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A Comparison of Murine and Human Immunoproteomes of *Helicobacter pylori* Validates the Preclinical Murine Infection Model for Antigen Screening

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Preclinical mouse infection models are widely used for *Helicobacter* vaccine development, but how well such models mimic important aspects of human infections is unknown. A comparison of *Helicobacter pylori* immunoproteomes of infected mice with previously reported patient data reveals a high agreement in the antigens recognized, suggesting that *H. pylori* in vivo protein composition and recognition by the host immune system are comparable in mice and humans. Murine *Helicobacter* models may thus be valid to screen antigens for human vaccination.

The gram-negative bacterium *Helicobacter pylori* is a major causative agent of chronic active gastritis as well as gastric and duodenal ulcers. Moreover, it contributes to the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. There has been extensive work to develop a *Helicobacter* vaccine. Most preclinical studies have been performed using mouse *Helicobacter* infection models because of the practical advantages of small-animal models (15). *H. pylori* does not normally colonize mice, but some mouse-adapted strains that can infect the murine stomach have been identified. Little work on in vivo gene expression of *H. pylori* has been done, but transcript levels of four important *Helicobacter* genes are similar for human biopsy samples and mouse samples (23). However, there is no detectable phase variation of Lewis antigen expression (27), and the important virulence factors CagA and VacA are lost during mouse colonization (11, 25). Pathological changes in the murine system include gastritis and in some cases follicle formation and even low-grade mucosa-associated lymphoid tissue lymphoma in the gastric mucosa (5, 17), whereas ulcer formation and adenocarcinoma have not been observed. Various vaccines that induce protective immunity against a *Helicobacter* challenge in the mouse model have been developed, but clinical trials have revealed a poor efficacy of such vaccines in humans (21), suggesting that murine *Helicobacter* models might be of limited value for vaccine development. The failure to transfer mouse vaccination strategies to humans could be due to potential differences in *Helicobacter* protein expression, antigen exposure to the host immune system, vaccine delivery, and protective immune mechanisms (8). To address the first two issues (protein composition and antigen exposure to the immune system,) which could particularly affect the screening of protective antigens, we compared the *H. pylori* immunoproteome in infected mice with previous data from infected human patients. *Helicobacter* antigens that induce specific antibody responses are obviously expressed in situ (4, 9, 26) and become exposed to the host immune system.

Female, 6- to 8-week-old, C57BL/6 mice were infected by three sequential oral inoculations of 5×10^9 *H. pylori* SS1 (16) cells as described previously (6). Mice were killed at 14 weeks postinfection, and *H. pylori* colonization was assessed by plating of stomach samples. Sera were obtained prior to infection and by terminal bleeding. Alternatively, mice were subcutaneously immunized with 550 μg of *H. pylori* P76 sonicate mixed with incomplete Freud's adjuvant and given two booster doses on days 14 and 28.

H. pylori SS1 proteins were resolved in two dimensions on small gels (7.0 by 8.5 cm) and blotted as described previously (12). The blots were incubated with mouse sera at a dilution of 1:200 and stained using a peroxidase-coupled polyvalent goat antibody to mouse immunoglobulins (Sigma) at a dilution of 1:10,000 and enhanced chemiluminescence detection (ECL kit; Amersham). To increase the detection range, films were exposed for 5 to 30 min. The 35 spots with the highest postinfection seroreactivity but low to nondetectable preinfection seroreactivity (specifically recognized) and the 14 spots with the highest preinfection seroreactivity (cross-reactive) were analyzed by matrix-assisted laser desorption ionization-mass spectrometry peptide mass fingerprinting using a minimum sequence coverage of 30% (12). Coomassie brilliant blue spot staining intensities were quantified using the gel analysis program TOPSPOT.

Among the several hundred detectable protein species of *H. pylori* strain SS1 (12), only a few were recognized by preinfection sera (Fig. 1A). Sera from the same individual mice obtained at 14 weeks postinfection reacted with a much larger number of proteins, and the overall staining intensity was higher (Fig. 1B), which agrees with previously published human data (7, 14, 19, 20). Despite the facts that all mice were

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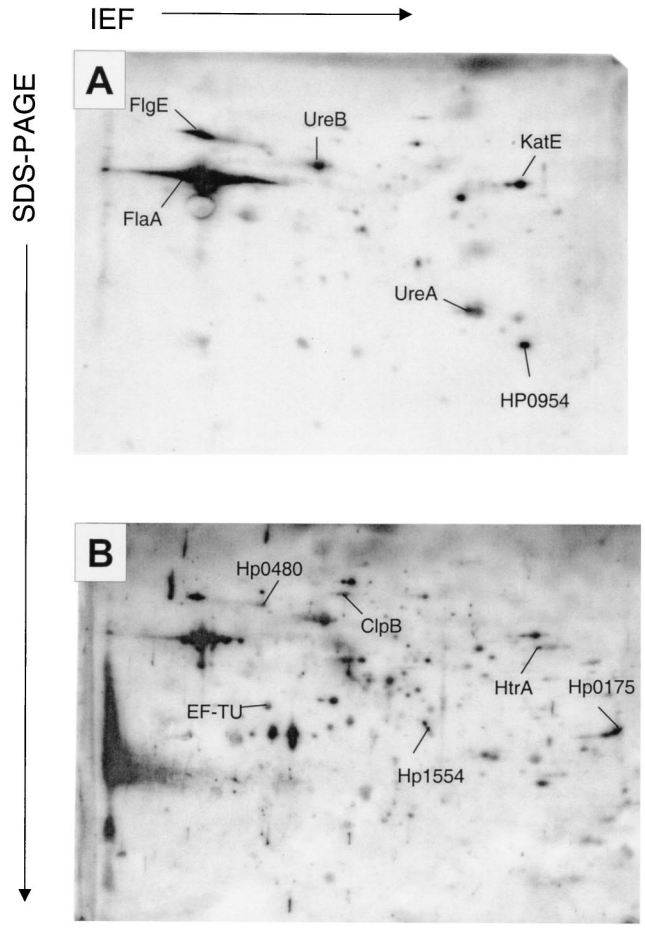

FIG. 1. Typical immunoproteome of *H. pylori* strain SS1 as revealed by two-dimensional gel electrophoresis and immunoblotting with sera from the same mouse obtained either prior to infection (A) or 14 weeks after infection (B). The immunoblots were stained with a peroxidase-coupled antibody to mouse immunoglobulins followed by chemiluminescence detection. To enhance the detection of weakly cross-reactive antigens, film A was exposed for a longer time period (15 min) than film B (5 min). Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; IEF, isoelectric focusing.

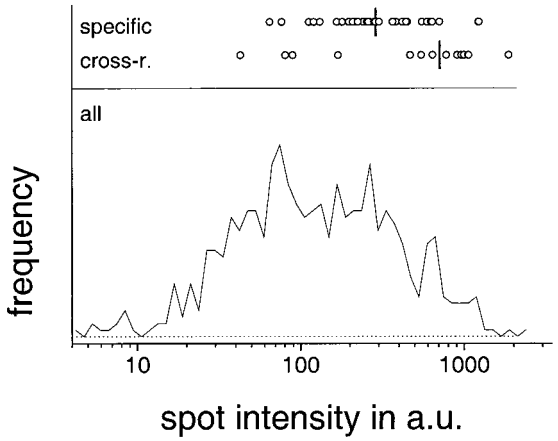

FIG. 2. Staining intensities of specifically recognized and cross-reactive (cross-r.) *H. pylori* antigens (top). The vertical lines represent the median values. For comparison, a histogram for the staining intensities of all detectable protein spots is shown at the bottom of the figure. Strongly immunogenic spots have higher staining intensities than those of all detectable spots ($P < 0.001$, t test), and cross-reactive species are significantly more intensely stained than specifically recognized species ($P < 0.01$, t test). a.u., arbitrary units.

from a genetically homogenous inbred background and all were infected with the same cultures of a single *H. pylori* strain, there were marked differences between the recognition patterns of the individual sera. Immunostaining intensities of each of the 587 recognized protein species were determined in mice using a semiquantitative scale and average values from eight infected mice. Of the 35 species that were most strongly recognized by sera from infected mice but not by preinfection sera (specific recognition), 31 species corresponding to 21 different proteins could be identified (Table 1). In addition to these specifically recognized proteins, 14 protein species that were already strongly recognized prior to infection (cross-reactive) were analyzed, resulting in the identification of 13 protein species that corresponded to 10 proteins (Table 1).

Among the total of 31 highly immunogenic proteins (21 specifically recognized and 10 cross-reactive) in the mouse model, 18 (58%) have been previously described as *H. pylori* antigens recognized by sera of infected patients, and similar levels of overlap are also observed for the specific and cross-reactive subsets, respectively (Table 1) (7, 14, 19, 20). Some differences between murine and human data sets are likely due to the different *H. pylori* strains. However, the level of agreement between the mouse data and the combined human data sets is still high compared to the rather large variation observed in the various human studies (only 34% of the seroreactive species were detected in more than one study). The majority of immunogenic *Helicobacter* antigens thus appears to be expressed both in infected murine and human stomachs and to be exposed to both immune systems (4, 9, 26).

Abundant *Helicobacter* proteins are overrepresented among the seroreactive antigens (Fig. 2), suggesting that dose-dependent responses may influence the recognition pattern. An alternative explanation is that spots containing rather small amounts of antigen might bind to a small amount of antibody and thus escape detection. However, on the basis of the least abundant species that was strongly recognized on the immunoblots (spot 59, a protein species yet to be identified), it can be estimated that this technical limitation probably affects only a minority of rare species (Fig. 2).

The 32 identified secreted and/or surface-associated proteins (2, 24) represent only 2% of the total *Helicobacter* proteome but 13 (18%) of the 71 seroreactive antigens (Table 1), suggesting that antigen localization may influence antigenicity. Secreted proteins and surface-exposed proteins that are sequestered by vesicle budding (13) penetrate the mucosa (18) and may thus more easily gain access to inductive sites. Interestingly, a predominance of surface-associated proteins among seroreactive antigens has recently also been demonstrated for *Staphylococcus aureus* (4).

It was thought that immunoblotting would be a good way to

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TABLE 1. *H. pylori* antigens recognized by murine and human sera

Antigen	Encoded protein[a]	Localization[b]	Recognition			
			Mouse	Human		
				A	B	C
HP0010	GroEL	ND	S	S	U	U
HP0011	GroES	ND	–	–	S	–
HP0027	Isocitrate dehydrogenase	ND	–	S	S	S
HP0072	UreB	ND	U	U	U	U
HP0073	UreA	Surf.	U	U	–	U
HP0109	DnaK	ND	–	S	S	S
HP0115	FlaB	ND	–	U	–	–
HP0153	RecA	ND	–	–	S	–
HP0154	Enolase	ND	–	S	–	–
HP0175	Cell binding factor 2	Sec., Surf.	S	S	–	–
HP0177	EF-P	ND	–	–	S	–
HP0192	FrdA	ND	S	–	–	S
HP0210	HtpG	ND	S	–	–	–
HP0231	Hypoth. protein	Sec., Surf.	–	–	–	S
HP0243	NapA	ND	–	S	S	–
HP0264	ClpB	ND	S	–	S	S
HP0305	Hypo. protein	ND	–	–	–	U
HP0318	Cons. hypo. protein	ND	U	–	S	U
HP0371	FabE	ND	–	–	S	–
HP0399	Rps1	ND	S	–	–	–
HP0400	LytB	ND	–	–	–	S
HP0410	HpaA	Surf.	S	–	–	S
HP0480	YihK	ND	S	–	–	–
HP0512	GlnA	ND	–	–	S	–
HP0522	Cag3	ND	–	–	–	S
HP0537	Cag16	Surf.	–	S	–	U
HP0547	CagA	ND	S	–	–	S
HP0589	Ferredoxin oxidoreductase α	ND	–	U	–	–
HP0599	HylB	ND	–	–	S	S
HP0601	FlaA	ND	U	U	U	–
HP0632	HydB	ND	S	–	–	–
HP0649	AspA	ND	–	–	S	–
HP0691	YxjD	ND	–	–	S	–
HP0752	FliD	ND	–	–	S	–
HP0779	AcnB	ND	–	–	S	–
HP0786	SecA	ND	S	–	–	–
HP0794	ClpB	ND	–	S	–	–
HP0795	Trigger factor	ND	S	–	S	S
HP0829	GuaB	ND	–	–	–	S
HP0870	FlgE	Sec.	U	–	–	–
HP0875	KatE	Surf.	U	S	–	U
HP0900	HypB	ND	–	–	S	–
HP0912	Omp20	ND	–	S	–	–
HP0913	Omp21	ND	U	–	–	–
HP0954	NAD(P)H nitroreductase	ND	U	–	–	–
HP1018	Hypo. protein	ND	–	S	–	–
HP1019	HtrA	Sec., Surf.	S	–	–	S
HP1037	Cons. hypo. protein	ND	–	–	S	–
HP1098	Cons. hypo. secreted protein	Sec., Surf.	–	–	–	U
HP1110	Pyruvate ferredoxin oxidoreductase α	ND	–	S	S	–
HP1125	Omp18	ND	–	–	U	–
HP1132	AtpD	ND	S	–	S	S
HP1134	AtpA	ND	S	–	–	S
HP1152	Ffh	ND	S	–	–	S
HP1173	Hypo. protein	Sec.	S	–	–	–
HP1193	Putative aldo-keto reductase	ND	U	–	–	–
HP1199	RP L7/L12	ND	–	U	U	U
HP1201	RP L1	ND	–	–	–	S
HP1205	EF-TU	ND	S	U	U	S
HP1213	Pnp	ND	S	–	–	–
HP1285	Cons. hypo. protein	Surf.	–	–	–	U
HP1293	RpoA	ND	–	–	S	U
HP1302	RP S5	ND	–	–	–	S
HP1307	RP L5	ND	–	–	–	S
HP1350	Protease	Surf.	–	S	–	S
HP1379	Lon	ND	S	–	–	–
HP1554	RP S2	ND	S	–	–	–
HP1555	EF-Ts	ND	–	–	S	–
HP1563	TsaA	ND	–	S	S	–
HP1564	Outer membrane protein	Surf.	–	–	–	S
HP1582	PdxJ	ND	U	–	–	U

[a] Abbreviations: Hypo., hypothetical; Cons., conserved.
[b] Experimental data from references 2 and 24. Abbreviations: ND, not detected; Surf., surface associated; Sec., secreted.
[c] Recognition of *H. pylori* antigens by mouse and human sera. Abbreviations: U, unspecific; S, specific; –, not detected. The human data are from published studies as follows: column A data are from references 19 and 20, column B data are from reference 14, and column C data are from reference 7.

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TABLE 2. Seroreactivity of protective *Helicobacter* antigens in mice and humans

Antigen	Seroreactivity[a]			
	Mice			Human[d]
	Infected	s.c.[b]	Oral[c]	
Urease A	U	+	+	U
Urease B	U	+	+	U
Catalase	U	+	–	U
HspA	–	–	–	–
HspB	S	+	+	U
VacA	–	+	–	–
Lipoprotein Lpp20	–	–	+	–
L7/L12 ribosomal protein	–	+	+	U
Hypothetical secreted protein HP1488	–	–	+	–
Hypothetical secreted protein HP1117	–	–	+	–
Hemolysin secretion protein precursor	–	+	+	S
Citrate synthase	–	+	–	–
NapA	–	+	–	S
CagA	S	+	–	S

[a] Seroreactivity of protective *Helicobacter* antigens in mice and humans. Abbreviations: U, unspecific; S, specific; +, positive (not analyzed for specificity); –, not detected.
[b] Data are from a single subcutaneously immunized mouse.
[c] Data are from orally immunized mice. Data are from reference 10.
[d] Data are from references 7, 14, 19, and 20.

select promising vaccine antigen candidates (10, 14, 19, 20), although cellular instead of humoral immune responses seem to be relevant for protection against *H. pylori* at least in the mouse model (1, 3, 22). Interestingly, the combined data from infected mice (this study) and differentially immunized mice (this study) (10) show that all known protective antigens can be recognized by antibodies (Table 2), supporting a correlation between seroreactivity and cellular immune responses which could be related to the fact that optimal antibody responses depend on help from T cells. Interestingly, many protective *H. pylori* antigens, including the well-characterized urease, are cross-reactive in noninfected mice and patients, suggesting that specificity is not a prerequisite for protective efficacy. A comparison between the recognition patterns of infected (nonprotected) versus lysate-immunized (protected) mice might yield interesting information about individual, potentially protective antigens, but our preliminary data indicate a large number of differentially recognized antigens, suggesting that relevant candidates might be difficult to identify (not shown).

In conclusion, the pattern of *H. pylori* proteins that are expressed in infected mice and become exposed to the mouse immune system appear to be similar to those in human *Helicobacter* infections, suggesting that the mouse infection model might be suitable for preclinical screening of antigen candidates.

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