We have previously shown that individuals infected with *Chlamydia trachomatis* can develop a robust antibody response to a *Chlamydia* type III secretion effector protein called Tarp and that immunization with Tarp induces protection against challenge infection in mice. The current study aimed to map the immunodominant regions of the Tarp protein by expressing 11 fragments of Tarp as glutathione S-transferase (GST) fusion proteins and detecting the reactivity of these fusion proteins with antisera from patients infected with *C. trachomatis* in the urogenital tract or in the ocular tissue and from rabbits immunized with *C. trachomatis* organisms. A major immunodominant region was strongly recognized by all antibodies. This region covers amino acids 152 to 302, consisting of three repeats (amino acids 152 to 201, 202 to 251, and 252 to 302). Each of the repeats contains multiple tyrosine residues that are phosphorylated by host cell kinases when Tarp is injected into host cells. Several other minor immunodominant regions were also identified, including those comprising amino acids 1 to 156, 310 to 431, and 582 to 682 (recognized by antisera from both humans and rabbits), that comprising amino acids 425 to 581 (recognized only by human antisera), and that comprising amino acids 683 to 847 (preferentially recognized by rabbit antiserum). This immunodominance was also confirmed by the observations that six out of the nine monoclonal antibodies (MAbs) bound to the major immunodominant region and that the other three each bound to one of the minor fragments, comprising amino acids 1 to 119, 120 to 151, and 310 to 431. The antigenicity analyses have provided important information for further understanding the structure and function of Tarp.

Infection with *Chlamydia trachomatis*, a species of obligate intracellular bacterial pathogens, imposes serious health problems in humans. The *C. trachomatis* organisms are categorized into four biovars on the basis of their tissue tropism: the trachoma biovar, which infects human ocular epithelial cells (20); the genital biovar, which infects human urogenital tract epithelial tissues, potentially leading to complications such as ectopic pregnancy and infertility (10, 17); the lymphogranuloma venereum biovar, which can cause systemic infections and from rabbits immunized with *C. trachomatis* organisms. A major immunodominant region was strongly recognized by all antibodies. This region covers amino acids 152 to 302, consisting of three repeats (amino acids 152 to 201, 202 to 251, and 252 to 302). Each of the repeats contains multiple tyrosine residues that are phosphorylated by host cell kinases when Tarp is injected into host cells. Several other minor immunodominant regions were also identified, including those comprising amino acids 1 to 156, 310 to 431, and 582 to 682 (recognized by antisera from both humans and rabbits), that comprising amino acids 425 to 581 (recognized only by human antisera), and that comprising amino acids 683 to 847 (preferentially recognized by rabbit antiserum). This immunodominance was also confirmed by the observations that six out of the nine monoclonal antibodies (MAbs) bound to the major immunodominant region and that the other three each bound to one of the minor fragments, comprising amino acids 1 to 119, 120 to 151, and 310 to 431. The antigenicity analyses have provided important information for further understanding the structure and function of Tarp.

**MATERIALS AND METHODS**

**GST fusion protein production.** For the purpose of mapping immunodominant regions, sequences for the full-length Tarp protein and 11 fragments were cloned from the *C. trachomatis* serovar D genome sequence (http://www.stdgen.lanl.gov/) into pGEX vectors (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). The 11 fragments were designated F1 to F11. The primers for cloning the

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* Corresponding author. Mailing address for Ping Yu: Xiangya School of Medicine, Central South University, Changsha 410078, China, and University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229.

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full-length protein and the 11 fragments were as follows (the restriction sites are underlined): for F1, 5′-CGG-GGATCC-ATGCAAAATTTGATGTTTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F2, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F3, the same primer as the F2 forward primer (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F4, the same primer as the F2 forward primer (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F5, the same primer as the F2 forward primer (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F6, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F7, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F8, the same primer as the F7 forward primer (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F9, the same primer as the F8 forward primer (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F10, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F11, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F12, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F13, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F14, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F15, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F16, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F17, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F18, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F19, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F20, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse); for F21, 5′-CGG-GGATCC-GAGGCTGCTTGTCTTGTGTG-3′ (forward) and 5′-CTTCTTATTTGCGGCCGCTTTATTCGTATTGGAT-3′ (reverse).

RESULTS AND DISCUSSION

Reactivity of Tarp fragments with antisera from STI and trachoma patients. Although we have previously shown that the Chlamydia trachomatis type III secretion effector protein Tarp is immunodominantly recognized by antisera from patients with Chlamydia trachomatis infection in the urogenital tract or ocular tissues (21), it is not known which regions of Tarp are responsible for its robust antigenicity and immunogenicity. We expressed the full-length Tarp protein (amino acids 1 to 1005) and three fragments, designated F1 (1-156), F2 (120-431), and F7 (425-1005), as GST fusion proteins and reacted these four fusion proteins with the antisera from 21 women urogenitally infected with Chlamydia trachomatis (STI antisera) and 21 trachoma patients (trachoma antisera) whose sera positively reacted with the full-length Tarp protein in an ELISA (Fig. 1). The binding of a given human serum sample to a given fusion protein with an OD four times above the background level was determined to be positive. F2 (120-431) was recognized by all 21 STI antisera, with a mean OD similar to that obtained using the full-length protein, while F1 (1-156) and F7 (425-1005) were recognized by 2 and 14 STI antisera, respectively. All of the 21 trachoma antisera recognized F2 (120-431) without binding to either F1 (1-156) or F7 (425-1005). Because all 42 human antisera that recognized the Tarp full-length protein also bound to F2, we can conclude that this region contains the most-immunodominant epitopes of Tarp. These results were consistent with the functional observations that F2, which includes three repeats, mediates the tyrosine phosphorylation of Tarp but that the recruitment of actin is mediated by the C-terminal domain [F7 (425-1005)] (1, 4–6, 8, 9, 11).

To more finely identify the immunodominant regions of the Tarp protein, we further divided F2 into four fragments, designated F3 to F6, and divided F7 into four fragments, F8 to F11, for expression as GST fusion proteins. These 11 fragments were further evaluated for their ability to be recognized by the various antisera (Fig. 2). The pooled STI sera reacted strongly with multiple Tarp fragments, including those comprising amino acids 1 to 156, 120 to 431, 120 to 309, 152 to 302, 310 to 431, 425 to 581, 582 to 682, and 683 to 847 but not that comprising amino acids 120 to 151 or 848 to 1005. In contrast,
the pooled trachoma sera strongly recognized only the fragments comprising amino acids 120 to 431, 120 to 309, and 152 to 302 and not any other fragments. F5 (152-302), which contains three repeats, each with ~50 amino acids (amino acids 152 to 201, 202 to 251, and 252 to 302), was the main region responsible for the reactivity of F2 with the STI antisera and trachoma antisera. The mapping data above suggest that the most immunodominant fragment of Tarp is the triple repeat region regardless of the site of infection (either in urogenital or ocular epithelial tissues). Three of the four fragments of F7, F8 (425-581), F9 (582-682), and F10 (683-847), were immunodominantly recognized by the pooled STI sera. However, none of these three fragments were as immunodominant as F7 itself. A previous analysis of C. trachomatis L2 Tarp showed that residues T425 to S825 within L2 Tarp were responsible for binding to and polymerizing actin (8). However, Tarp-mediated actin polymerization is not simply the product of a stable Tarp-actin compound but is a more complex association involving other domains of Tarp, and the actin-binding domain and polymerization domain are separated and located in different residues. In comparison to C. trachomatis L2, C. trachomatis serovar D contains sequences similar to the L2 actin-binding domain and proline-rich oligomerization domain, which are located between residues A626 to K636 and S503 to N528, respectively. Therefore, our results are consistent with the reported functional domain analysis of Tarp. However, the previous data showed that the C. trachomatis Tarp protein was even more immunodominant in patients with C. trachomatis infection in ocular tissues than in those with C. trachomatis infection in the urogenital tract. In comparison to other known immunodominant antigens encoded by the C. trachomatis genome, including CopN, HSP60, IncA, CT529 (Inc), CT813 (Inc), MOMP, and CPAF, Tarp was recognized by trachoma patient antibodies as dominantly as CPAF, the most immunodominant antigen in STI patients among all chlamydial proteins analyzed so far (12, 16, 21). However, F5 (152-302), the tyrosine-phosphorylated region, was the only region responsible for the reactivity of Tarp with the trachoma sera. To explain the different recognition of Tarp fragments by STI and trachoma antisera, we compared the amino acid sequences of

FIG. 1. Reactivity of 21 Tarp-positive STI (A) and trachoma (B) antisera with full-length Tarp and three fragment GST fusion proteins. After 1:500 dilution, each of the 21 STI and 21 trachoma human antisera (displayed along the y axis of panel a) was reacted with each of the four GST fusion proteins (listed along the x axis) immobilized onto 96-well microplates. Human antibody binding was detected with a secondary goat anti-human IgG antibody conjugated with HRP plus a soluble substrate. The results are expressed as OD readings obtained at a wavelength of 405 nm. Any given reaction with an OD reading 4-fold above the value of the control well (coated with GST alone) was determined to be positive, which is represented with a horizontal bar in panel a. The total number of human serum samples that positively recognized a given fusion protein is summarized in panel b. The average OD readings calculated by dividing the total OD values by 21 are displayed in panel c.
Tarp homologues from urogenital serovar D and trachoma serovars A, B, and C and found greater than 97% amino acid identity among all Tarp homologues. Both urogenital serovar D and trachoma serovars A, B, and C possess three repeats in the N terminus that contain multiple tyrosine residues that are phosphorylated by host cell kinases when Tarp is injected into host cells, and all Tarp proteins of the ocular serovars contain sequences similar to the actin-binding domain and the proline-rich oligomerization domain in the C terminus of Tarp of the L2 and D serovars. Thus, lack of recognition of F1 and F7 by trachoma antisera is not likely due to Tarp sequence variation between the genital and ocular serovars but might be due to a lack of antibodies that bind to epitopes in these regions in the trachoma antiserum.

Reactivity of Tarp fragments with antisera from rabbits immunized with *C. trachomatis* serovar D organisms. To determine the antigenicity and immunogenicity of the Tarp protein in small animals, four rabbits were immunized with *C. trachomatis* serovar D organisms, and the reactivity of immunized rabbit sera with Tarp was tested. Three out of the four rabbits developed substantial antibody responses against Tarp protein, which indicates that the Tarp protein is immunogenic in rabbits immunized with inactivated *Chlamydia*. The immunodominant regions on the Tarp protein that induced an Ab response in rabbits were further determined by detecting the reactivity of 11 Tarp fragments with the three pooled Tarp-positive rabbit sera (Fig. 3). The pooled rabbit sera reacted strongly with multiple Tarp fragments, including those comprising amino acids 1 to 156, 120 to 431, 120 to 309, 152 to 302, 310 to 431, 425 to 1005, 582 to 682, and 683 to 847 but not those comprising amino acids 120 to 151, 425 to 581, and 848 to 1005. The region comprising amino acids 152 to 302, which consists of three repeats, strongly reacted with the pooled rabbit sera, suggesting that this major immunodominant region of Tarp induces Ab responses not only in humans but also in rabbits. The facts that the region comprising amino acids 425 to 581 was recognized only by human antisera and that the region comprising amino acids 683 to 847 was preferentially recognized by rabbit antiserum indicate that even though the phosphorylated region was the common immunodominant region in both humans and rabbits, other major antigenic sites of the Tarp protein may function differently in humans and rabbits.

FIG. 2. Mapping immunodominant regions of Tarp by reacting the full-length Tarp and 11 fragment GST fusion proteins with the pooled STI (B) and trachoma (C) antisera in an ELISA. The GST fusion proteins were precipitated with glutathione-agarose beads from bacterial lysates, and the precipitates were checked with an SDS-polyacrylamide gel for quality (A). The same lysates were used as the source of fusion proteins in ELISA. The 21 STI and 21 trachoma serum samples were pooled at equal ratios and assayed for reactivity against each of the fusion proteins at 5-fold serial dilutions, as indicated in the figure. The Tarp-GST fusion proteins and GST alone were immobilized onto a glutathione-coated microplate, and the antiserum samples were added to the plates. The antibody binding to antigens was visualized with goat IgG conjugated to HRP and the substrate ABTS. The OD values are displayed along the y axis.

FIG. 3. Mapping immunodominant regions of Tarp by reacting full-length Tarp and 11 fragment GST fusion proteins with pooled rabbit sera, which were obtained after immunization with *C. trachomatis* serovar D organisms in an ELISA. The three Tarp-positive rabbit serum samples were pooled at equal ratios and assayed at 1:2,000 dilutions against each of the fusion proteins.

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Monoclonal antibody binding to Tarp fragments. The recombinant full-length, F1 (1-156), F2 (120-431), and F7 (425-1005) GST-Tarp proteins were used as the immunogen for the production of MAbs. Splenocytes from BALB/c mice immunized with purified GST-Tarp proteins were fused with murine myeloma cells to generate hybridomas. In total, nine MAbs were produced. Seven MAbs (R4D5, R5B6.2, R2H1.2, R12B12, R2H7, R5G8.1, and R8G7.2) were raised from full-length-protein-immunized mice, one MAb (N5B11) was from F1 (1-156)-immunized mice, one MAb (M4F4) was from F2 (120-431)-immunized mice, and none were from F7 (425-1005)-immunized mice.

To determine the epitope specificities of the MAbs, we detected the reactivity of the 11 Tarp fragments with each MAb by ELISA (Fig. 4). All MAbs were reactive with specific fragments of the Tarp protein and the full-length Tarp protein. R4D5 was reactive with F1 only, which suggested that R4D5 recognized epitopes in the region comprising amino acids 1 to 119 of the Tarp protein. N5B11 was reactive with F2, -3, and -4, which suggested that it recognized epitopes in the region comprising amino acids 120 to 151. R5B6.2 was reactive with F1, -2, -3, and -5, which suggested that it recognized epitopes in the region comprising amino acids 152 to 156. Five MAbs (R2H1.2, R2H7, R5G8.1, R8G7.2, and R12B12) were reactive with F2, -3, and -5, which suggested that these MAbs recognized epitopes in the region comprising amino acids 152 to 302. M4F4 was reactive with F2, -3, and -6, which suggested that it recognized epitopes in the region comprising amino acids 310 to 431. None of the MAbs bound to the region comprising amino acids 425 to 1005.

In summary, the region consisting of three repeats has been identified as the most immunodominant region of the Tarp protein in the present study. Several other minor immunodominant regions were also identified, including those comprising amino acids 1 to 156, 310 to 431, and 582 to 682 (recognized by antisera from both humans and rabbits), that comprising amino acids 425 to 581 (recognized only by human antisera), and that comprising amino acids 683 to 847 (preferentially recognized by rabbit antisera). It will be interesting to further study the role of the immunodominant regions of the Tarp protein and the Abs elicited by these regions in the pathology of C. trachomatis infection and to examine whether they are protective against C. trachomatis infection.

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