Genome Scale Identification of *Treponema pallidum* Antigens

Matthew McKevitt,¹ Mary Beth Brinkman,¹ Melanie McLoughlin,² Carla Perez,¹
Jerrilyn K. Howell,² George M. Weinstock,⁴ Steven J. Norris,^{2,3}
and Timothy Palzkill^{1*}

Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030¹;
Department of Pathology and Laboratory Medicine² and Department of Microbiology and Molecular Genetics,³
University of Texas—Houston Medical School, 6431 Fannin Street, Houston, Texas 77030; and
Human Genome Sequencing Center, Baylor College of Medicine,
One Baylor Plaza, Houston, Texas⁴

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Antibody responses for 882 of the 1,039 proteins in the proteome of *Treponema pallidum* were examined. Sera collected from infected rabbits were used to systematically identify 106 antigenic proteins, including 22 previously identified antigens and 84 novel antigens. Additionally, sera collected from rabbits throughout the course of infection demonstrated a progression in the breadth and intensity of humoral immunoreactivity against a representative panel of *T. pallidum* antigens.

Syphilis is a multistage sexually transmitted disease that remains a public health problem, with an estimated 12 million new cases per year worldwide (13). If untreated, syphilis can evolve from localized primary lesions (chancres) to disseminated, chronic infections, including secondary, latent, and tertiary forms (32). Primary, latent, and occasional secondary manifestations can be reproduced in experimental animal models, including the rabbit (25). Although no vaccine for the prevention of syphilis is currently available (20, 34), the immunization of rabbits with recombinantly expressed antigens prior to infection has resulted in attenuated lesion development (4, 6, 7, 35). Therefore, a combination of recombinantly expressed antigens may provide protection in rabbits and, hopefully, humans. Systematic screening of antigens for reactivity with antibodies from *Treponema pallidum*-infected animals represents a logical approach for identifying new, potentially protective antigens.

An immunoassay that utilizes a protein array was employed to identify antigenic proteins (21, 28). Of the 1,039 predicted genes in the *T. pallidum* genome, 882 were fused to glutathione *S*-transferase (GST) by using the Univector Cre-*loxP* recombination system (19), and the fusion proteins were tested for antibody binding. Protein arrays were generated by using the GST fusion protein set deposited into 96-well enzyme-linked immunosorbent assay plates coated with glutathione. Construction of the GST clone set and the techniques used to generate the protein arrays have been described previously by McKevitt et al. (21). Rabbit sera were derived from three rabbits that were each infected with 4×10^8 *Treponema pallidum* Nichols strain organisms by intratesticular injection. Sera were collected 2 days before infection, as well as 7, 14, 28, 56, and 84 days postinfection. All animal procedures were reviewed and approved by the University of Texas Health Sci-

ence Center Animal Welfare Committee. Sera from the three rabbits were pooled for use in the enzyme-linked immunosorbent assay experiments to detect antigenic proteins. Rabbit serum (45 μ l) that had been preabsorbed to *Escherichia coli* protein lysates (0.70 ml) (21) was diluted in 12 ml phosphate-buffered saline (pH 7.4) and 0.05% Tween 20 (buffer A) for a final serum dilution of 1:282. In order to systematically identify antigenic proteins, 110 μ l of the preabsorbed, diluted sera pooled from three infected rabbits was incubated in each well of the protein arrays and washed five times with buffer A. Proteins reactive with antibodies in the sera were identified by chemiluminescence with an anti-rabbit immunoglobulin G antibody conjugated to horseradish peroxidase as described previously (21) (Fig. 1). Light emission from each plate well was

FIG. 1. Identification of antigenic proteins in the *T. pallidum* proteome. The proteins arrayed for the immunoassay are presented numerically along the x axis according to ORF numbers TP0001 through TP1041. Chemiluminescence from a secondary anti-rabbit antibody conjugated to horseradish peroxidase was used to monitor rabbit antibody binding to GST fusion proteins and is measured in relative light units, shown along the y axis. *T. pallidum* fusion proteins that exhibited the highest levels of antibody binding are labeled.

* Corresponding author. Mailing address: Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Phone: (713) 798-5609. Fax: (713) 798-7375. E-mail: timothy@bcm.tmc.edu.

TABLE 1. *T. pallidum* proteins that bind serum antibodies from rabbits at 84 days postinfection

ORF; putative function (gene) ^a	Mean light units	SD	Reference(s) for previously identified antigen ^b	Signal sequence prediction ^c
TP0684; methylgalactoside ABC transporter (<i>mgIB-2</i>)	28,320	7,063	14	II
TP0435; lipoprotein, 17 kDa (<i>tpp17</i>)	25,255	2,497	9, 24	II
TP0993; rare lipoprotein A, putative	21,389	1,758		I
TP0574; carboxypeptidase, 47 kDa	19,924	1,704	9, 24	II
TP0257; glycerophosphoryldiester phosphodiesterase (<i>glpQ</i>)	19,080	1,757	30	II
TP0768; membrane protein (<i>tmpA</i>)	17,304	1,050	24	II
TP0957; Tp33 protein	15,477	781	3	I
TP0625; hypothetical protein	12,112	690		CYT
TP0956; hypothetical protein	10,614	477		II
TP0100; thioredoxin, putative	9,651	702	30	I
TP0463; hypothetical protein	8,941	355		CYT
TP0567; conserved hypothetical protein	8,719	599		TMH
TP0772; hypothetical protein	8,340	384		CYT
TP0225; leucine-rich repeat protein TpLRR	8,039	536	29	I
TP0971; membrane antigen, pathogen specific (<i>tpd</i>)	7,938	556	16, 24	II
TP0326; outer membrane protein	7,415	305	6	I
TP1016; basic membrane protein (<i>tpn39b</i>)	7,320	752	12, 24	I
TP0693; hypothetical protein	6,118	401		II
TP0954; conserved hypothetical protein	4,989	192		II
TP0398; flagellar hook-basal body complex protein (<i>fliE</i>)	4,593	260		CYT
TP0727; flagellar hook protein (<i>flgE</i>)	4,512	184		CYT
TP0292; outer membrane protein (<i>tpn50</i>)	4,478	383	11	CYT
TP0319; membrane lipoprotein (<i>tmpC</i>)	4,468	244	24, 27	II
TP1002; conserved hypothetical protein	4,245	296		I
TP0133; hypothetical protein	3,875	204		TMH
TP0486; antigen, p83/100	3,775	163		CYT
TP0183; hypothetical protein	3,724	232		I
TP0965; membrane fusion protein, putative	3,598	141		I
TP0618; hypothetical protein	3,458	141		I
TP0277; carboxyl-terminal protease (<i>ctp</i>)	3,070	584		CYT
TP0633; protein-methionine-S-oxide reductase (<i>msrA</i>)	2,991	108		CYT
TP0789; hypothetical protein	2,930	112		I
TP0434; hypothetical protein	2,913	162		CYT
TP0469; conserved hypothetical protein	2,736	198		I
TP0821; lipoprotein (<i>tpn32</i>)	2,604	190		CYT
TP0136; hypothetical protein	2,487	160		TMH
TP1001; hypothetical protein	2,465	194		TMH
TP0468; conserved hypothetical protein	2,405	73		CYT
TP0862; pept-prolyl <i>cis-trans</i> isomer, FKBP type, 22 kDa (<i>fkIB</i>)	2,068	146		II
TP0895; hypothetical protein	1,913	112		II
TP0444; conserved hypothetical protein	1,891	91		CYT
TP0307; conserved hypothetical protein	1,786	93		TMH
TP0088; conserved hypothetical protein	1,715	178		II
TP0453; hypothetical protein	1,685	138		II
TP0303; DNA mismatch repair protein (<i>mutL</i>)	1,672	61		CYT
TP0769; outer membrane protein (<i>tmpB</i>)	1,621	125	24, 26	I
TP0433; hypothetical protein	1,615	119		I
TP0660; flagellar hook-associated protein 1 (<i>flgK</i>)	1,491	58		CYT
TP0823; desulfoferrodoxin (<i>rbo</i>)	1,476	83		CYT
TP0171; lipoprotein, 15 kDa (<i>tpp15</i>)	1,473	123	9, 24	II
TP0470; conserved hypothetical protein	1,421	99		I
TP0327; cationic outer membrane protein (<i>ompH</i>)	1,412	47		CYT
TP0841; periplasmic serine protease Do (<i>htrA-2</i>)	1,355	72		CYT
TP0751; hypothetical protein/laminin binding protein	1,348	42	5	II
TP0750; hypothetical protein	1,294	54		I
TP0509; alkyl hydroperoxide reductase (<i>ahpC</i>)	1,267	51		CYT
TP0583; hypothetical protein	1,233	56		CYT
TP0163; ABC transporter, periplasmic binding protein (<i>troA</i>)	1,054	81	2, 15	I
TP0942; hypothetical protein	1,052	37		CYT
TP0298; exported protein (<i>tpn38b</i>)	1,023	37	1, 24	II
TP0395; integrase/recombinase (<i>xprB</i>)	1,012	78		CYT
TP0456; hypothetical protein	996	32		I
TP0072; conserved hypothetical protein	983	34		CYT
TP0122; phosphoenolpyruvate carboxykinase (<i>pckA</i>)	967	60		CYT
TP0056; oxaloacetate decarboxylase, subunit alpha (<i>oadA</i>)	965	20		CYT
TP0507; ATP-dependent Clp protease proteolytic component (<i>clpP-1</i>)	944	44		CYT
TP0571; Tp70 protein	943	39		CYT

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TABLE 1—Continued

ORF; putative function (gene) ^a	Mean light units	SD	Reference(s) for previously identified antigen ^b	Signal sequence prediction ^c
TP0247; <i>N</i> -acetylmuramoyl-L-alanine amidase (<i>amiA</i>)	927	57		TMH
TP0216; heat shock protein 70 (<i>dnaK</i>)	925	63	24	CYT
TP0817; enolase (<i>eno</i>)	920	61		CYT
TP0365; chemotaxis protein (<i>cheX</i>)	918	47		CYT
TP0329; serine hydroxymethyltransferase (<i>ghyA</i>)	889	37		CYT
TP0127; hypothetical protein	871	129		I
TP0474; conserved hypothetical protein	852	24		CYT
TP0220; anti-sigma F factor antagonist (<i>spoIIAA-1</i>)	814	40		CYT
TP0622; hypothetical protein	793	51		CYT
TP0604; ribosome recycling factor	788	28		CYT
TP0222; hypothetical protein	783	85		I
TP0037; D-specific D-2-hydroxyacid dehydrogenase	736	61		CYT
TP0663; outer membrane protein, putative	712	58	10, 15	I
TP0767; translation elongation factor G (<i>fusA-2</i>)	692	29		CYT
TP0369; hypothetical protein	684	35		II
TP0655; spermidine/putrescine ABC transporter (<i>potD</i>)	668	24		II
TP0546; periplasmic serine protease, putative	650	77		CYT
TP0856; hypothetical protein	631	37		I
TP0858; hypothetical protein	628	23		I
TP0240; ribosomal protein L7/L12 (<i>rplL</i>)	601	35		CYT
TP0030; heat shock protein (<i>groEL</i>)	601	28	17, 24	CYT
TP0606; ribosomal protein S2 (<i>rpsB</i>)	599	32		CYT
TP0465; hypothetical protein	564	40		CYT
TP1010; nucleoside-diphosphate kinase (<i>ndk</i>)	559	39		CYT
TP0437; hypothetical protein	549	10		CYT
TP0085; PTS nitrogen regulatory IIA component (<i>ptsN-1</i>)	545	36		CYT
TP0494; conserved hypothetical protein	524	30		CYT
TP0490; conserved hypothetical protein	521	24		CYT
TP0412; conserved hypothetical protein	510	23		CYT
TP0399; flagellar basal-body M ring protein (<i>fliF</i>)	503	25		CYT
TP0537; triosephosphate isomerase (<i>tpi</i>)	494	29		CYT
TP0047; conserved hypothetical protein	481	11		CYT
TP0029; UDP- <i>N</i> -acetylglucosamine 1-carboxyvinyltrans (<i>murA</i>)	465	36		CYT
TP1038; bacterioferritin/TpF1	457	25	24, 33	CYT
TP0368; hypothetical protein	456	20		I
TP1017; alanyl-tRNA synthetase (<i>alaS</i>)	385	52		CYT
TP0315; hypothetical protein	379	18		CYT
TP1040; lysyl-tRNA synthetase (<i>lysS-2</i>)	373	26		CYT
TP1039; adenine phosphoribosyltransferase (<i>apt</i>)	364	32		CYT

^a FKBP, FK506 binding protein; pept, peptidyl; PTS, phosphotransferase system.

^b Reference(s) in which antigen was previously identified. A literature search was conducted to compile a list of all previously identified antigenic *T. pallidum* proteins. It is possible that proteins may have been overlooked in the literature, but the goal was to include all antigens.

^c Prediction of type I or II (I or II) signal sequence or N-terminal transmembrane helix (TMH) or cytoplasmic (CYT) location. The predictions were made using the LipoP 1.0 server (18).

monitored at 5- and 15-minute intervals with a Genios plate reader (Tecan, Durham, North Carolina) for 200 milliseconds; the results at these two time points were comparable and were averaged. When the reactivity of the fusion protein was significantly greater than the background reactivity of the immunoassay, as judged by comparing the experimental fusion protein data with the GST protein-only negative control data by using the Student *t* test, the ability of a protein to elicit an immune response was inferred. The experiment was repeated twice, and the results were combined and used for statistical analysis. Analysis of the immunoassay data collected from 882 arrayed proteins indicated that 106 proteins exhibited reactivity with antibodies at higher-than-background levels (Fig. 1; Table 1).

Examination of *T. pallidum* literature generated a list of 29 proteins known to elicit an immune response in humans or rabbits. Of these 29 proteins, 27 were included in the array, and 22 of these were detected as antigens (Table 1). Reactivity of

81% of the known immunogenic proteins indicates that the assay provides a representative view of the immunoproteome. The identified proteins, however, are likely to be an incomplete set of all the antigens that react with serum antibodies from infected rabbits, for several reasons. First, only 882 of the 1,039 predicted proteins in the *T. pallidum* proteome were assayed. Second, some of the *T. pallidum* proteins may be poorly expressed in the system. For example, the TprK protein is known to elicit a strong humoral and cell-mediated immune response (8, 22, 23) but was not identified in the antigenic screen (Table 1). Examination of expression of the GST-TprK fusion protein by immunoblotting with anti-GST antibody indicated that the fusion is very poorly expressed in *E. coli*, suggesting that the lack of reactivity with rabbit sera is due to the absence of the protein in the lysate. Thus, TprK is a false negative in these experiments. Finally, DNA sequence information for 106 clones and a total of 72,187 bp in the clone set

TABLE 2. Rabbit humoral immune response over the course of a *T. pallidum* infection

ORF ^a	GST-ORF/GST ratio ^b						Putative gene function (gene)
	Preinfection	7 days	14 days	28 days	56 days	84 days	
TP444	0.83	0.81	1.16	1.29	0.99	1.83	Conserved hypothetical protein
TP222	0.91	0.89	0.94	1.09	1.11	1.88	Hypothetical protein
TP474	0.91	0.89	0.98	0.83	0.83	1.98	Conserved hypothetical protein
TP434	0.92	0.98	1.10	1.07	0.90	1.99	Hypothetical protein
TP663*	0.94	0.99	1.19	1.51	1.21	2.05	Outer membrane protein, putative
TP583	1.00	1.00	1.11	4.87	3.21	2.10	Hypothetical protein
TP30*	1.38	1.30	1.29	1.42	1.14	2.15	Heat shock protein (<i>groEL</i>)
TP72	0.88	0.94	1.08	2.02	1.47	2.21	Conserved hypothetical protein
TP37	0.99	1.12	1.99	2.35	1.69	2.22	D-Specific D-2-hydroxyacid dehydrogenase
TP456	0.80	0.92	0.98	1.00	0.80	2.36	Hypothetical protein
TP1001	0.76	0.81	0.87	1.89	1.33	2.40	Hypothetical protein
TP56	0.81	0.92	1.02	0.90	0.82	2.41	Oxaloacetate decarboxylase, subunit alpha (<i>oadA</i>)
TP88	0.86	0.87	0.94	2.15	1.50	2.49	Conserved hypothetical protein
TP247	0.80	0.80	1.07	1.98	1.37	2.54	N-Acetylmuramoyl-L-alanine amidase (<i>amiA</i>)
TP817	0.76	0.82	0.99	0.86	0.86	2.55	Enolase (<i>eno</i>)
TP216*	0.79	0.73	1.23	2.12	1.56	2.62	Heat shock protein 70 (<i>dnaK</i>)
TP509	0.98	0.89	1.05	1.05	0.89	2.64	Alkyl hydroperoxide reductase (<i>ahpC</i>)
TP163	0.88	0.88	1.02	1.44	1.27	2.70	ABC transporter, periplasmic binding protein (<i>troA</i>)
TP942	0.91	0.83	1.03	0.83	0.77	3.01	Hypothetical protein
TP369	1.23	1.00	1.10	1.14	0.91	3.07	Hypothetical protein
TP571	0.81	0.83	0.98	1.12	0.99	3.19	Tp70 protein
TP750	0.92	0.89	1.55	4.85	3.10	3.42	Hypothetical protein
TP470	0.78	0.81	0.86	0.92	0.73	3.42	Conserved hypothetical protein
TP365	0.99	0.92	1.01	3.91	2.38	3.61	Chemotaxis protein (<i>cheX</i>)
TP823	0.85	0.86	1.05	0.90	0.78	3.68	Desulfoferrodoxin (<i>rbo</i>)
TP171*	0.77	0.87	0.97	1.43	1.04	4.11	Lipoprotein, 15 kDa (<i>tpp15</i>)
TP841	0.95	1.00	4.62	6.78	4.28	4.15	Periplasmic serine protease DO (<i>htrA-2</i>)
TP433	0.83	0.76	0.89	1.08	0.82	4.26	Hypothetical protein
TP298	0.95	0.87	0.99	1.46	1.17	4.40	Exported protein (<i>tpn38b</i>)
TP751*	1.13	1.00	1.14	3.09	2.13	4.40	Hypothetical protein/laminin binding protein
TP957*	0.86	0.84	1.07	1.43	1.07	4.42	Tp33 protein
TP769*	1.80	1.59	2.52	6.31	4.27	4.73	Outer membrane protein (<i>ompB</i>)
TP327	0.94	0.88	0.87	1.00	0.76	4.90	Cationic outer membrane protein (<i>ompH</i>)
TP329	5.82	4.86	6.37	3.80	2.61	5.08	Serine hydroxymethyltransferase (<i>glyA</i>)
TP660	1.11	1.02	1.13	1.66	1.26	5.27	Flagellar hook-associated protein 1 (<i>flgK</i>)
TP398	0.87	0.87	4.58	9.32	5.61	5.95	Flagellar hook-basal body complex protein (<i>fljE</i>)
TP468	0.80	0.80	0.95	2.98	1.87	6.13	Conserved hypothetical protein
TP862	0.83	1.01	1.39	5.98	3.49	6.37	Peptidyl-prolyl <i>cis-trans</i> isomerase, FKBP type
TP453	0.89	0.86	0.96	1.29	1.10	6.86	Hypothetical protein
TP895	0.92	0.93	1.16	1.97	1.54	6.95	Hypothetical protein
TP307	0.87	0.82	1.26	2.56	1.79	7.57	Conserved hypothetical protein
TP633	0.85	0.86	1.01	2.58	1.80	7.78	Protein-methionine-S-oxide reductase (<i>msrA</i>)
TP136	0.90	0.90	1.09	3.32	2.23	8.20	Hypothetical protein
TP469	0.83	0.86	1.38	2.64	1.87	8.20	Conserved hypothetical protein
TP965	0.90	1.16	1.84	4.10	2.60	8.38	Membrane fusion protein, putative
TP133	0.73	0.81	1.13	4.75	2.97	8.89	Hypothetical protein
TP821	1.23	1.26	1.52	4.42	2.73	8.94	Lipoprotein (<i>tpn32</i>)
TP486*	0.90	0.99	1.59	3.91	2.50	8.95	Antigen, p83/100
TP618	0.86	0.83	12.06	9.95	6.44	9.43	Hypothetical protein
TP789	1.17	1.11	1.58	5.39	3.56	9.83	Hypothetical protein
TP693	0.89	0.84	1.32	2.15	1.50	10.95	Hypothetical protein
TP954	0.99	0.97	1.33	2.05	1.62	12.08	Conserved hypothetical protein
TP277	0.87	0.85	1.24	7.14	4.76	12.66	Carboxyl-terminal protease (<i>ctp</i>)
TP183	0.79	1.12	3.16	18.66	11.89	13.45	Hypothetical protein
TP727	0.98	1.58	4.69	12.94	7.94	15.06	Flagellar hook protein (<i>flgE</i>)
TP292*	0.86	0.87	1.19	3.80	2.50	16.49	Outer membrane protein (<i>tpn50</i>)
TP1002	1.83	1.86	2.29	8.09	4.72	16.83	Conserved hypothetical protein
TP319*	0.85	2.16	13.54	21.08	12.83	19.54	Membrane lipoprotein (<i>tmpC</i>)
TP567	0.98	0.98	3.38	13.91	8.96	20.66	Conserved hypothetical protein
TP225*	0.89	0.98	1.44	6.37	3.87	21.90	Leucine-rich repeat protein TpLRR
TP1016*	1.07	0.98	2.97	12.76	7.76	22.02	Basic membrane protein (<i>tpn39b</i>)
TP463	0.86	0.84	6.39	14.65	9.25	22.85	Hypothetical protein
TP971*	0.79	5.10	23.90	20.41	12.78	23.78	Membrane antigen, pathogen specific (<i>tpd</i>)
TP625	0.86	0.90	1.30	2.07	1.35	23.83	Hypothetical protein
TP772	0.84	0.91	1.62	5.57	3.33	24.73	Hypothetical protein
TP257*	0.88	0.97	1.90	7.05	4.09	25.19	Glycerophosphoryldiester phosphodiesterase (<i>glpQ</i>)
TP326	0.84	1.09	6.49	15.35	9.48	29.45	Outer membrane protein
TP956	1.26	1.13	1.17	1.41	1.04	30.88	Hypothetical protein
TP100*	0.94	0.92	2.17	15.03	9.45	32.90	Thioredoxin, putative
TP574*	0.87	5.59	25.04	25.13	15.43	42.50	Carboxypeptidase, 47 kDa
TP768*	0.85	1.21	12.50	28.16	20.07	48.41	Membrane protein (<i>tmpA</i>)
TP993	0.97	1.35	5.24	13.21	8.16	52.31	Rare lipoprotein A, putative
TP684*	0.88	9.09	29.15	26.99	17.49	54.84	Methylgalactoside ABC transporter (<i>mgIB-2</i>)
TP435*	1.31	1.31	6.46	30.88	19.53	65.95	Lipoprotein, 17 kDa (<i>tpp17</i>)

^a Asterisks indicate proteins previously identified as antigens.^b GST fusion protein/GST ratios of ≥ 2.0 are in bold.

indicated one error per 2,673 bp (generated during PCR amplification of the genes prior to insertion into the Univector plasmid); therefore, some genes are likely to be false negatives due to PCR-generated mutations (21). The occurrence of false negatives, however, does not invalidate the positive identification of the 106 antigens identified in Table 1.

Functional classifications have been assigned to 56 of the 106 antigens identified (Table 1). Proteins associated with the cell envelope are the most represented class (35%). Twenty-four of the 35 proteins (69%) exhibiting the highest reactivity were predicted to encode signal peptides or an N-terminal transmembrane helix (18); 10 of these were predicted or experimentally verified lipoproteins, consistent with prior observations that lipoproteins are among the immunogenic proteins of *T. pallidum* (Table 1).

The GST fusion proteins were next used to monitor the development of the humoral immune responses in rabbits against *T. pallidum* infection (Table 2). Sera from three rabbits were collected and pooled prior to the inoculation of *T. pallidum* and again at 7, 14, 28, 56, and 84 days after intratesticular inoculation. For this analysis, 74 proteins that are representative of the 106 reactive proteins identified in the global screen were selected (Table 2).

With the exception of the protein encoded by TP0329 (Table 2), little reactivity between serum antibodies from uninfected rabbits and *T. pallidum* proteins was observed. At 7 days postinfection, however, antibody binding at levels twofold higher than the level of binding to the negative control, GST protein alone, was observed for the following proteins: TP0319 membrane lipoprotein TmpC, TP0971 membrane antigen Tpd, TP0574 47-kDa carboxypeptidase, and TP0684 methylgalactoside ABC transporter. These proteins bound high levels of antibody at all postinfection time points (Table 2). The early and continuous antibody response is a characteristic that would make these proteins useful as diagnostics of *T. pallidum* infection.

The humoral response broadened at 14 days postinfection in that antibodies bound to 19 of the 74 proteins arrayed at levels twofold higher than the binding level of GST alone (Table 2). The most reactive proteins at this time point corresponded with those seen at 7 days postinfection, including MglB-2, the 47-kDa carboxypeptidase, and TmpC. By 28 days postinfection, the immune response was robust, with significant antibody binding to 47 of the 74 proteins tested. The response continued to expand, however, and by 84 days postinfection, 70 of the 74 proteins assayed exhibited twofold-higher levels of antibody binding than the GST-only control.

In general, proteins with relatively low reactivity at 84 days did not elicit a humoral response until late in the infection, while those proteins that exhibited high levels of antibody binding at 84 days elicited a response early that continued to increase up to the 84-day level (Table 2). An exception is the hypothetical protein encoded by TP0956 that exhibited a 30-fold increase in antibody binding between 56 and 84 days. The significance of this finding is that rabbits typically exhibit only partial immunity to reinfection 1 to 2 months after intratesticular inoculation of *T. pallidum*. However, by 3 months postinoculation, rabbits exhibit chancre immunity (31). The increase in antibodies against the TP0956 protein correlates with the

timing of chancre immunity, and therefore, it is an interesting candidate as a potential protective antigen.

This study utilized a comprehensive clone set consisting of ~90% of *T. pallidum* open reading frames (ORFs) (21) to conduct the first systematic search for antigenic proteins in this organism. A large set of antigens which were further characterized with respect to the timing of the immune response was identified. Proteins that elicit an early response that continues throughout experimental rabbit infection may be useful for immunodiagnosis of humans. Additional studies with sera from individual rabbits and humans to distinguish antigens that consistently elicit an antibody response in patients with syphilis or other treponemal infections are needed. Several of the novel antigens identified in these experiments are currently being tested in rabbits for the ability to provide protective immunity.

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