Chaperonin-containing T-complex Protein 1 Subunit ζ Serves as an Autoantigen Recognized by Human Vδ2 γδ T Cells in Autoimmune Diseases*

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Human γδ T cells recognize conserved endogenous and stress-induced antigens typically associated with autoimmune diseases. However, the role of γδ T cells in autoimmune diseases is not clear. Few autoimmune disease-related antigens recognized by T cell receptor (TCR) γδ have been defined. In this study, we compared Vδ2 TCR complementarity-determining region 3 (CDR3) between systemic lupus erythematosus (SLE) patients and healthy donors. Results show that CDR3 length distribution differed significantly and displayed oligoclonal characteristics in SLE patients when compared with healthy donors. We found no difference in the frequency of Jδ gene fragment usage between these two groups. According to the dominant CDR3ζ sequences in SLE patients, synthesized SL2 peptides specifically bound to human renal proximal tubular epithelial cell line HK-2; SL2-Vm, a mutant V sequence of SL2, did not bind. We identified the putative protein ligand chaperonin-containing T-complex protein 1 subunit ζ (CCT6A) using SL2 as a probe in HK-2 cell protein extracts by affinity chromatography and liquid chromatography-electrospray ionization-tandem mass spectrometry analysis. We found CCT6A expression on the surface of HK-2 cells. Cytotoxicity of only Vδ2 γδ T cells to HK-2 cells was blocked by anti-CCT6A antibody. Finally, we note that CCT6A concentration was significantly increased in plasma of SLE and rheumatoid arthritis patients. These data suggest that CCT6A is a novel autoantigen recognized by Vδ2 γδ T cells, which deepens our understanding of mechanisms in autoimmune diseases.

T lymphocytes are classified structurally into two types according to either T cell receptor (TCR)αβ or γδ. The γδ T cells represent a minor population of human peripheral blood T lymphocytes and are involved in tumor immunosurveillance and immune defense against pathogenic infection (1).

Functionally, without negative selection during thymus development, γδ T cells take advantage of their limited diversity to recognize stress-induced self-protein antigens, such as MHC class I chain-related molecules A and B (2), UL-16-binding proteins (3, 4), heat shock proteins (HSPs) (5, 6), ectopically expressed mitochondrial ATPase (7), and human mutS homolog 2 (hMSH2) (8). Thus, γδ T cells play vital roles in autoimmune diseases linked to these autoantigens. A clear understanding of the roles of γδ T cells in autoimmune diseases remains unknown and even contradictory (9). Some studies have shown that γδ T cells display negative effects such as damaging tissue and aggravating autoimmune diseases (10, 11). In contrast, we and another group have shown that γδ T cells have regulatory roles in preventing an autoimmune response and relieving autoimmune disease symptoms (12, 13).

Understanding how human γδ T cells recognize autoimmune disease-related antigens could clarify the role of γδ T cells in autoimmune diseases.

We previously established a novel technical strategy to identify human TCRγδ Recognized proteins based on the binding specificity of complementarity-determining region 3 in TCRδ chain (CDR3δ) (8). We identified a DNA mismatch repair protein, hMSH2, as a stress-induced antigen, ectopically expressed on the cytomebrane of tumor cells and recognized by γδ T cells (14, 15). In this study, we investigated CDR3ζ characteristics in peripheral blood γδ T cells from systemic lupus erythematosus (SLE) patients, a common autoimmune disease that causes severe immune system dysregulation. We observed several distinct oligoclonal characteristics in the length of Vδ2 CDR3 of patients when compared with healthy controls. We found that γδ T cells infiltrated lupus nephritis tissues. Our results demonstrate that CDR3ζ peptide SL2, synthesized...
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according to the dominant CDR3 sequences in SLE patients, specifically bound to a human renal proximal tubular epithelial cell line HK-2, and blocked the cytotoxicity of γδ T cells to target cells. In addition, we show that the chaperon-containing T-complex protein 1 subunit ζ (CCT6A) is a target for peptide SL2 via SL2-mediated affinity chromatography and liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) analysis. Immunoprecipitation assay was performed to validate the binding of SL2 peptide to CCT6A in 293T Tet-Off cells. Furthermore, we found that CCT6A expressed on the surface of HK-2 cells. Cytotoxicity of γδ T cells to HK-2 cells was blocked by anti-CCT6A antibody, suggesting that CCT6A is a novel ligand for γδ T cells. Finally, we observed a significant increase in CCT6A concentration in the plasma from SLE and rheumatoid arthritis (RA) patients. In summary, we discovered a novel ligand related to autoimmune diseases for human γδ T cells. These findings highlight a γδ T cell-mediated mechanism in autoimmune diseases.

Experimental Procedures

Autoimmune Disease Patients and Healthy Control Subjects—Thirty-seven Chinese patients with SLE (30 females and 7 males, age 19–61 with an average age of 38 ± 12 years) and 36 patients with RA (31 females and 5 males, age 25–77 with an average age of 51 ± 11 years) were enrolled in this study. All patients were inpatients under the treatment as shown in Table 4. (SLE: 92% glucocorticosteroids; 76% immunosuppressor; 27% antimalarials; 16% recombinant antibody; 57% others. RA: 67% immunosuppressor; 53% glucocorticosteroids; 33% non-steroid anti-inflammatory drug; 53% antimalarials; 11% recombinant antibody; 75% others.) Forty-two healthy volunteers were enrolled as controls (29 females and 13 males, age 25–75 with an average age of 47 ± 11 years). Peking Union Medical College Hospital ethical committee approved the study.

Gene Scan and Random Sequence Analysis—Total RNA from 1 × 10⁷ peripheral blood mononuclear cells (PBMCs) was isolated from SLE patients and healthy controls with TRIzol reagent (Promega). cDNA was synthesized using oligo(dT) (Promega) and Moloney murine leukemia virus reverse transcriptase (Promega). For gene scan analysis, the CDR3 regions of β2 chain were amplified by PCR using FAM-marked primers as follows: forward, 5’-GCACCATCAGAGAGATGAAGGG-3’; reverse, 5’-FAM-AAACGGATGGTTTGG-TATGAGGC-3’. Amplified DNA was subjected to agarose gel electrophoresis, fluorescence intensity was scanned by ABI sequencer Model 3700 and analyzed by GeneScan software. For CDR3 sequencing, the entire V region of β2 chain was amplified by the following primers: forward, 5’-GCCATTGA-GTTGTTGCCTGAAACAC-3’; reverse, 5’-AACCGGATGGT-TTTGATGAGGC-3’. Amplified DNA was cloned into the pGEM-T easy vector (Promega) and sequenced with the ABI automatic sequencer 377.

Peptide Synthesis—Four CDR3 peptides were synthesized. SL1 and SL2 (SL1, ACDSVTLGDGTGTDKLIFGKG; SL2, ACD-TLVSTDKLIFGRG) corresponded to the dominant sequences derived from SLE patients. Two structurally similar peptides, SL1-Vm and SL2-Vm, were synthesized with mutations in the V gene segment (QEHSVTLGDGTGTDKLIFGKG and TQEHL-VSTDKLIFGRG, respectively); amino acids in CDR3 for each CDR3 peptide were used as controls. All peptides were biotinylated using a EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit for detection. Peptides were synthesized by the Academy of Military Medical Sciences, Beijing, China. The purity of each peptide was 85% in HPLC analysis.

Cell Culture and PBMC Preparation—The human renal proximal tubule epithelial cell line HK-2 was maintained in DMEM/F-12 medium supplemented with 10% fetal calf serum (Invitrogen). Fresh PBMCs, separated from peripheral blood of healthy donors by density gradient centrifugation on a Ficoll-Hypaque (Dakewe, Beijing, China), were grown in RPMI1640 (Gibco BRL) medium with 10% FCS, IL-2 (200 units/ml) in 24-well culture plates. γδ T Cell Expansion with Recombinant CCT6A—Fresh isolated PBMCs from healthy donors were cultured in RPMI1640 (Gibco BRL) medium with 10% FCS, IL-2 (200 units/ml) in 24-well culture plates with immobilized recombinant CCT6A protein (CUSABIO, 10 µg/ml). Plates with immobilized BSA (Sigma, 10 µg/ml) were used as a control. After 2 weeks of culture, the purity of γδ T cells was analyzed by flow cytometry with TCRγδ staining. CD69 staining was applied to detect the activation of γδ T cells at the same time.

Flow Cytometry and Confocal Microscopy Assay—CDR3 peptide-mediated binding activity to target cells (HK-2 cells/PBMCs) and CCT6A expression on HK-2 cells/PBMCs were measured with immunofluorescence assays. For flow cytometry assay, cells were incubated with biotinylated SL2 (20 µg) goat anti-human CCT6A polyclonal antibody (Santa Cruz Biotechnology). FITC-conjugated streptavidin (Pierce) or FITC-conjugated donkey anti-goat IgG antibody (Pierce) was then added and incubated for 30 min at 4°C; SL2-Vm and goat IgG (Zhongshan, Beijing, China) were used as controls, respectively. The cells were analyzed on a FACSAria flow cytometer (BD Biosciences). For confocal microscopy, cells were fixed on slides in 2% cold paraformaldehyde and incubated with goat anti-human CCT6A polyclonal antibody and FITC-conjugated donkey anti-goat IgG antibody. Cells incubated with goat IgG served as controls. Slides were examined with a confocal laser microscope (LSM 510; Carl Zeiss).

ELISA—The 96-well plates were coated with HK-2 cells (4 × 10⁵) or the total cell lysis of HK-2 cells (10 µg) or the plasma (10 µl) of SLE patients and healthy controls. After blocking with 3% BSA solution, plates were incubated with biotinylated CDR3 peptides (10 µg/well) and HRP-conjugated streptavidin (0.01 µg/well) and then read on a microplate reader (LabSystems) at 450/630 nm. Vδ2 γδ T cells (purity over 90%), amplified from PBMCs by anti-pan-TCRγδ monoclonal antibody (Immuno-tech), were rested in the medium without IL-2 for 24 h before stimulation with C3TA6 protein (CUSABIO, 5 µg/ml) or BSA (Sigma, 5 µg/ml) for 24 h. Human IL-2 and IFN-γ ELISA kits (Beijing 4A Biotech) were used to measure the concentration of IL-2 and IFN-γ in the supernatant. Human C3TA6 ELISA kit (CUSABIO) was used for detection of CCT6A concentration in human plasma. Antibody-coated wells were incubated with 100 µl of plasma from SLE patients, RA patients, and healthy donors (100 × dilution) and then detected according to the user manual. CCT6A standards were used for quantification.
Cytotoxicity and Blocking Assay—HK-2 target cells were added to 96-well plates at a density of $3 \times 10^4$ cells/well, incubated with SL2 peptide (40 μg) for 2 h at 37 °C. γδ T cells, amplified from PBMCs by immobilized anti-pan-TCRγδ monoclonal antibody, were added to the plate as effector cells at effector/target ratios (E:T) of 1:1, 10:1, and 20:1, respectively, and each condition was plated in triplicate. In addition, αβT cells, Vδ1 T cells, and Vδ2 T cells were sorted and subjected as effector cells in antibody blocking assay to determine the cytotoxicities to tumor cells of which effector cells are mediated by CCT6A (E:T = 5:1, anti-CCT6A antibody: 0.8 μg mock: equivalent amount of irrelevant isotype antibody). The four control groups used were: maximal cpm release group, volume-corrected group, background group, and spontaneous cpm release group. The cytotoxicity assay was conducted according to manufacturer's instructions (Cyto Tox 96 nonradioactive cytotoxicity assay reagent kit (Promega)).

Affinity Chromatography—SL2 peptide (5 mg) was coupled to a 1-ml HiTrap NHS-activated HP column (Amersham Biosciences) through its C terminus hydroxyl group in coupling buffer. The excess active groups uncoupled to SL2 peptide were deactivated according to manufacturer's instructions. SL2-coupled column was then equilibrated with binding buffer (20 mM PBS, pH 7.0). After HK-2 cell total proteins were added to the column, the OD value of the outflow was detected at 280 nm and elution buffer (1% formic acid) was changed until the A$_{280}$ returned to the baseline. The fractions containing eluted proteins were collected and neutralized quickly with 1 M Tris-HCl (pH 9.1). Throughout the process, the flow rate was kept under 0.2 ml/min.

Western Blotting—The enriched proteins from affinity chromatography were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The biotinylated SL2 or SL2-Vm peptide (10 μg/ml) was used as the primary antibody, and HRP-streptavidin was used as the secondary antibody. Chemiluminescent HRP substrate (Pierce) was added, and the blot was exposed to x-ray film.

Database Search and Protein Identification—The in-gel trypsin digestion and MS analysis of specific SDS-PAGE protein bands were conducted at the Beijing Proteome Research Center, China. LC ESI-MS/MS analyses were performed using an LTQ system (Thermo Finnigan). Tryptic peptide mixtures were loaded onto a capillary column (15-cm length, 75-μm inner diameter). The elution from the reverse phase liquid chromatography (RPLC) column was analyzed by MS. MS/MS spectra were searched using the SEQUEST algorithm-based BioWorks version 3.2 (Thermo Finnigan) against the International Protein Index (IPI) human database 3.19, which had 60,397 entries. Proteins with at least two unique “identity” score peptides were considered unambiguously identified, and the single peptide-matched proteins were rejected.

CCT6A Overexpression in 293T Tet-Off Cells—The Gateway cloning system was used to produce overexpression of CCT6A in 293T Tet-Off cells. CCT6A cDNA fragment was transferred from an entry clone into a destination vector generating the expression clone through the LR Clonase reaction. The entry clone (which included a CCT6A cDNA fragment) destination vector (75 ng), LR Clonase mix (Invitrogen, 2 μl), and TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0) were added to a 1.5-ml microcentrifuge tube and mixed. Reactions were incubated for 3 h at 25 °C. 1 μl of the proteinase K solution (Invitrogen) was added to the reactions to terminate the reaction. Sample was incubated at 37 °C for 10 min. The product was transformed to trans5-α, and positive clones were confirmed by restriction enzyme digestion. After transfection to 293T Tet-Off cells, the whole cell lysis solution was analyzed by Western blotting using anti-GFP (Abmart) primary antibody to identify the overexpression of CCT6A in 293T Tet-Off cells.

Immunoprecipitation Assay—Biotinylated ST2 (50 μg) and ST2-Vm (50 μg) peptides were incubated with 293T Tet-Off whole cell lysate (200 μg) overnight at 4 °C with rotation. 20 μl of streptavidin-conjugated Sepharose beads were added to the system and incubated for 3 h at 4 °C with rotation. Pellets were washed five times with cell lysis buffer. The pellet was resuspended with 20 μl of 1× SDS sample buffer and vortexed, and the sample was heated to 95–100 °C for 2–5 min. The sample was loaded on a 8% gel for SDS-PAGE. The sample was analyzed by Western blotting using anti-GFP primary antibody.

Statistical Analysis—All data were analyzed using SPSS version 13.0 software. One-way analyses of variance were used to compare data with normal distribution and homogeneity of variance. Independent sample t tests were used to compare means between two groups. p values < 0.05 were considered significant. All tests were two-tailed.

Results

The TCR Vδ2 CDR3 Regions in SLE Patients and Healthy Controls. A, representative results of PCR amplification. M, DL2000; lanes 1 and 2, the whole V region of δ2 chain (~300 bp) for CDR3δ sequencing; lanes 2 and 4, the TCR Vδ2 CDR3 region (~170 bp) for gene scan analysis; lanes 1 and 3, SLE patients; lanes 2 and 4, healthy controls. B, representative results of gene scan analysis for CDR3δ length distribution. SLE1–SLE5, five different SLE patients; C1–C5, five different healthy controls.
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tiple peaks. However, CDR3δ in SLE patients was focused at a certain length, showing a single peak or several peaks in spectra typing profiles (Fig. 1B). This suggests disease-related oligoclonal expansion of γδ T cells in SLE patients. However, we note that due to the huge individual differences in the specific length among SLE patients, we found no significant difference in CDR3δ length distribution when comparing all patients and controls (Table 1).

A total of 221 clones in 10 SLE patients and 137 clones in 7 healthy controls were randomly selected and sequenced to identify the dominant SLE-related Vδ CDR3 sequences. We identified five dominant SLE-related Vδ2 CDR3 sequences (SL1, SL2, SL3, SL4, and SL5) (Table 2). Two CDR3δ peptides, SL1 and SL2, were synthesized according to their sequences. We also synthesized two peptides, SL1-Vm and SL2-Vm, with mutant V segment sequences for controls in the following tests.

The SL1 and SL2 Peptides Did Not Specifically Bind to the Plasmas and PBMCs of SLE Patients—To investigate CDR3δ peptide binding specificities, we analyzed the binding of SL1 and SL2 peptides to the plasmas and PBMCs of SLE patients. We found that SL1 and SL2 peptides both bound to the plasmas of SLE patients, whereas control peptides SL1-Vm and SL2-Vm did not (Fig. 2A). However, binding was not SLE-specific; both SL1 and SL2 peptides bound to the plasmas of healthy controls as well. Flow cytometry shows nonspecific binding of SL2 peptide to PBMCs of SLE patients (Fig. 2B). Taken together, our data suggest that the synthesized CDR3δ peptides bind to certain targets in the plasma of both SLE patients and healthy controls.

SL2 Bound To and Blocked the Cytotoxic Effect of γδ T Cells on HK-2 Cells—We analyzed the infiltration of γδ T cells in frozen renal biopsy tissues of SLE patients. Results confirm the infiltration of γδ T cells in the kidney (data not shown), suggesting that γδ T cells recognize certain antigens in renal epithelial cells. Given the infiltration of γδ T cells in the kidney of lupus nephritis patients, we used the human renal proximal tubule epithelial cell line HK-2 to validate SL2 binding specificity.

We measured the binding of SL2 peptides to the human renal proximal tubule epithelial cell line HK-2 by flow cytometry, with PBMC as control cells. We found that SL2 bound HK-2 cells, but SL2-Vm did not (Fig. 3A). Binding was in a dose-dependent manner (Fig. 3B).

### TABLE 1

| Transcript length (bp) | Subjects |
|-----------------------|----------|
|                       | Healthy controls (n = 9) | SLE (n = 5) | p value |
| <130                  | 0 ± 0%   | 0.13 ± 0.4% | NS       |
| 130–140               | 33.83 ± 8.02% | 37.76 ± 14.12% | NS       |
| 141–150               | 35.54 ± 21.68% | 37.53 ± 21.68% | NS       |
| 151–160               | 30.16 ± 11.97% | 22.17 ± 13.02% | NS       |
| 161–170               | 0.48 ± 0.96% | 2.41 ± 5.11% | NS       |

### TABLE 2

The usage of TCR Vδ2 CDR3 J segments derived from SLE patients and healthy controls

| J segment usage (%) | Subjects |
|---------------------|----------|
|                     | Healthy controls (n = 7) | SLE (n = 5) | p value |
| Vδ2                 | 93.10 ± 5.54% | 91.90 ± 8.87% | NS       |
| J1                  | 1.63 ± 2.14%  | 0.48 ± 1.51%  | NS       |
| J2                  | 4.89 ± 5.73%  | 6.70 ± 7.51%  | NS       |
| J3                  | 0.39 ± 1.02%  | 0.33 ± 1.05%  | NS       |
| J4                  | 8.00 ± 8.00%  | 8.00 ± 8.00%  | NS       |

### TABLE 3

Dominant CDR3 sequences of TCRδ2 chain in SLE patients

| Name | V | N | J | J6 segment | Peptide synthesized |
|------|---|---|---|------------|---------------------|
| SL1  | ACD | SVTLGDTG | TDKLIFGK | J1 | Y |
| SL1-Vm | QEH | SVTLGDTG | TDKLIFGK | J1 | Y |
| SL2  | ACID | LVS | TDKLIFGK | J1 | Y |
| SL2-Vm | TQEH | LVS | TDKLIFGK | J1 | Y |
| SL3  | ACD | KLGEPY | TDKLIFGK | J1 | Y |
| SL4  | ACID | VTTGGYGETN | TDKLIFGK | J1 | Y |
| SL5  | ACID | LGDTS | TDKLIFGK | J1 | Y |

*The sequences of synthesized control CDR3δ peptides with a mutant V segment. Y, yes.
Consistent with flow cytometry results, we found that SL2 specifically bound to HK-2 cells and HK-2 cell total protein extracts in SL2-mediated ELISA, whereas SL2-Vm did not (Fig. 3C). These data indicate that the SL2 peptide, derived from dominant SLE patients, specifically binds HK-2 cells.

γδ T cells lyse target cells via a TCR-mediated pathway. We determined whether binding of synthesized SL2 to HK-2 cells affects the cytotoxicity of γδ T cells to HK-2 cells using a peptide-blocking cytotoxicity assay. The purity of γδ T cells was above 80% after a 2-week culture with anti-pan-TCRγδ monoclonal antibody, with the purity more than 85% in flow cytometry analysis, used as effector cells in a cytotoxicity assay (E). A Cyto Tox 96 nonradioactive cytotoxicity assay reagent kit was used to evaluate the cytotoxicity inhibition of γδ T cells against HK-2 cells after preincubation with SL2 peptide. SL2 peptide (40 μg/well) was incubated with HK-2 cells for 2 h before adding γδ T cells. E:T stands for the ratio of effector cells to target cells. Results are representative of three independent experiments. *, p < 0.05, the OD value of SL2 peptide-coated wells when compared with that of wells without SL2 on the same E:T.

Identification of SL2 Peptide-bound Proteins in HK-2 Cells—We screened the total cell lysate of HK-2 cells by SL2-mediated affinity chromatography. The 1-ml HiTrap NHS-activated HP column was coupled with 4.5 mg of SL2 with a coupling ratio of 90% (data not shown). The elution peak was collected after washing with elution buffer (Fig. 4A). The protein fractions were pooled and concentrated. Western blotting analysis indicated two specific protein bands, with the 35–40-kDa protein band being SL2-specific (Fig. 4B). We analyzed the specific band by LC ESI-MS/MS and found a total of 20 proteins with at least two unique peptides using a SEQUEST search. T-complex protein 1 subunit ζ had a highly similar unique peptide count and a higher cover percentage. We obtained the CCT6A cDNA by the gateway system, and successfully overexpressed the CCT6A protein in 293T Tet-Off cells (Fig. 4C). Immunoprecipitation experiments were carried out using the CCT6A overexpression cell model. The results show that CCT6A protein can be specifically precipitated by SL2 peptide (Fig. 4D), showing a direct interaction between SL2 and intracellular CCT6A protein.

Surface-expressed CCT6A May Be a Novel Antigen Recognized by Vδ2 γδ T Cells—T-complex protein 1, also named CCT, is the most unique and complex eukaryotic cytosolic chaperonin. It is involved in the folding of only a small set of proteins. CCT is composed of two superimposed rings, each
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**Discussion**

Although γδ T cells belong to a minor subset of total T cell pool in the peripheral blood, γδ T cells play important roles in host defense against microbial infections, monitoring of tumorigenesis, immunoregulation, and development of autoimmunity (17). However, reports are limited for TCRγδ-recoognized antigenic proteins. It is noted that γδ T cells recognize some of the stress-inducible self-protein antigens (18).

We previously developed a novel and specific strategy to identify antigens recognized by γδ T cells based on the binding specificity of the CDR3δ peptide. We identified DNA mismatch repair protein hMSH2 as a tumor-related antigen recognized by γδ T cells (8). Further research showed that some of the tumor cells express mutant hMSH2 on the cell surface, implying an interaction with TCRγδ. Ectopically expressed hMSH2 in a malignant environment, including virus infection, oxidative stress, and tumorigenesis, created a signal alerting the immune system in immunosurveillance via γδ T cells (14, 15). Further, engineered antibodies, based on the CDR3δ sequence, showed tumor binding specificity and antitumor effects in mouse xenograft models (19, 20). In this study, we demonstrated that a novel autoimmune disease-associated antigen CCT6A is recognized by γδ T cells. This result further validates our specific CDR3δ-based immunological biochemical strategy.

The key step in our CDR3δ-based strategy was to choose a suitable CDR3δ probe by following two basic standards. First, the CDR3δ sequence must be the dominant sequence, including the highest frequency and the most shared by disease samples. Second, and more importantly, the synthetic CDR3δ peptides must specifically bind to disease tissues, cells, or molecules.

We chose two candidate CDR3δ sequences and synthesized two peptides, SL1 and SL2, and two control peptides, SL1-Vm and SL2-Vm, with the V region mutation. We used ELISA and flow cytometry to verify the binding specificity of these peptides to plasma and PBMCs of SLE patients. CDR3δ derived from tumor infiltrate lymphocytes specifically binds to tumors (21). However, we found that SL1 and SL2 bound to plasma of SLE patients and healthy controls. This is likely due to the source of the CDR3δ sequence. The tumor-specific CDR3δ was derived with eight different subunits (CCTα, -β, -γ, -δ, -ε, -ζ, -η, and -θ; CCT1–CCT8). CCT6A is the ζ subunit of CCT (16).

To further validate the expression and function of CCT6A, we measured CCT6A expression on the cell surface of HK-2 cells by immunofluorescence assays. Our confocal images show that CCT6A antibody stained HK-2 cells on the surface and in the cytoplasm, whereas the isotype antibody did not. We did not observe this in PBMC samples (Fig. 5A). Flow cytometry results show that 14% of HK-2 cells were positive for surface staining for CCT6A, when compared with less than 2% in normal PBMCs (Fig. 5B). Immobilized CCT6A protein could significantly expand γδ T cells from PBMC when compared with BSA. The expression of activation marker CD69 was also significantly increased on the CCT6A expanded γδ T cells (Fig. 5, C and D). The levels of IFN-γ and IL-2 were significantly higher in the supernatant of CCT6A-stimulated γδ T cells when compared with BSA control (Fig. 5E). In our cytotoxicity blocking assay, we found that the CCT6A antibody blocking effect was only specific for the Vδ2 T cells and that there was no significant change in the cytotoxicity of αβ T cells and Vδ1 T cells to HK-2 cells after CCT6A antibody blocking (Fig. 5F). These findings suggest that surface CCT6A is a newly described antigen that mediates cytotoxicity of Vδ2 γδ T cells to CCT6A-expressing target cells.

**High Concentration of Plasma CCT6A in Autoimmune Disease**—Given that the SL2 peptide sequence is derived from the dominant CDR3δ of SLE patients, we investigated a connection between CCT6A and autoimmune diseases SLE and RA. The concentrations of CCT6A in the plasmas of 42 healthy controls, 37 SLE patients, and 36 RA patients were detected by sandwich ELISA. Results show wide individual differences in plasma CCT6A concentration among these samples (Fig. 6). CCT6A concentration in healthy controls was 33.26 ± 17.34 ng/ml, significantly lower when compared with total patients (75.07 ± 31.60 ng/ml). SLE patients (55.89 ± 11.21 ng/ml), and especially RA patients (94.79 ± 33.71 ng/ml). Because all patients were inpatients and were treated with a variety of medicines, we analyzed whether different treatments affected the levels of plasma CCT6A. However, no significant difference was observed in the levels of plasma CCT6A among different treatments (Table 4). The results suggest that a high concentration of plasma CCT6A is associated with the pathological functions of γδ T cells in autoimmune diseases. The detailed mechanism needs to be further researched.
from tumor infiltrate lymphocytes of tumor patients, not PBMCs. For this reason, to identify a more specific CDR3 probe for T cells, we should analyze CDR3 sequences of T cells in damaged tissues of patients with autoimmune diseases such as the synovial fluid of damaged joints in RA patients or kidneys of lupus nephritis patients. The dominant CDR3 sequence was analyzed from randomly picked clones. The results did not fully reflect the CDR3 repertoire. Future studies will require immune repertoire sequencing techniques, based on specific PCR amplification and high-throughput sequencing (22, 23), for a comprehensive and systematic analysis.

CCT is a eukaryotic cytosolic chaperonin with large oligomers composed of eight subunits. Chaperonins are a ubiquitous family of proteins that assist the folding of other proteins, and are split into two main groups. One is GroEL proteins, which are present in eubacteria and in the eukaryotic organelles of endosymbiotic origin (mitochondria and chloroplast) and which are the most well characterized (24, 25). The other is...
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CCT proteins, which are found in archaeabacteria and in the cytosol of eukaryotic organisms. Although CCT proteins share little sequence homology with eubacterial counterparts, they are more heterogeneous in structure and composition (16). CCT is primarily involved in the folding of actin and tubulin, but data suggest additional cellular roles. CCT is composed of eight different, albeit homologous, subunits (CCTα, β, γ, δ, ε, ζ, η, and θ; CCT1–CCT8). Previous studies have focused on the chaperone function, including the folding mechanism with its main substrates actin (26) and tubulin (27). Some research has focused on the role of CCT in diseases caused by damage to cytoskeleton structure, including tumors (28, 29) and some neurodegenerative diseases (30). CCT6A is a self-protein that also exists in the normal human plasma, but is not expressed on the PBMC surface. This explains our results showing that the SL2 peptide binds to both normal plasma and plasma of SLE patients, but not to both PBMCs.

HSPs, a major chaperone family, are up-regulated under stressful conditions, and are involved in immune responses to various pathogens and tumors. They are also the target antigens for γδ T cells (5, 6). In this study, we demonstrated that the molecular chaperone CCT6A is recognized by γδ T cells. There are two key problems to be solved regarding the interaction of CCT6A and γδ T cells. First, given that CCT6A is one of the subunits of the CCT complex, can γδ T cells recognize only CCT6A, or can they also identify other CCT subunits? Second, HSPs are stress-induced proteins that can be ectopically expressed on the surface of target cells, and that are shed from the cell surface, presenting in the serum of cancer patients as a soluble form under stress conditions. Does the eukaryotic CCT family have stress-induced characteristics as well? In this study, we found that CCT6A is expressed on the surface of some HK-2 cells, but not PBMCs. In addition, we found high concentrations of CCT6A in plasma from a considerable number of healthy individuals. However, the detailed mechanism is still unclear.

Autoimmune diseases, including SLE and RA, are characterized by abnormal immune responses to self-antigens. Although the pathogenesis of most autoimmune diseases is not yet fully elucidated, it is generally accepted that they are induced by environmental factors on a genetically susceptible background, leading to abnormality in antigen recognition, antigen presentation, and T/B lymphocyte activation and differentiation, and subsequently resulting in enhanced production of proinflammatory cytokines and autoantibodies, which eventually cause damage to specific organs and tissues.

The roles of γδ T cells in the pathogenesis of autoimmune diseases have attracted attention in recent years (9). As a subset of T cells that bridge innate and adaptive immunity, γδ T cells display different functions in the pathogenesis of autoimmune diseases including antigen presentation, proinflammatory, and B cell helper functions. Much evidence suggests that γδ T cells confer immunoregulatory functions by interacting with regulatory T cells. We previously demonstrated a novel human regulatory γδ T cell with CD27+CD45RA−Vα1 phenotype and demonstrated their suppressive effect on the proliferation of autologous naive CD4+ T cells (12).

In the present study, we identified an autoimmune disease-associated antigen, CCT6A, recognized by Vδ2 γδ T cells. We found that CCT6A concentration in plasma of autoimmune disease patients was significantly higher when compared with healthy controls. The increased CCT6A in the pathologic process may be due to the significant increase of autoantibody titers against CCT (31). Here, we offer a hypothesis to explain the relationship between an increase in plasma CCT6A and γδ T cell function during the pathogenesis of autoimmune disease. Given immunoregulation disruption, γδ T cells recognize CCT6A expressed on tissue cells, leading to tissue damage. Damaged tissue cells release more soluble CCT6A and significantly elevate the plasma CCT6A concentration. A high concentration of plasma CCT6A may have two opposing mechanisms for further development of disease. On the one hand, soluble CCT6A competitively binds to γδ T cells, hindering the binding of γδ T cells to CCT6A-expressing tissue cells and forming a negative feedback to tissue damage. On the other hand, soluble CCT6A maintains a sustained activation of more

**TABLE 4**

The plasma CCT6A concentration of SLE/RA patients under different treatments

| Treatment               | SLE (n = 37) | Case | CCT6A (ng/ml) | RA (n = 36) | Case | CCT6A (ng/ml) |
|-------------------------|--------------|------|---------------|-------------|------|---------------|
| Glucocorticosteroids    | 34           | 55.7 ± 11.5 |                | 19          | 96.1 ± 34.2   |
| Immunosuppressor        | 28           | 54.2 ± 11.4 |                | 24          | 101.9 ± 32.9  |
| Antimalarials           | 10           | 60.8 ± 8.7  |                | 19          | 84.1 ± 31.2   |
| Recombinant antibody    | 6            | 58.3 ± 5.3  |                | 4           | 85.8 ± 15.4   |
| Others                  | 21           | 56.8 ± 9.7  |                | 12          | 80.4 ± 31.7   |
|                         |              |       |               | 27          | 91.8 ± 32.8   |
γδ T cells responding to other self-antigens and causes more serious pathological damage. Although γδ T cells are involved in the first line of defense, playing a role in the early stages of tissue injury, CCT6A is likely to change in the early stages of autoimmune diseases. Plasma CCT6A concentration may be related to the process of autoimmune diseases, and can be used as a marker for early immune diagnosis of autoimmune diseases. In ongoing research, we are trying to clarify the detailed mechanisms and further verify with a larger number of clinical samples.

In summary, we successfully identified an autoimmune disease-related antigen, CCT6A, recognized by human Vβ2 γδ T cells. This is a novel finding for a γδ T cell–recognized autoimmune and will further our understanding of the role of γδ T cells in autoimmune diseases.

Author Contributions—H. C. conducted the experiments on clinic samples, and wrote the paper. H. Y. conducted the experiments on functional validation of CCT6. L. W. conducted the experiments on CCT6 identification using CDR3 peptide. Z. X. provided all patient samples used in the paper. W. H. and J. Z. conceived the idea for the project, and all of the experiments were on the direction of them.

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