Identification of Outer Membrane Porin F Protein of *Yersinia enterocolitica* Recognized by Antithyrotopin Receptor Antibodies in Graves' Disease and Determination of Its Epitope Using Mass Spectrometry and Bioinformatics Tools

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Context: In addition to genetic susceptibility, *Yersinia enterocolitica* (YE) infection played an important causative role in the pathogenesis of Graves' disease (GD) through molecular mimicry. However, the specific YE proteins and epitopes recognized by anti-TSH receptor (TSHR) autoantibodies (TRAb) have not been fully clarified, resulting in conflicting results from clinical research.

Objective: Our aim was to explore the roles of YE in the pathogenesis of GD and identify the YE proteins and epitopes that are similar to the TSHR and are recognized by TRAb.

Design: Assays of YE antibodies, TRAb, thyroglobulin antibodies, and thyroid microsomal antibodies as well as cross-absorption and two-way immunodiffusion were performed in patients with GD. Using mass spectrometry and the bioinformatics tools of protein structure modeling and epitope prediction, we identified the YE protein and its epitope, which was recognized by TRAb and was similar to TSHR.

Results: Our study demonstrated for the first time that the YE protein outer membrane porin F protein (ompF) shared cross-immunogenicity with a leucine-rich domain of TSHR. The epitope recognized by antihuman TSHR antibody is located within the ompF region of amino acids 190–197, and the polyantibody against ompF protein showed TSAb activity.

Conclusions: Our results suggest that YE ompF is involved in the production of TRAb and the pathogenesis of GD through molecular mimicry. These findings are potentially important for understanding the role molecular mimicry plays in the disturbance of immune tolerance and the induction of autoimmunity to the TSHR. **(*J Clin Endocrinol Metab* 95: 4012–4020, 2010)**

Graves' disease (GD) is the main type of autoimmune thyroid disease (AITD). It afflicts up to 30 per 100,000 of the population (1). Thyroid-specific autoantibodies are frequently found in the serum of GD patients, whereas greater than 90% of patients with GD have anti-TSH receptor (TSHR) autoantibodies (TRAb). Some TRAb are thyroid-stimulating antibodies (TSAb) that can bind to the TSHR and stimulate the thyroid to produce excessive thyroid hormone resulting in hyperthyroidism. TSAbs are considered to be the primary cause of thyroid

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Abbreviations: AITD, Autoimmune thyroid disease; CHO-TSHR, CHO cells expressing TSHR; 3D, three dimensional; GD, Graves' disease; h, human; HT, Hashimoto's disease; LRD, leucine-rich domain; mAb, monoclonal antibody; ompF, outer membrane porin F protein; TFC, thyroid follicular cell; TGAb, thyroglobulin antibody; TMAb, thyroid microsomal antibody; TRAb, TSHR autoantibodies; TSAb, thyroid-stimulating antibodies; TSHR, TSH receptor; YE, *Yersinia enterocolitica*; YEAb, antibodies to YE.

dysfunction and also the most important autoantibody and immunological hallmark in GD (2–4).

Although a lot of information is known regarding the characteristics of TRAb in patient serum and on GD pathology, the mechanism of loss of immune tolerance to TSHR and how TRAbs are induced are not fully understood. Many studies have suggested that susceptibility is determined by genetic factors (*e.g.* human leukocyte antigen-D-related and cytotoxic T lymphocyte associated antigen-4), which play important roles in the pathogenesis of GD, similar to other autoimmune diseases. Nevertheless, twin studies have shown that monozygotic twins are not always in synchrony in their presentation of AITD. Whereas bizygotic twins have a low concordance rate for AITD of about 2%, monozygotic twins have a concordance rate of only about 20–40% rather than 100%. In addition, the study of 756 twin pairs indicated that only 60–70% of the risk for AITD were genetic (5–7). All of this evidence certainly suggests that genetics cannot totally account for susceptibility to GD, and genes are not the only factor in the disturbance of autoimmune tolerance to TSHR and the induction of autoantibodies resulting in GD (8, 9). Nongenetic and environmental factors including infection, iodine, smoking, and stress may have secondary causative roles that are likely very important. However, the detailed aspects of how these genetic and environmental factors cause the immune system to attack autologous tissue, and organs are not fully understood. Along with environmental factors, infection has long been implicated in the pathogenesis of AITD. A potential mechanism for the induction of autoantibodies by infection could be molecular mimicry, in which there is immunological cross-reactivity between the autoantigen and the exogenous antigens of a bacterium or virus (10, 11).

Yersinia enterocolitica (YE) is a common intestinal infection with symptoms of myocarditis, reactive arthritis, and erythema nodosum. Since the 1970s, evidence has suggested a role for YE in the pathogenicity of GD. This includes: 1) the relatively high prevalence of antibodies to YE (YEAb) in patients with GD (12), 2) the presence of stable TSH binding sites on YE that are recognized by immunoglobulins in GD patient serum (13), 3) cross-reactivity between the TSHR protein and the YE proteins (14, 15), 4) and a twin study, which indicated a close relationship between YE infection and GD (16). Although many studies suggested that YE might be implicated in the pathogenesis of GD, most have been descriptive and have failed to provide definitive answers. Even if some studies showed that envelope proteins and lipoproteins of YE were involved in GD, there is no direct evidence showing the structural similarity of epitopes recognized by TRAb between TSHR and YE. Furthermore, studies of more in-depth questions, such as the function of TRAb induced by the above-mentioned epitopes have not yet been reported. Thus, although there have been many studies of GD and YE, the role of YE in the pathogenesis of GD remains controversial.

More detailed studies of the structure of TSHR and YE proteins and epitopes on YE proteins that are recognized by TRAb and their immunological effects are crucial for clarifying the roles of YE in GD and in the prognosis and immunotherapy of GD. In this study, we examined the roles of YE in the pathogenesis of GD based on clinical and experimental data gathered using mass spectrometry, protein structure modeling, and epitope prediction. The results indicated that the prevalence of YEAb in GD patients was significantly higher than normal and YEAb correlated with TRAb. We also demonstrated that cross-immunoreactivity exists between YE and TSHR. These results were consistent with previous research. Moreover, the outer membrane porin F protein (ompF) in YE was found to be recognized by TRAb in GD patients and to have cross-immunogenicity with human (h) TSHR. The region of amino acids 147–228 of TSHR, especially amino acids 198–205, was very similar to region 190–197 of ompF and the polyantibody raised in rabbit against ompF showed TSAb activity. It is therefore very likely that ompF could induce TSAb by molecular simulation and lead to GD.

Patients and Methods

Ethical statement

The studies in this paper have been approved by the Ethics Committee of Shandong University.

Patients and grouping

There were 81 healthy people in the control group. The 203 thyroid disease patients were confirmed using clinical manifestations and laboratory examinations and were divided into four groups including GD, Hashimoto's disease (HT), simple goiter, and thyroid neoplasms. The GD group was divided into three subgroups including *de novo*, remission, and relapse patients. The information of people in all groups was shown in Table 1.

TABLE 1. The information of people in all groups

Group	Male	Female	Age range (yr)
Control	33	48	17–68
HT	3	33	24–58
Simple goiter	4	12	17–65
Thyroid neoplasms	2	8	30–72
GD			
de novo patients[a]	9	27	15–65
Remission[b]	13	42	23–63
Relapse[a]	10	40	18–62

[a] T_3: 5.3 ± 2.4 nmol/liter; T_4: 263 ± 200 nmol/liter.

[b] T_3: 2.4 ± 0.8 nmol/liter; T_4: 150 ± 59 nmol/liter.

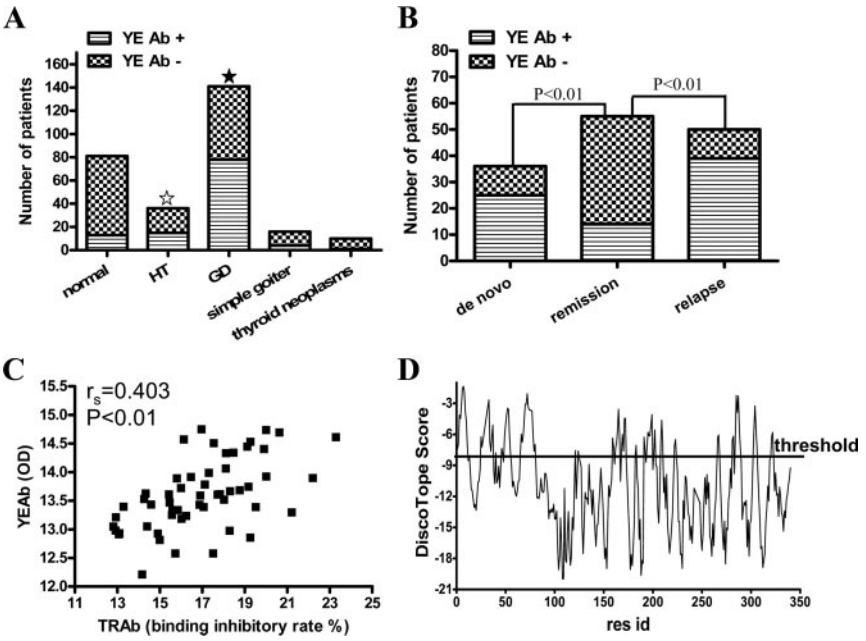

FIG. 1. The YEAb and TRAb assay and the prediction of the B-cell epitope on the ompF. A and B, The prevalence of YEAb in patients with different types of thyroid disease (A) and GD (B). *P* values are labeled on the figures. ⋆, $P < 0.01$ *vs.* normal, simple goiter and thyroid neoplasms groups; ☆, $P < 0.01$ *vs.* normal, simple goiter, and thyroid neoplasms groups. C, Spearman's rank correlation analysis for TRAb and YEAb. *P* and r_s values are labeled on the figures. D, The prediction of the B-cell epitope on the ompF protein using protein 3D structures as described by Andersen *et al.* (29). res id, Amino acid residues ID.

Bacteria, reagents, and antibodies

O:3 YE and its antiserum were provided by the National Institute for the Control of Pharmaceutical and Biological Products. Homemade anti-ompF polyantibody was prepared by rabbit immunization with purified ompF protein according to the standard procedure. The soluble porcine TSHR protein was supplied with the TRAb radioreceptor assay kit (ALPCO, Salem, NH). The commercial anti-hTSHR monoclonal antibodies (mAbs; A9, A10, and 2C11 against amino acid 147–228, 22–35, and 355–358 of TSHR, respectively) used in the study were bought from Abcam (Cambridge, MA) and Santa Cruz Biotechnology (Santa Cruz, CA).

Cross-absorption and two-way immunodiffusion assays

A total of 100 μl of YE proteins or soluble porcine TSHR was added to 300 μl of GD patient serum and incubated for 1 h. After centrifuging, the concentrations of TRAb and YEAb were assayed in the supernatants, 5% BSA was used as the control of YE proteins and soluble porcine TSHR. Two-way immunodiffusion assays were performed using standard procedures. All experiments were performed in triplicate.

YEAb, TRAb, thyroglobulin antibody (TGAb), and thyroid microsomal antibody (TMAb) assays

YEAb was assayed using ELISA. A positive result was regarded as an OD of the tested sample that was greater than the mean + 3 SD of the control. TRAb assays were performed using the TRAb radioreceptor assay kit, and the results are represented by the binding inhibitory rate of the tested serum to ^{125}I-bTSH. TGAb and TMAb were assayed by RIA (normal: TGAb <30%, TMAb <20%). Assays were performed in triplicate. The two-sided paired *t* test, χ^2, and rank correlation analyses were used for statistical analysis using SPSS 10.5 (SPSS, Chicago, IL).

Preparation of YE total proteins, Western blot, and mass spectrometric identification

The protein extraction from YE was separated on sodium dodecyl sulfate gel and then electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). The membranes were blocked and incubated with different antisera. After washing and incubating with the corresponding secondary antibodies, the membranes were developed by enhanced chemiluminescence. The common positive bands immunoblotted by the GD patients' sera were excised and cut into pieces. After digestion and desalting, the peptide mixture was analyzed with 4800 mass spectrometry (Applied Biosystems Inc., Foster City, CA) (17). Identifications were done with a GPS and ProteinPilot (Applied Biosystems Inc., Foster City, CA), and a confidence interval of greater than 99.999% was accepted.

cAMP assay

Primary human thyroid follicular cells (TFCs) were prepared from surgical specimens as described previously (18–20). The immunofluorescence was performed with anti-TSHR mAb as previously described (21). For cAMP assay, CHO cells expressing TSHR (CHO-TSHR) (kindly provided by Dr. Basil Rapoport, University of California, Los Angeles, CA) and TFCs were cultured, respectively, in Ham's F-12 medium and 5H serum-free medium (6H without hTSH) for 48 h. Then cells were incubated with hTSH (50 mU/ml), anti-TSHR mAb A9 (10 μg/ml), homemade anti-ompF polyantibody (50 μg/ml), or normal rabbit IgG (50 μg/ml) for 24 h in the presence of 3-isobutyl-1-methylxanthine (1 mM). The measurement of intracellular cAMP, performed in triplicate, was performed using cAMP ELISA kit (Millipore) according to the manual.

Cloning, expression, and purification of full-length and truncated ompF and the leucine-rich domain (LRD) of hTSHR

The ompF gene was amplified by PCR, and the deletion mutations of ompF (deleting amino acids 190–197) and TSHR LRD (deleting amino acids 198–205) were made using an inverse PCR strategy (22). Then the full-length and truncated genes were cloned into the pET-46 Ek/LIC vector, respectively, as described in the manual. All inserts were confirmed by sequencing. The recombinant 6His-tagged fusion proteins were purified using the purification kit (Merck, Whitehouse Station, NJ). Then they were electrophoresed and immunoblotted by anti-hTSHR mAb (A9) and different antisera, respectively.

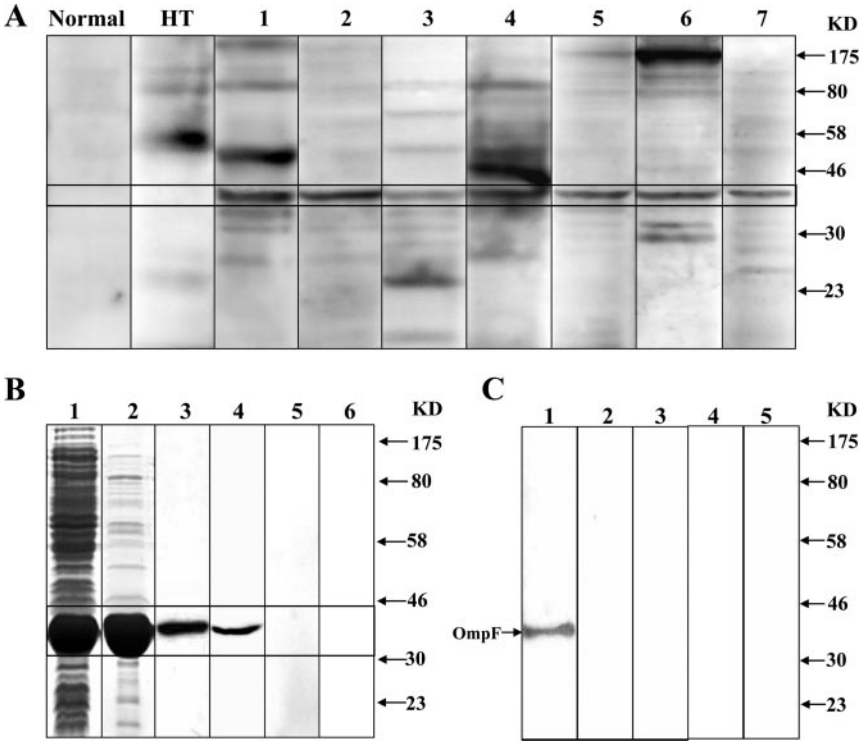

FIG. 2. Identification and confirmation of ompF cross-immunogenic to TSHR. A, The representative Western blot of total YE proteins. Lanes 1–7 represent Western blot of total YE proteins using antisera from seven GD patients. Normal and HT indicated the Western blot of total YE proteins using antisera from healthy donors or HT patients. KD, kilodaltons. B, The purified recombinant His-ompF was immunoblotted with different antisera. Lane 1, Total protein electrophoresis of *Escherichia coli* expressing ompF protein; lane 2, the electrophoresis of purified ompF protein; lane 3, Western blot of purified ompF immunoblotted by anti-His antibody; lanes 4–6, Western blot of purified His-ompF protein immunoblotted by GD patient, HT patient, and healthy donors' sera, respectively. C, The purified recombinant full-length ompF protein was immunoblotted with anti-hTSHR mAb A9 (lane 1), A10 (lane 2), or 2C11 (lane 3). The purified truncated ompF (deleting amino acids 190–197) (lane 4) and truncated TSHR LRD (deleting amino acids 198–205) (lane 5) were immunoblotted with anti-hTSHR mAb A9, which recognized the epitope from TSHR amino acid residues 147–228.

Results

YEAb and TRAb assay

The positive rates of YEAb in the GD and HT patients were significantly higher than in normal and other thyroid disease patients ($P < 0.01$). However, the differences of YEAb between other thyroid disease patients and normal group as well as between GD and HT did not reach statistical significance (Fig. 1A). Correlation studies were done by the rank correlation analysis (Spearman). The results showed that TRAb correlated significantly with YEAb ($r = 0.403$, $P < 0.01$) (Fig. 1C), but the correlations between YEAb and TMAb, TGAb, T_3, or T_4 were not significant. Additionally, the prevalence of YEAb in remission GD patients (25.5%) was significantly lower than in *de novo* (69.4%) and relapsing GD patients (78%) ($P < 0.01$) (Fig. 1B). These results were consistent with many previous studies that implied that YEAb induced by YE infection may play an important role in the production of TRAb and the development of GD.

Analysis of cross-immunogenicity of TSHR and YE

After preincubation with total protein from YE, the concentration of TRAb (expressed as binding inhibition rate) in the serum of GD patients was significantly decreased (36.1%, $P < 0.01$). Vice versa, the concentration of YEAb was also decreased significantly (53.1%, $P < 0.01$) after preincubation with porcine TSHR. Additionally, the two-way immunodiffusion assay of YEAb and TSHR showed obvious immunoprecipitation lines. The results indicated that some proteins of YE shared cross-immunogenicity with TSHR.

Identification and confirmation of ompF cross-immunogenic to TSHR

The total proteins of YE were separated by SDS-PAGE and immunoblotted with antisera from healthy donors and HT and GD patients. A common positive band was observed at approximately 40 kDa in only 41 of 50 GD patients (Fig. 2A). These positive bands were identified as ompF (theoretical molecular mass: 41.72 kDa) in YE (Fig. 3A). To confirm this result, the purified recombinant ompF protein was again immunoblotted with the antisera from GD and HT patients and healthy donors, and the positive band was still present at the same molecular weight position in GD group but not in HT and healthy donor groups (Fig. 2B). This result confirmed that ompF could be recognized only by the antisera of GD patients and that the epitope could be recognized by TRAb.

TSAb activity of the polyantibody against ompF

The result of immunofluorescence stained with anti-TSHR mAb proved the purity of primary human TFCs (>99%) (Fig. 4, A and B). Then we examined the effects of anti-ompF polyantibody on intracellular cAMP levels in TFCs and CHO and CHO-TSHR cells. Stimulation with anti-ompF polyantibody and hTSH (positive control) significantly increased cAMP levels in TFCs and CHO-TSHR cells, whereas normal rabbit IgG and PBS (negative control) had no effect. The anti-TSHR mAb A9 slightly raised cAMP levels (not significant) compared with nor-

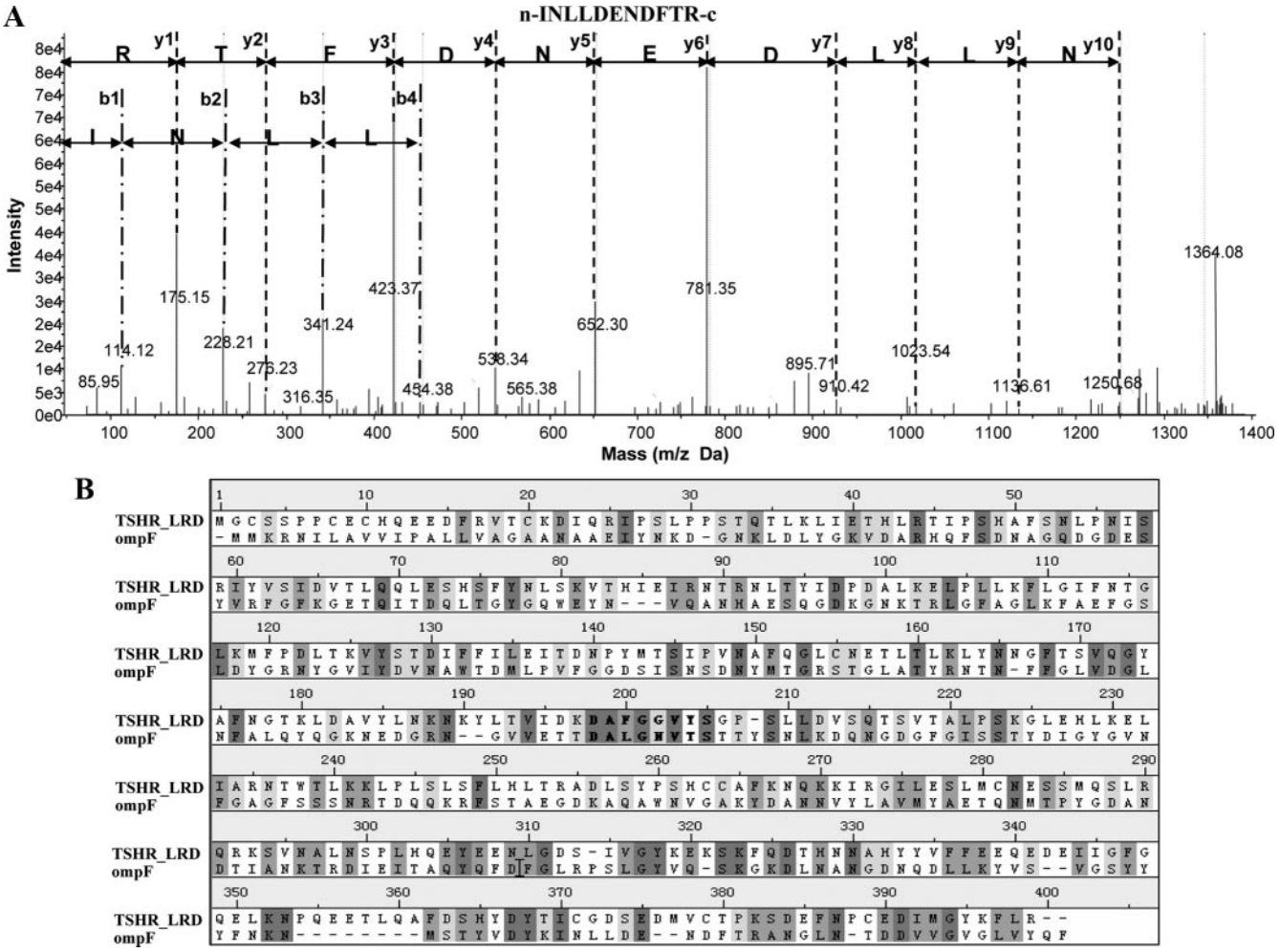

FIG. 3. Mass spectrometry identification and sequence alignment. A, Identification of ompF by tandem mass spectrometry. The common positive band at about 40 kDa shown in Fig. 2A was digested on-membrane and analyzed by 4800 mass spectrometry (Applied Biosystems). The product ion spectrum of the precursor ion 1364.1 Da showed that the common positive band was outer membrane porin F (gi:123441874). B, The sequence alignment of ompF and hTSHR LRD. The *bold letters* indicate the most identical amino acid residues between ompF and hTSHR LRD. The *background color* represents the similarity of amino acid residues with the *darker color* to denote higher similarity and sequence conservation.

mal rabbit IgG or PBS. Normal CHO cells did not respond to any of these treatments (Fig. 4C). These results indicated that anti-ompF polyantibody had TSAb activity.

Homology modeling of ompF and TSHR

To identify these epitopes shared by ompF and hTSHR, the sequences of ompF and hTSHR (gi:123441874 and gi:64085121) were aligned, and the results demonstrated that the sequence of ompF shared only 10.6 and 12% identity with hTSHR and hTSHR LRD, respectively. Although more than four consecutive identical amino acid residues were not found, there were some local amino acid residues clustered in ompF that were highly similar to the sequence of TSHR LRD. However, it was possible that the spatial location of those residues would be adjacent, resulting in epitope identity with TSHR LRD (Fig. 3B). The three-dimensional (3D) structure of ompF was built using 1OSM as the template, which shared 59.4% identity with the ompF sequence as described previously (23, 24). The crystal structure of the TSHR LRD was determined according to the published literature (25, 26) (Fig. 5, A and B).

Determination of the epitopes on ompF protein of YE recognized by TRAb

For the purpose of finding epitopes in ompF that were similar to TSHR LRD and were recognized by TRAb, the primary structure analysis and the spatial structure information for ompF and TSHR LRD and the properties of the amino acid residues were comprehensively analyzed. The results indicated that the sequence of amino acids 190–197 of ompF (DALGNVTS) was very similar to amino acids 198–205 of TSHR LRD (DAFGGVYS). The two sequences were located at the surface of the proteins and in a prominent position with good accessibility (Fig. 5, C and D). Furthermore, the epitopes of ompF were predicted using different bioinformatics tools. According to the method described by Larsen *et al.* (27), the sequence of

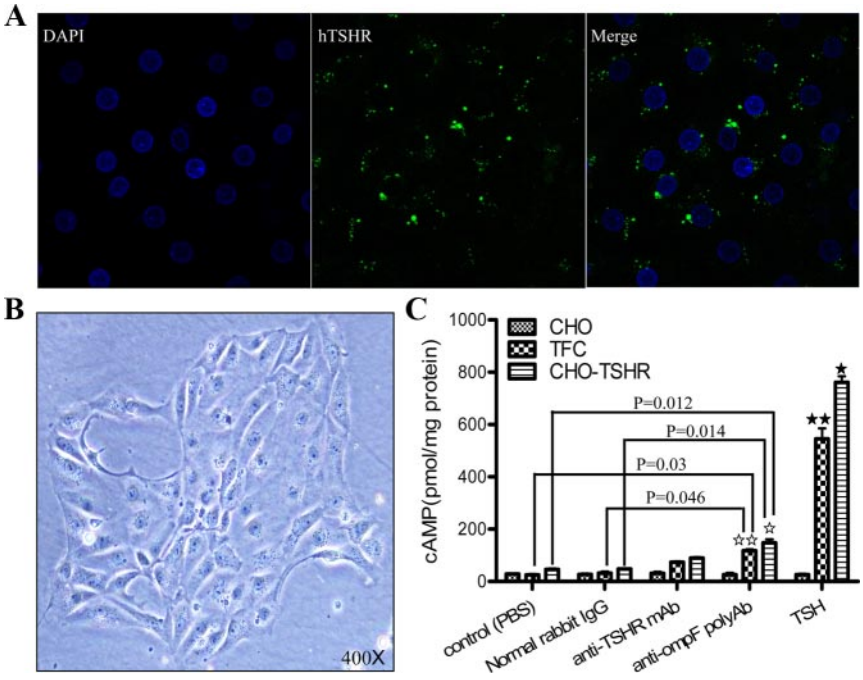

FIG. 4. Assays of the purity of primary human TFCs and the bioactivity of anti-ompF polyantibody. A, The cellular immunofluorescence of TSHR in TFCs. B, A light microscopic view of TFCs cultured in a 6H medium based on DMEM/F12 medium containing 5% fetal bovine serum. C, TFCs and CHO and CHO-TSHR cells were treated with normal rabbit IgG, anti-TSHR mAb, TSH, or homemade anti-ompF polyantibody for 24 h in the presence of IMBX (1 mM). Data are mean ± SEM. ★ and ★★, $P < 0.0001$ *vs.* all other four groups (ANOVA and n = 3); ☆ and ☆☆, $P < 0.05$ *vs.* control (PBS) and rabbit IgG groups (ANOVA and n = 3). *P* values are labeled on the figures. The differences of anti-TSHR mAb *vs.* control (PBS), rabbit IgG, and anti-ompF polyantibody did not reach statistical significance in TFCs and CHO-TSHR cells. The differences in all groups of CHO cells were not statistically significant.

ompF, DALGNVTS, scored 1.07 (specificity >98.5%). The results of B-cell epitope prediction in the ompF sequence demonstrated that it was very possible that DALGNVTS was a B-cell epitope (accuracy >76%) (28). The prediction of the B-cell epitope on the ompF protein using protein 3D structures, as described by Andersen *et al.* (29), also indicated that the scores of the DALGNVTS peptide significantly exceeded the threshold (−8.2) and that it was very likely to be a B-cell epitope (Fig. 1D). To verify these results, a commercial anti-hTSHR mAb A9 against amino acids 147–228 of TSHR, including TSHR LRD from amino acid residues 198–205, DAFGGVYS, was chosen as the primary antibody, and mAbs recognizing other regions of TSHR were used as controls. The purified recombinant ompF protein was then immunoblotted with the aforesaid antibodies. The results demonstrated that the mAb A9 could recognize only ompF but not truncated ompF (deleting amino acids 190–197) and truncated TSHR LRD (deleting amino acids 198–205), whereas the mAbs against amino acids 22–35 and 355–358 of TSHR could not recognize the full-length and truncated ompF (Fig. 2C). All these results imply that the DALGNVTS sequence of ompF could induce the production of anti-TSHR LRD antibody through molecular mimicry.

Discussion

GD is a AITD characterized by the production of autoantibodies that bind to the TSHR and cause hyperthyroidism. Many studies have suggested an association between the pathogenesis of GD and infection with YE (8–11, 30, 31). Our clinical study results also supported this conclusion. A potential mechanism of TRAb induction could be molecular mimicry and namely the YE-specific immune response cross-reacts with thyroid-specific components resulting in GD (11). Therefore, identifying YE proteins cross-immunogenic with TSHR became the key step of the research. To prove this hypothesis, various studies were performed and demonstrated that there was epitope homology and cross-immunogenicity between proteins of YE (*e.g.* lipoproteins and envelope proteins) and the TSHR (12, 14, 15). However, traditional technology was not convenient enough to identify a new protein antigen in YE. With the progress of immunoproteomic technology, the process of characterizing unknown cross-reactive protein antigens can be done more conveniently. With the help of these techniques, we found and confirmed that the protein ompF in YE was recognized by TRAb in the serum of GD patients. This association was previously unreported in the literature. ompF contains 379 amino acid residues and is an integral membrane protein that forms a trimer in the outer membrane of YE. These features of ompF make it easily recognized by the immune system, facilitating the potential induction of TRAb. We did not find the cross-antigens in YE reported previously (lipoproteins, envelope proteins, or Yersinia outer membrane protein). This is most likely the result of several factors. First, it is possible that there are multiple proteins of YE that can induce TRAb, and their interactions and effects on GD need to be further studied. Second, due to the impact of different factors (such as age, area, diet, *etc.*) on GD, the anti-YE antibodies in GD patient sera may be different in the studies previously reported. In addition, the concentration of SDS-PAGE, the time of electrophoresis, and other different experimental conditions

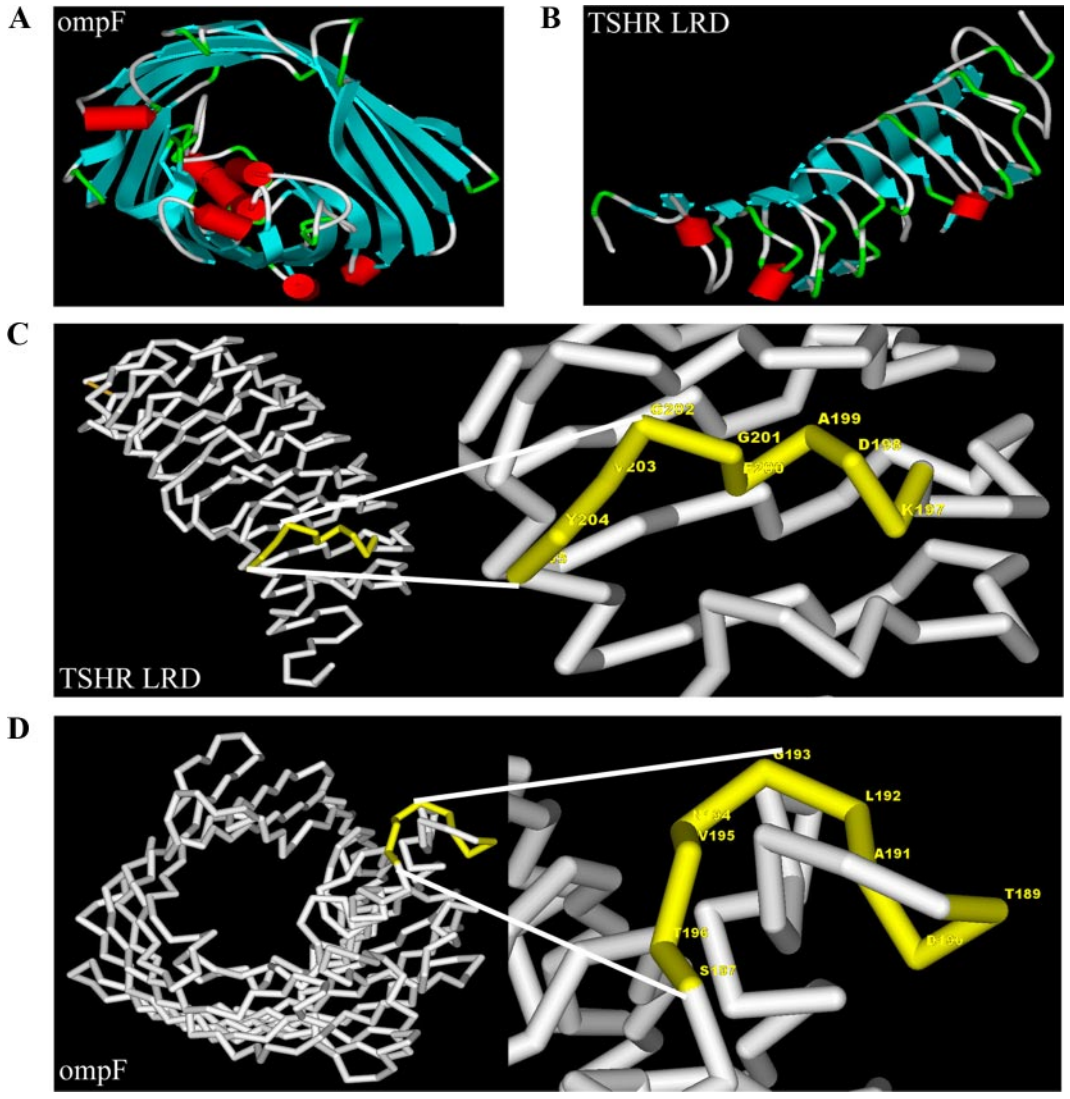

FIG. 5. Assays of the protein crystallographic structures and determination of the epitope on ompF (A and B). The protein crystallographic structures of ompF (A) and TSHR LRD (B) were homology modeled from 1OSM and 1XWD as well as the published literature, respectively. C and D, The protein crystallographic structures of sequences from amino acids 198–205 of TSHR LRD (DAFGGVYS) (C) and 190–197 of ompF (DALGNVTS) (D) indicated that the two sequences are located at the surface of the proteins and in a prominent position with good accessibility.

may also affect the final results (*e.g.* the molecular masses of lipoproteins and envelope proteins were very low, <15 kDa).

Determination of the epitope structure recognized by TRAb using epitope mapping can provide crucial information about the interaction between YE proteins and TRAb for characterizing and clarifying the role of YE infection in the pathogenesis of GD at the molecular level. Many traditional epitope-mapping techniques have limitations, and these limitations reduce the rate of success of epitope mapping (32). Moreover, it is more difficult to identify the epitopes that cross-immunoreact with hTSHR in the bacterial proteins that have evolved very differently from human proteins. Although the sequence homology was very low between the ompF protein of YE and the human TSHR, the length of the epitopes varied considerably, and B-cell epitopes are perhaps only five to seven amino acids. In addition, some nonadjacent amino acid sequences of ompF might be in a spatial position that would result in an epitope with identity to sequence regions within TSHR LRD as a result of protein folding, leading to cross-immunoreactivity with TSHR.

The N-terminal LRD of hTSHR, located in the extracellular region, is strongly immunogenic, and it is the specific binding domain of the TSH/TRAb. The epitopes in TSHR recognized by TRAb are mainly located in this region and lead to hyperthyroidism and GD (33). Therefore, we focused on the analysis of LRD. The spatial structures of ompF and TSHR LRD were constructed by homology modeling. Based on these protein structures, similar epitopes in ompF and TSHR LRD were determined using various predictive algorithms. According to these results, the corresponding commercial anti-hTSHR mAbs were chosen to

verify the predicted epitopes. This simplified the experiments and improved the efficiency and success rate of epitope mapping. This strategy may be helpful in the study of cross-reactive antigens in other autoimmune diseases.

Although it is well known that infections play essential roles in the development of autoimmune diseases, we have little knowledge of the molecular mechanisms involved at present. Although many studies of GD and YE have been carried out since the 1970s, the role of YE in the pathogenesis of GD remains controversial (16, 34–36). One important reason is the lack of the detailed studies of molecular mechanisms. Most of the experimental studies previously reported showed a class of protein antigens in YE with shared cross-immunogenicity with TSHR and were recognized by TRAb. However, the epitopes in these exogenous protein antigens of YE recognized by TRAb were not identified. Thus, it was considered that these results were inconclusive. In this paper, we found that the YE protein ompF shared cross-immunogenicity with the TSHR LRD using mass spectrometry and the bioinformatics tools of protein structure modeling and epitope prediction. The epitope recognized by anti-hTSHR antibody was located within this region at amino acids 190–197 of ompF. The anti-ompF polyantibody showed TSAb activity. The levels of cAMP in TFCs and CHO-TSHR cells stimulated by anti-ompF polyantibody were higher than that stimulated by anti-TSHR mAb A9. This result implied that other sequences of ompF might also share cross-immunogenicity with hTSHR, and this warrants further study. Therefore, the role of YE in the pathogenesis of GD is very complex, and our results might provide clues for future studies. With the help of epitope extraction and epitope excision techniques based on affinity purification and mass spectrometry, the more precise and detailed studies of the epitopes in ompF recognized by the anti-hTSHR antibodies and the effects of various antibodies induced by the aforesaid epitopes on thyroid function are ongoing. Additionally, the interactions of ompF with lipoproteins, envelope proteins, and YOP, which shares cross-immunogenicity with TSHR, and the exact roles it plays in inducing the production of TRAb should be studied more extensively. Only on the basis of further in-depth molecular studies will the roles of YE in the development of GD be fully understood.

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