantially fewer lymphocytes were purified from spleen (8 to 12%) than from lymph node (25 to 35%) cells. The nonadherent cells were examined with specific goat antiserum to guinea pig T cells (ATS) or specific rabbit antiserum to guinea pig immunoglobulins, together with a fluorescein isothiocyanate-labeled antibody specific for goat or rabbit immunoglobulins (Cooper Biomedical, Inc., West Chester, Pa.), by indirect immunofluorescence tests. The ATS was prepared in our laboratory by a protocol similar to that reported previously for rabbit thymocytes (33). After sequential absorption of the inactivated antiserum with guinea pig erythrocytes, bone marrow, macrophages, and L2C guinea pig B plasmacytoma cells (7), the antiserum was cytotoxic for approximately 89% of unfractionated thymocytes, 55% of lymph node cells, and 27% of spleen cells. The absorbed antiserum did not react by immunofluorescence with the L2C leukemia cells, which contain surface receptors characteristic of bone marrow-derived or B cells (7). The L2C cells were kindly provided by U. Rudofsky of the Wadsworth Center for Laboratories and Research and were maintained in our laboratory by serial passage in strain 2 guinea pigs.

The mitogenic response of the T-cell-enriched preparation was examined in microplates in parallel with the original pool of lymphoid cells previously purified by centrifugation through Ficoll-Hypaque as previously described (32), using 4  $\mu$ g of concanavalin A (Sigma Chemical Co., St. Louis, Mo.), 0.1  $\mu$ g of phytohemagglutinin (Burroughs Wellcome Co., Research Triangle Park, N.C.), or 10  $\mu$ g of lipopolysaccharide (Difco Laboratories, Detroit, Mich.) per  $2 \times 10^5$  cells. Control cultures received only medium. Cultures were incubated for 3 days in a 5% CO<sub>2</sub> incubator. The counts per minute (cpm) of [<sup>3</sup>H]thymidine of triplicate samples were averaged, and a stimulation index (SI) was determined as follows: SI = mean cpm of stimulated cultures/mean cpm of nonstimulated cultures.

Macrophages were identified by the latex technique (6) and by nonspecific esterase staining with alpha-naphthyl acetate as the substrate (Histozyne Kit 90-A1; Sigma) (9).

**Mixed leukocyte culture.** Intrastrain histocompatibility differences in C4D guinea pigs were examined by the mixed leukocyte culture technique by using Ficoll-Hypaque-purified lymph node lymphocytes from 10 guinea pigs each of strains C4D, 2, and Hartley A (Albany line); the last two strains were used as additional allogeneic controls (32). One

week before the test all animals were injected in the footpad with 0.5 ml of an equal volume of phosphate-buffered saline and complete Freund adjuvant containing killed *Mycobacterium smegmatis*.

Triplicate cultures containing 0.1 ml of responder cells and 0.1 ml of irradiated (2,000 R) partially syngeneic or allogeneic stimulator cells were cultured for 5 days at 37°C in a 5% CO<sub>2</sub> incubator. The cultures were pulsed with 0.5  $\mu$ Ci of [<sup>3</sup>H]thymidine and harvested 24 h later. The cpm of triplicate cultures were averaged, and an SI was calculated as follows: SI = mean cpm of allogeneic cultures/mean cpm of partially syngeneic cultures.

For a maximum stimulatory effect, lymph node cells from each individual strain 2 and C4D animal were exposed to a pool of irradiated cells of the 9 or 10 remaining allogeneic donors.

**Passive transfer experiments.** Although experiments with strains 2 and C4D were done at different times, both are described simultaneously. Young adult male (3 to 4 months old) guinea pigs were given an inoculum of  $10^8$  (strain 2) or  $2 \times 10^8$  (C4D) purified T cells in 1 ml of RPMI 1640, injected intravenously. After 24 h each animal was infected i.d. at one site in the pubic area with 0.2 ml of a suspension containing  $10^8$  treponemes. In a previous study this dose caused typical symptomatic lesions in essentially all animals (32).

Three C4D guinea pigs were infused with TPI cells which had been treated for 2 h at 37°C with the optimal amount (dilution, 1:5) of ATS and complement and washed with abundant RPMI 1640. As a control, three C4D animals were infused with TPI cells which had been treated similarly except that the serum was inactivated normal goat serum absorbed with guinea pig erythrocytes.

Special care was taken to ensure that all recipient animals, experimental and control, were of the same age (young adult) and were infected at approximately the same time with similar doses of *T. pallidum*. The animals were closely examined for clinical symptoms. The presence of treponemes at the injection site and in lymphoid organs was determined in randomly selected animals, and blood was collected periodically for serologic assays.

**Serologic assays.** The fluorescent-treponemal-antibody (FTA) test was performed as described previously (33) except that the first 1:5 dilution was done in sorbent. The Sera Tek microhemagglutination treponemal-antibody test was performed in accordance with the instructions of the manufacturer (Ames Div., Miles Laboratories, Inc., Elkhart, Ind.) but with a starting serum dilution of 1:5 instead of 1:20. Enzyme-linked immunosorbent assays (30) were carried out in Immulon I flat-bottom microplates (Dynatech Laboratories, Inc., Alexandria, Va.) with slight modifications as described elsewhere (8a). The antigens were sonicated *T. pallidum* and *T. phagedenis* Reiter at concentrations of 5 and 3  $\mu$ g/ml, respectively. The probe was an alkaline phosphatase-labeled rabbit immunoglobulin G against guinea pig immunoglobulin G, with *p*-nitrophenyl phosphate as the substrate. A<sub>410</sub> was determined in a Microelisa Minireader (Dynatech). An absorbance of  $\geq 0.2$ , which was  $>2$  standard deviations above the mean determined for 50 normal sera, was considered positive. The endpoint titer was therefore the reciprocal of the last dilution giving an absorbance of  $\geq 0.2$ . Results were evaluated statistically by Student's *t* test.

**Histopathology.** Punch biopsies of dermal lesions or organ specimens were placed in 10% neutral-buffered Formalin. Paraffin-embedded sections were stained with hematoxylin

TABLE 1. Detection of differences in histocompatibility among strains of guinea pigs by mixed leukocyte culture test

Leukocyte culture		SI (mean [range])
Responder strain	Stimulator strain <sup>a</sup>	
2	2	0.78 (0.34–0.90)
	C4D	4.82 (2.9–5.8)
	Hartley A	2.27 (1.7–2.85)
C4D	C4D	1.03 (0.64–1.5)
	2	3.63 (2.27–5.25)
	Hartley A	3.52 (2.5–4.5)

<sup>a</sup> Irradiated leukocytes.

and eosin for histology and by the Warthin-Starry silver method for detection of treponemes.

## RESULTS

The mild histocompatibility differences within C4D guinea pigs were confirmed by the low blastogenic proliferation elicited by a pool of irradiated C4D leukocytes compared with that evoked by similar pools of allogeneic leukocytes (strain 2 and Hartley A; Table 1).

**Characterization of purified lymphocytes.** The T-cell-enriched preparations were shown by indirect immunofluorescence to contain  $96 \pm 4\%$  (mean  $\pm$  standard deviation) cells reactive with ATS and  $3 \pm 2\%$  cells reactive with antiserum to guinea pig immunoglobulins. Histochemical analysis showed  $2 \pm 1\%$  esterase-positive cells; this result was confirmed by the latex test. The viability of the cells ranged from 92 to 98%.

Lymphoproliferative responses of the T-cell-enriched preparations showed that the uptake of [<sup>3</sup>H]thymidine in response to T- and B-cell mitogens was substantially reduced by fractionation, probably due to removal of the adherent cells. However the SI was sharply increased with concanavalin A and phytohemagglutinin but significantly diminished with lipopolysaccharide (Table 2)

**Effect of passive transfer on course of *T. pallidum* infection.** The results of experiments with the various groups of strain 2 and C4D recipients of TPI, TRI, and ITFI cells and NGP cells subsequently challenged with *T. pallidum* are presented in Table 3. For each strain the results for the experimental group were compared with those for the various syngeneic control groups.

A delayed-type erythematous reaction 3 to 8 mm in diameter developing within 24 to 48 h after challenge and lasting up to 5 days was observed in guinea pigs infused with TPI, TRI, or ITFI cells but not in animals infused with NGP cells or in untreated controls (data not shown). Typical papular or chancrelike lesions developed in approximately 2 to 3 weeks in all strain 2 animals treated with TRI, ITFI, or

NGP cells and in 9 of 10 untreated animals but in none of the animals infused with TPI cells.

Of 11 strain 2 guinea pigs in the TPI group, 2 developed an erythematous reaction 3 and 4 months postchallenge. One of these animals was positive for treponemes by dark-field examination, and the other was negative. Histologic examination of the treponeme-positive reaction showed qualitative changes similar to those in lesions of unprotected controls: acanthosis of the epidermis and infiltration of the dermis by polymorphonuclear leukocytes, macrophages, and a moderate number of lymphocytes. Some necrosis of the collagen fibers, edema, and capillary proliferation were also seen. The number of treponemes visualized by silver staining, however, was substantially lower than in cutaneous lesions of unprotected controls.

Among the C4D animals, except for those infused with TPI cells, all control groups, including three animals given TPI cells pretreated with ATS and complement, developed chancrelike lesions 9 to 12 days postinfection which lasted up to 3 months. Only one of the eight animals infused with TPI cells showed an erythematous reaction at 2 months postinfection: a flat area approximately 5 mm in diameter (Fig. 1A) positive for *T. pallidum* by the silver staining procedure.

The late appearance, nature, size, and short duration of the late cutaneous reaction of 3 of 19 protected guinea pigs were in sharp contrast to the typical ulcerative syphilitic lesions (chancres) of control guinea pigs (Fig. 1B to D). The time of appearance of the syphilitic lesions was not consistent among the control groups or even within a group. All lesion aspirates examined by dark-field microscopy showed the presence of treponemes.

Despite careful i.d. administration of an identical volume of infective inoculum at a single site in the pubic area, the size of the lesions varied within each group. Therefore, we did not consider the size of the lesions a reliable parameter for comparison (Table 3).

The late (2 to 4 months postchallenge) cutaneous reaction in 3 of 19 guinea pigs infused with TPI cells (Table 3) suggested that treponemes may be arrested for a long time at the injection site but in numbers insufficient to produce symptomatic infection. To investigate this possibility, infectivity tests were performed at 3 months postchallenge on five asymptomatic, adoptively immune guinea pigs (three strain 2 and two strain C4D) and on three actively immune recipients of NGP cells whose lesions had been healed for at least 3 weeks. Extracts from skin (at the injection site), inguinal and mesenteric lymph nodes, and spleens were aseptically removed. The tissues were separately minced and extracted in a small volume of sterile phosphate-buffered saline. After centrifugation at  $150 \times g$  for 10 min to remove the cellular debris, the extracts were individually injected i.d. at 2 or 3 sites on the depilated back of two normal rabbits. For comparison, another group of normal rabbits were infected

TABLE 2. Lymphoproliferative responses to T- and B-cell mitogens by unfractionated and fractionated lymphoid cells from donor guinea pigs

Mitogen	Unfractionated cells		Fractionated cells	
	Uptake of [ <sup>3</sup> H]TdR (cpm [mean $\pm$ SD]) <sup>a</sup>	SI	Uptake of [ <sup>3</sup> H]TdR (cpm [mean $\pm$ SD]) <sup>a</sup>	SI
None	4,906 $\pm$ 1,477		418 $\pm$ 257	
Concanavalin A	103,416 $\pm$ 20,624	21.07	65,362 $\pm$ 7,363	156.36
Phytohemagglutinin	170,006 $\pm$ 70,701	34.65	76,811 $\pm$ 10,166	183.75
Lipopolysaccharide	64,088 $\pm$ 16,846	13.06	678 $\pm$ 242	1.62

<sup>a</sup> Data from five experiments. [<sup>3</sup>H]TdR, [<sup>3</sup>H]thymidine.

TABLE 3. Ability of TPI cells to confer resistance against subsequent challenge with *T. pallidum*

Guinea pig strain	No. of animals	Donor cells	Characteristics of lesions			
			No. of animals	Time (day) of appearance	Duration (days)	Diam (mm)
2	11	TPI	2	105 ± 21 <sup>a</sup>	6	3.5 ± 0.7
	5	TRI	5	14 ± 3.7	27.5 ± 1.2	6.4 ± 1.3
	7	ITFI	7	15 ± 3.8	27.8 ± 8.4	9 ± 1.1
	5	NGP	5	15 ± 4	29.4 ± 9.5	7.6 ± 2.3
	10	None	9	12.2 ± 4.7	29.8 ± 8	7.1 ± 2.9
C4D	8	TPI	1	60	6	5
	3	ATS-treated, TPI	3	10 ± 1.7	71.6 ± 16	11 ± 1.7
	5	TRI	5	9.6 ± 0.5	71.4 ± 12	9.8 ± 0.8
	6	ITFI	6	10.3 ± 1.2	79.8 ± 12	11.5 ± 1
	5	NGP	5	10.1 ± 1.3	78 ± 13	11.4 ± 5.4
	10	None	10	10.7 ± 1.2	75 ± 10	11 ± 3

<sup>a</sup> Mean ± standard deviation.

i.d. with various concentrations of treponemes ( $10^2$  to  $10^6$ ) freshly obtained from orchitic rabbit testes. All organ extracts from strain 2 and C4D animals were negative for treponemes when examined by dark-field microscopy, and none of them produced cutaneous lesions in the recipient rabbits. Serologic (FTA) examination of the rabbits up to 10 weeks postinjection, however, showed that while all tissue extracts from adoptively immune C4D guinea pigs were negative, two of three skin extracts and one of three inguinal lymph node extracts from adoptively immune strain 2 guinea pigs were infectious, as were all tissue extracts from recipients of NGP cells (Table 4). The FTAs developed approximately 4 weeks after injection. At 10 weeks the FTA titers were two- to fourfold higher in rabbits injected with extracts from NGP cell recipients than with extracts from TPI cell recipients. Two control rabbits infected i.d. with  $10^2$  *T. pallidum* organisms did not develop lesions but had FTA titers of 20 and 40 at 4 weeks postinfection and 160 and 320, respectively, at 10 weeks postinfection.

**Humoral response in recipient guinea pigs.** All recipient guinea pigs (except C4D recipients of NGP cells) were

individually monitored at 2, 4, 6, 8, 12, and 16 weeks after treponemal challenge for production of antitreponemal antibodies. Although strain 2 recipients of TPI cells showed lower humoral responses than did syngeneic control nonimmune animals in all serologic tests (FTA, microhemagglutination treponemal antibody, and enzyme-linked immunosorbent assay [antigen, sonicated *T. pallidum*]), the differences were significant ( $P < 0.01$ ) only for the FTA test (Fig. 2A). The differences in titers between adoptively immune and nonimmune guinea pigs for all tests were much more pronounced in the C4D animals than in the strain 2 animals (Fig. 2B).

## DISCUSSION

Identification of the immune effector mechanisms involved in protection against venereal syphilis has been delayed for lack of readily available inbred rabbits, the model of choice for experimental syphilis. Recently, however, the suitability of the guinea pig as an alternative model for venereal syphilis has been gradually but steadily established.

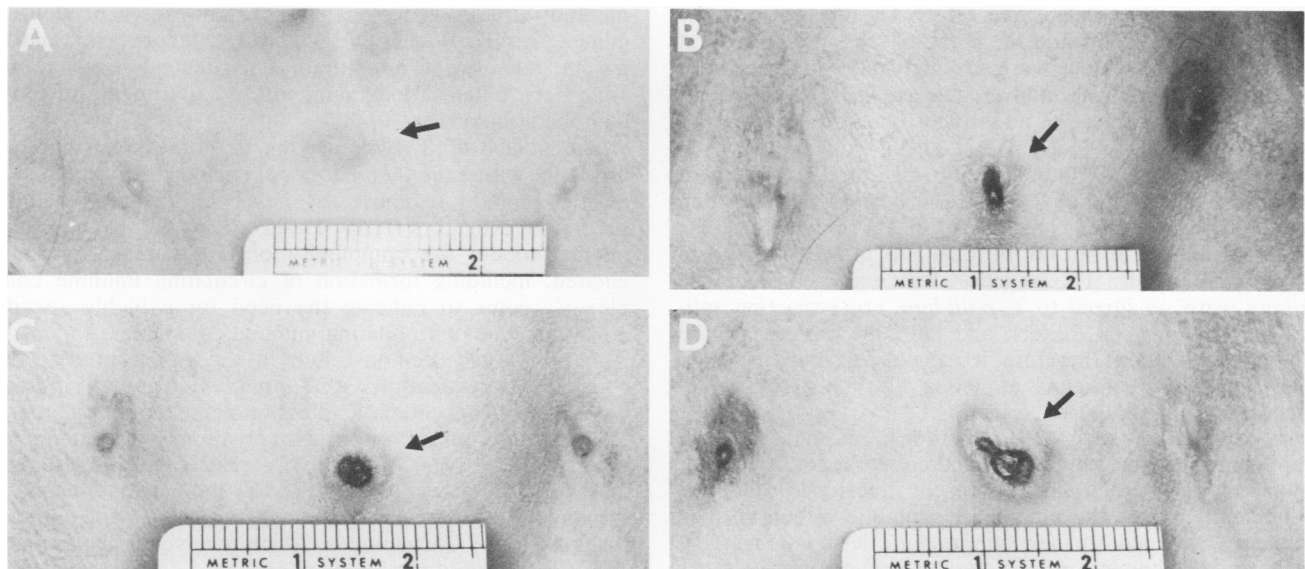


FIG. 1. Cutaneous reaction or lesions in C4D guinea pigs 2 months postchallenge. (A) Recipient of TPI cells; (B) recipient of TRI cells; (C) recipient of ITFI cells; (D) untreated control.

TABLE 4. Infectivity tests in rabbits injected with tissue extracts prepared from strain 2 and C4D guinea pigs infused with immune or normal lymphocytes and challenged with *T. pallidum*

Guinea pig strain (n)	Infused cells	Organ examined <sup>a</sup>	FTA titer in rabbits <sup>b</sup>		No. of infectious organs
			4 wk	10 wk	
2 (3)	TPI	SK	20 ± 0 <sup>c</sup>	60 ± 28	2
		ILN	20	40	1
		MLN	<5	<5	0
		SP	<5	<5	0
C4D (2)	TPI	SK	<5	<5	0
		ILN	<5	<5	0
		MLN	<5	<5	0
		SP	<5	<5	0
2 (3)	NGP	SK	40 ± 0	133 ± 46	3
		ILN	33 ± 11	266 ± 92	3
		MLN	53 ± 23	133 ± 46	3
		SP	33 ± 15	186 ± 122	3

<sup>a</sup> SK, Skin at injection site; ILN, inguinal lymph node; MLN, mesenteric lymph node; SP, spleen. All organs were negative for treponemes by dark-field microscopy.

<sup>b</sup> No cutaneous lesions developed.

<sup>c</sup> Mean ± standard deviation.

The results of this study provide direct evidence for an important protective role for T cells against venereal syphilis.

Although C4D is not strictly an inbred strain, differences in histocompatibility within the strain seem to be negligible, as indicated by the mixed leukocyte culture results and by the prolonged survival of cells adoptively transferred (up to 6 months posttransfusion) in the present study and in two preliminary experiments (data not presented). Graft-versus-host reactions are very mild or unnoticeable in guinea pigs (29). Webster et al. (31) reported the establishment of a successful allogeneic chimeric state in C4D guinea pigs (Hartley→C4D) for more than 1 year.

Because the C4D animals are larger and develop a more severe cutaneous response to syphilitic infection than do strain 2 animals (32), we used twice as large an inoculum of donor cells without deleterious effect. On the contrary, the resulting clinical signs and the humoral response consistent with a state of resistance were strikingly different from those of unprotected controls and were more defined than the signs and humoral response of adoptively immune strain 2 animals.

Pretreatment of TPI cells with ATS and complement prevented the transfer of resistance. A delayed-type reaction appeared shortly after challenge in most recipients of TPI, TRI, and ITFI cells but not in recipients of NGP cells or in untreated controls. This reaction was expected since the donors were sensitized to *T. pallidum*, cross-reacting antigens (*T. phagedenis* Reiter), and ITF, the antigens in the challenge inoculum; therefore, it was not considered specific for *T. pallidum* antigens as Pavia and Niederbuhl (15) suggested after similar experiments.

In contrast to the cross-protection between pathogenic treponemes reported by Turner and co-workers (25, 27, 28), injections of TRI cells in the present investigation did not significantly affect the course of syphilitic infection. The animals developed smaller symptomatic lesions (Fig. 1B), but the size of the lesions and the humoral response did not differ significantly from those of NGP cell recipients or untreated controls (Fig. 2). This is consistent with the fact

that animals immunized with *T. phagedenis* Reiter are not protected against *T. pallidum* infection (14).

The humoral response to *T. pallidum* antigens in strain 2 recipients of TPI cells was lower than in controls by all serologic tests, but the difference was significant only for the FTA test. The decrease in humoral response was consistently more pronounced and extended to *T. phagedenis* Reiter (enzyme-linked immunosorbent assay; data not shown) in passively immune C4D guinea pigs, possibly reflecting the larger inoculum of TPI cells. For hamsters adoptively immune against *T. pertenuis*, Guerraz et al. (8) reported similarly low FTA titers, whereas Schell et al. (21), using the microhemagglutination treponemal antibody test, did not observe differences in the humoral response between adoptively immune animals and unprotected controls.

We interpret our results as consistent with a state of resistance, confirmed by the restricted growth of the pathogen at the injection site and by its limited systemic dissemination.

Partial protection against venereal syphilis has been reported by Metzger and Smogor (13), who used unfractionated lymphoid cells from allogeneic rabbits, and more recently by Pavia and Niederbuhl (15), who used unfractionated lymphoid cells or T- or B-cell-enriched preparations from chancre-immune syngeneic guinea pigs. In the latter study, and contrary to our results, significantly high levels of antitreponemal antibodies were detected immediately (1 week postchallenge) by the microhemagglutination treponemal antibody test in guinea pigs treated with TPI unfractionated or purified T or B cells.

We cannot explain this discrepancy, but the assumption by Pavia and Niederbuhl that the high humoral response in the recipients of the T-cell-enriched population reflected an effective helper function is incompatible with the procedure used for its preparation. The anti-Ia alloantiserum used by these investigators to eliminate B cells should also have reacted with and killed a large population of T cells. Unlike T cells in humans and mice, most guinea pig T cells bear Ia antigen and react with the conventional anti-Ia alloantiserum and with monoclonal antibodies to Ia antigens (3, 10, 37).

Moreover, one of the criteria used to evaluate susceptibility or resistance to infection with *T. pallidum*, the development of cutaneous lesions, is sex- and age-dependent in guinea pigs (K. Wicher, V. Wicher, A. Jakubowski, and R. Gruhn, manuscript submitted). This dependence may not have been taken into account in the experiments of Pavia and Niederbuhl (15).

The ability of T lymphocytes to confer resistance to challenge with *T. pallidum* does not exclude the participation of the humoral response, as has been demonstrated by several investigators (2, 16, 23, 26). However, in the experimental model the complexity of the humoral response elicited, including formation of circulating immune complexes, seems to indicate the need for a highly specific antiserum free of circulating immune complexes.

As previously demonstrated in the rabbit model (12), resistance to reinfection with *T. pallidum* in guinea pigs may be relative. Asymptomatic infection persisted for as long as 3 months after challenge of passively or actively immunized strain 2 guinea pigs. Alternatively, resistance to reinfection may be dose related, as suggested by the negative results of the infectivity test and by the substantially lower humoral response in the C4D animals, which received twice as many TPI cells as did strain 2 guinea pigs.

Magnuson et al. (12) demonstrated dissemination of treponemes to lymph nodes in chancre-immune rabbits.

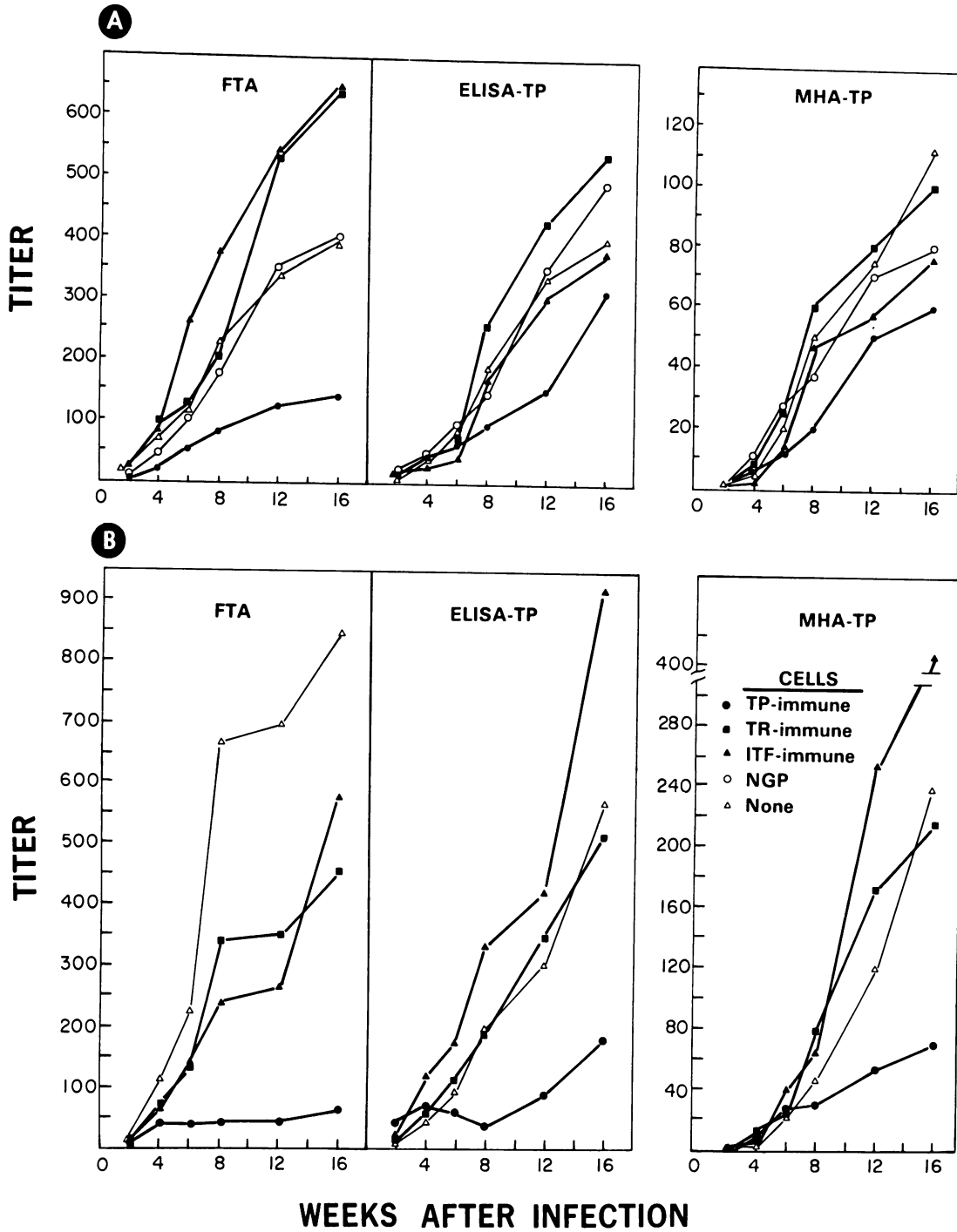


FIG. 2. Antitreponemal antibody titers in adoptively immune and unprotected control guinea pigs. (A) Strain 2; (B) strain C4D. Each datum point represents the group mean. Sera from C4D recipients of NGP cells were not tested. ELISA-TP, Enzyme-linked immunosorbent assay (antigen, sonicated *T. pallidum*); MHA-TP, microhemagglutination treponemal antibody test.

More recently, Sell et al. (22) reported the persistence of treponemes for up to 6 days at the injection site in chancre-immune rabbits. These workers suggested that treponemes may escape immune surveillance by hiding in epidermal structures, such as hair follicles, erector pili, and nerves.

Theoretically, it seems feasible that injection of a sufficiently large inoculum of immune cells could prevent infection, and relevant studies are under way in our laboratory. If

this does not prove to be true, our understanding of the immune process in syphilis may have to wait until the role of specific antibodies, independently or in conjunction with purified T cells, is delineated.

As indicated in this report and in the pertinent literature cited herein, the immunology of *T. pallidum* infection in the guinea pig mimics critical aspects of the early stage of natural infection in humans and experimental infection in

rabbits, providing an adequate model for exploration of various aspects of syphilis.

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