Anti-CD69 Autoantibodies Cross-React with Low Density Lipoprotein Receptor-Related Protein 2 in Systemic Autoimmune Diseases

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We investigated whether autoantibodies to CD69, one of the earliest markers of lymphocyte activation, exist in the sera of patients with systemic autoimmune disease. Serum samples were obtained from patients with rheumatoid arthritis (RA), systemic lupus erythematosus, and Behcet’s disease, and were tested for the presence of anti-CD69 autoantibodies by ELISA and Western blotting using rCD69 fusion proteins. IgG-type autoantibodies to CD69 were detected in the sera of 38.3% of the RA patients, 14.5% of the systemic lupus erythematosus patients, and 4.0% of the patients with Behcet’s disease. Among those with RA, the anti-CD69 autoantibody-positive patients had a higher serum level of rheumatoid factors and a more accelerated erythrocyte sedimentation rate than the anti-CD69 autoantibody-negative patients. Further, the predominant epitope on the CD69 molecule to which most of the anti-CD69 autoantibody-positive serum samples exclusively reacted, was mapped at the C terminus of CD69. Of interest, this epitope is homologous to a stretch of amino acids in the protein sequence of low-density lipoprotein receptor-related protein 2 (LRP2), which is a receptor for multiple ligands including β₂-low density lipoprotein and is also an autoantigen responsible for Heymann nephritis in rats. The anti-CD69 autoantibody cross-reacted to LRP2 through the homologous amino acid sequence. To our knowledge, this is the first evidence of the existence of anti-CD69 autoantibodies. This autoantibody may modulate the function of CD69- and LRP2-expressing cells. The Journal of Immunology, 2001, 166: 1360–1369.

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lymphocytes in the synovial fluid and synovial membranes, although CD69 is not present on the surface of circulating PBLs (26, 27). The level of CD69 expression on synovial T cells in RA is correlated with disease activity (28). The T cells of patients with SLE exhibit decreased or defective induction of CD69 upon stimulation (29). Further, a low CD69 to CD3 ratio on the surface of PBL is reported to be correlated with high disease activity in SLE (30). The T lymphocytes of patients with HIV also exhibit abnormal CD69 expression (31).

In this study, we demonstrated that autoantibodies to CD69 exist in the sera of patients with various autoimmune diseases, using a rCD69 molecule and that the anti-CD69 autoantibodies bound to a native form of CD69 on lymphocytes. Interestingly, we found that there is only one dominant autoepitope on the CD69 molecule. This epitope is homologous to a portion of low-density lipoprotein receptor-related protein 2 (LRP2), autoimmunity to which is reported to cause nephritis in rats (Heymann nephritis) (32). Further, we show that the autoantibody to CD69 cross-reacts to LRP2.

Materials and Methods

Human sera

Serum samples were obtained from a total of 137 patients with systemic autoimmune disease (110 females and 27 males; mean age 50.5 years, ranging between 20 and 79 years), which included 60 patients with RA (45 females and 15 males; mean age 57.2 years, ranging between 22 and 79 years), 55 patients with SLE (51 females and 4 males; mean age 42.7 years, ranging between 20 and 72 years), and 22 patients with Behcet’s disease (14 females and 8 males; mean age 50.9 years, ranging between 24 and 78 years). Each patient was diagnosed according to the standard criteria of the respective disease (33–35). The patients were being treated at the hospital of the University of Tokyo or the hospital of St. Marianna University School of Medicine. Control sera were obtained from 75 healthy donors (58 females and 17 males; mean age 49.7 years, ranging between 22 and 82 years). All serum samples were stored at −20°C until assay. Age and sex-matched control samples were used for each disease category.

Preparation of CD69 cDNA

PCR was performed on cDNA prepared from the lymphocytes of a healthy donor, to amplify CD69 cDNA. Based on the previously reported nucleotide (N) sequence of human CD69 (18), the primers, 5'-TTTGgaatcATGACCTGAAATGGTTTTGCT-3' and 5'-TTTGgaatcTTATTTGGTGA-3' were synthesized and used to amplify the DNA fragment (600 bp) that encodes the entire protein coding region (nucleotides 74–673) of CD69. The conditions of PCR were denaturation at 94°C for 1 min, annealing at 54°C for 2 min, and extension at 72°C for 1 min, for 35 cycles.

Construction of the expression plasmids of the entire and partial CD69 molecules

The cDNA fragment which encodes the entire CD69 molecule was subcloned into the EcoRI/SalI site of the pMAL-c expression vector (New England Biolabs, Beverly, MA) to form pMAL-CD69 full as previously described (36). The inserted cDNA was expressed as a maltose binding protein (MBP) hybrid protein. The DNA restriction enzymes were purchased from Takara Shuzo (Kyoto, Japan).

To investigate the distribution of autoepitopes on the CD69 molecule, we prepared three overlapping peptides of CD69 (encoded by F1, F2, and F3), which covered the entire protein-coding region of CD69 (Fig. 1f). The F1, F2, and F3 were each amplified from pMAL-CD69 full by PCR with the following primers: F1 (NT47-352), 5'-TTTGgaatcATGAGCTCTTGAATATTGTGTCTG-3' and 5'-TTTGgaatcTTATTTGGTGAG-3'; F2 (NT347-511), 5'-TTTGgaatcATGAGCTCTTGAATATTGTGTCTG-3' and 5'-TTTGgaatcTTATTTGGTGAG-3'; and 5'-TTTGgaatcATGAGCTCTTGAATATTGTGTCTG-3' and 5'-TTTGgaatcTTATTTGGTGAG-3'. Each of these amplified fragments was subcloned into pMAL-c to produce pMAL-CD69 F1, pMAL-CD69 F2, and pMAL-CD69 F3. Each amplified DNA fragment was expressed as a fusion protein with MBP in E. coli. CYT, Cytoplasmic region; TM, transmembrane region. b, Preparation of the rCD69 protein expressed in E. coli. The purified rCD69 and MBP were loaded onto three lanes of 10% SDS-PAGE, transferred onto nitrocellulose membranes, and then stained with Ponceau S (left), anti-MBP Ab (middle) and anti-CD69 Ab (right).

FIGURE 1. a, Mapping of the human CD69 gene. Map of the cDNA clone of CD69 is shown at the top. The span of amino acid residues expressed by the entire CD69 and the truncated CD69 fragments F1, F2, and F3. F3, The span of amino acid residues expressed by the F3a, F3b, F3c, and F3d fragments. Each of these DNA fragments was expressed as a fusion protein with MBP in E. coli. F1, F2, F3. b, Preparations of the rCD69 protein expressed in E. coli. The purified rCD69 and MBP were loaded onto three lanes of 10% SDS-PAGE, transferred onto nitrocellulose membranes, and then stained with Ponceau S (left), anti-MBP Ab (middle) and anti-CD69 Ab (right).

Expression and purification of the recombinant fusion protein

Escherichia coli (DH5α; ToYoBo, Tokyo, Japan) was transformed with each of these recombinant pMAL plasmids, and then grown in 2 × YT medium containing 100 μg/ml ampicillin at 30°C. To induce expression of the fusion protein, isopropyl-1-thio-β-D-galactoside was added to the medium to a final concentration of 0.3 mM, and the E. coli were incubated at 25°C for 7 h. Purification of fusion protein was performed as described elsewhere (36). Briefly, the cells were harvested by centrifugation at 4°C for 10 min. The cells were suspended in column buffer (10 mM sodium phosphate, 0.5 M NaCl, 1 mM sodium azide, 10 mM 2-ME, 1 mM sodium dodecyl sulfate, 1 mM 2-mercaptoethanol).
samples. The fusion protein was then stored at −20°C until use.

**ELISA**

Ninety-six-well microtiter plates (Cook Dynatech, Alexandria, VA) were coated by placing in each well 50 μl of 10 mg/ml purified fusion protein or MBP (as a background) in carbonate buffer (50 mM sodium carbonate, pH 9.6) at 4°C overnight. After washing with PBS containing Tween 20 (0.1%) three times, the plates were washed with PBS-Tween 20 (0.1%) 3 times. To absorb the reactivity of the serum sample to bacterial proteins and MBP, each serum sample was incubated with 20 μg/ml of bacterial lysate containing nonrecombinant pMAL-c product in 5% BSA-PBS-Tween 20 (0.1%) at room temperature for 2 h before being placed in the wells coated with recombinant protein. Fifty microliters of each serum sample diluted with 3% BSA-PBS-Tween 20 (0.1%), was placed in each well at 4°C overnight. After washing four times with PBS-Tween 20 (0.1%), the plates were incubated in 3% BSA-PBS-Tween 20 (0.1%) three times, the plates were incubated in 3% BSA-PBS-Tween 20 (0.1%) at room temperature for 2 h before being subject to ELISA.

For the inhibition experiments, the serum sample was incubated with various concentrations of the inhibitor for 2 h at room temperature, before being subject to ELISA.

**Adsorption of rheumatoid factors (RFs) from sera of patients with RA**

Sera were heat-inactivated at 56°C for 30 min. Then, sera diluted at 1:20 were incubated with denatured rabbit IgG-coated latex particles (Fujirebio, Tokyo, Japan) for 1 h at room temperature. After centrifugation, supernatants were subjected to the second adsorption in the same manner. Finally, titers of RFs in the serum samples were measured using an RA particle-agglutination (RAPA) test kit (SERODIA®-RA; Fujirebio).

**Western blotting**

Western blotting was performed as described previously (37). Briefly, 5 μg of each purified fusion protein or MBP (as a control), was separated by 10% SDS-PAGE, and then transferred onto a nitrocellulose membrane. After blocking with PBS containing 3% BSA and 0.1% Tween 20 for 1 h at room temperature, each membrane was incubated in the wells coated with recombinant protein. Fifty microliters of each serum sample diluted with 3% BSA-PBS-Tween 20 (0.1%), was placed in each well at 4°C overnight. After washing four times with PBS-Tween 20 (0.1%), the plates were incubated in 5000-fold-diluted HRP-conjugated goat anti-human IgG Ab for 8 h at 4°C, and then washed four times with PBS-Tween 20 (0.1%). Color development was achieved by adding 100 μl of the peroxidase substrate, which consisted of 0.04% o-phenylenediamine and 0.01% hydrogen peroxide in 0.1 M citrate/0.2 M Na2HPO4 (pH 5.0) to each well. After 15 min, the color reaction was stopped by adding 50 μl of 6 N H2SO4 to each well. The absorbance was measured with an ELISA microplate photometer at 492 nm. Each sample was measured in duplicate.

The reactivity to the fusion protein in ELISA was expressed in units according to the following formula: binding unit = ODsample/100(mean ODsample ± 3 SD of normal sera)/ODfusion protein − ODsample). For each sample, the OD value of MBP was subtracted from the OD value of the fusion protein to obtain ODsample. According to this formula, 100 binding units is the cut-off point.

**Homology search**

The CD69 cDNA sequence and its deduced amino acid sequence were analyzed with SDC-GENETYX Genetic Information Processing Software (Software Development, Tokyo, Japan), using the database of the National Biomedical Research Foundation and the SWISS-PROT protein sequence database of the European Molecular Biology Laboratory.
Flow cytometry

Binding of the anti-CD69 autoantibodies to native CD69 molecules on lymphocytes was investigated by indirect immunofluorescence. To this end, Jurkat cells nonstimulated or stimulated with 10 ng/ml of PMA (Sigma, St. Louis, MO) for 18 h at 37°C were used as CD69-negative and -positive cells respectively. After the Jurkat cells with or without PMA stimulation were washed in a staining buffer (PBS containing 2% BSA), the cells were incubated with anti-CD69 autoantibody-positive patients' serum samples diluted 1:1 by staining buffer for 30 min on ice. After additional washing in the staining buffer, the cells were incubated with PE-conjugated goat anti-human IgG (heavy and light chain) (Beckman Coulter, Fullerton, CA). To determine expression of CD69 on the untreated or PMA-stimulated Jurkat cells, PE-conjugated mouse anti-human CD69 mAb (Beckman Coulter) was used. To specify the binding of anti-CD69 autoantibodies in the serum samples, the serum samples were preincubated with 2.5 μM of MBP-CD69 (full length) or MBP (as a control) for 1 h at room temperature before reacting with the Jurkat cells. The fluorescence intensity was measured by FACScalibur (Becton Dickinson, Mountain View, CA).

Statistical analysis

Laboratory parameters are expressed as the mean ± SEM. The Mann-Whitney U test and Fisher’s exact test were used to examine the significance of the difference of the laboratory parameters of the RA patients with and without anti-CD69 autoantibody. Values of \( p < 0.05 \) were considered to be statistically significant.

Results

Expression of the rCD69 molecules

The DNA fragment encoding the entire human CD69 molecule was obtained by PCR using the cDNA of the PBL of a healthy donor (Fig. 1a). The recombinant full-length CD69 was then produced as a fusion protein with MBP in E. coli. We obtained a sufficient amount of the fusion protein which had the expected molecular mass. The nucleotide sequence of the fusion protein was identical with the previously reported sequence (18). Further, we confirmed that the rCD69 protein was stained with anti-MBP Ab and also with anti-CD69 Ab by Western blotting (Fig. 1b). These data demonstrated that the rCD69 protein was correctly produced.

Reactivity of the sera of patients with various systemic autoimmune diseases to the rCD69 protein

We investigated whether autoantibodies to CD69 exist in the sera of patients with various systemic autoimmune diseases by ELISA using the above rCD69 molecules. As shown in Fig. 2, IgG-type anti-CD69 autoantibodies were detected by ELISA in the sera of 28 of the 137 (20.4%) patient serum samples. In contrast, autoantibodies to rCD69 were not detected in the sera of any of the healthy donors. The prevalence of anti-CD69 autoantibodies in

Table I. Reactivity of anti-CD69 autoantibody-positive serum samples to full length and the three truncated recombinant proteins of CD69, as tested by ELISA and Western blotting

<table>
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<th>F3</th>
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</tr>
<tr>
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<td>+</td>
</tr>
<tr>
<td>RA-44</td>
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<td>–</td>
<td>–</td>
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</tr>
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<td>5</td>
<td>5</td>
<td>28</td>
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E: ELISA; W: Western blotting.
Each disease category was as follows: 19 of the 60 (31.6%) patients with RA, 8 of the 55 (14.5%) patients with SLE, and 1 of the 22 (4%) patients with Behcet’s disease (Table I).

Each serum sample was also tested for the presence of anti-CD69 autoantibodies by Western blotting. Eighteen of the 28 samples that were positive by ELISA reacted to rCD69 on Western blotting. However, four samples that were negative by ELISA reacted to rCD69 by Western blotting. From the results of Western blotting, the prevalence of anti-CD69 autoantibodies in each disease category was as follows: 17 of the 60 (28.3%) patients with RA, 8 of the 55 (14.5%) patients with SLE, and 1 of the 22 (4%) patients with Behcet’s disease (Table I).

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Table II. Reactivity of anti-CD69 autoantibody-positive serum samples, which had reacted to the F3 protein by Western blotting, to the four truncated F3 proteins by Western blotting

<table>
<thead>
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<th>Disease</th>
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<th>F3b</th>
<th>F3c</th>
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<tr>
<td></td>
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*a* +, Positive reactivity; ±, Weakly positive reactivity; –, Negative reactivity.
RA, 4 of the 55 (7.3%) patients with SLE, and 1 of the 22 (4%) patients with Behcet's disease. None of the tested healthy serum samples reacted to the rCD69 by Western blotting. Thus, taking together the results from ELISA and Western blotting, anti-CD69 autoantibodies were detected in 32 of the 137 (23.4%) patients with a systemic autoimmune disease. By disease category, anti-CD69 autoantibodies were detected in the sera of 23 of the 60 (38.3%) patients with RA, 8 of the 55 (14.5%) patients with SLE, and 1 of the 22 (4%) patients with Behcet’s disease (Table I).

Reactivity of the anti-CD69 autoantibody-positive sera to CD69 fusion proteins

To map the autoepitopes on the CD69 molecule, three plasmids with the F1, F2 and F3 cDNA fragments were constructed (pMAL-CD69F1, pMAL-CD69F2, and pMAL-CD69F3; Fig. 1a). As shown in Fig. 3a, the F1, F2, and F3 proteins migrated to form clear bands on electrophoresis. Then, the reactivity of the 32 serum samples to each fragment was examined by ELISA and Western blotting. As summarized in Table I, two of the 32 serum samples (6.3%) reacted to the proteins encoded by all three fragments by ELISA or Western blotting, four serum samples (12.5%) reacted to the proteins encoded by two fragments, and two samples reacted to the full-length CD69 protein but not to any of the proteins encoded by the three fragments. Importantly, 28 of the 32 samples (87.5%) reacted to the F3 protein, and 20 of the 28 (71.4%) reacted exclusively to the F3 protein. Thus, the F3 fragment is considered to contain a distinct dominant epitope of the CD69 molecule. The F1,
FIGURE 8. Reactivity of the anti-CD69 autoantibodies with the native form of CD69 expressed on the PMA-stimulated Jurkat cells. a, Jurkat cells expressed CD69 by stimulation with PMA. Untreated or PMA-stimulated Jurkat cells were stained with PE-conjugated mouse anti-human CD69 mAb, and then were analyzed by FACS. b, Anti-CD69 autoantibody-positive serum samples reacted to the PMA-stimulated Jurkat cells. Serum samples that contained anti-CD69 autoantibodies, diluted at 1:1 in staining buffer (2% FCS-PBS), were incubated with untreated or PMA-stimulated Jurkat cells, and then the bound Abs were detected by PE-conjugated anti-human IgG. A representative result from SLE17 is shown. c, The reactivity detected in b was reduced by removing the autoantibodies to the rCD69. The identical serum samples as used in b were preadsorbed with 2.5 μg of MBP-CD69 (full length) or MBP alone. Then the reactivity to Jurkat cells with or without PMA stimulation was analyzed. The shift of the MFI detected in b was defined as 100%. The y-axis indicates the difference of MFI according to the following formula: (MFI of serum to the PMA-stimulated Jurkat cells) – (MFI of serum to the PMA-unstimulated Jurkat cells). A representative result from SLE17 is shown.

F2, and F3 proteins were recognized by 5 (15.6%), 5 (15.6%) and 28 (87.5%), respectively, of the 32 patients’ sera. Representative results of Western blotting are shown in Fig. 3b. To confirm the monoreactivity to F3 in the majority of the tested serum samples, serially diluted serum samples were similarly tested by ELISA. Representative results are shown in Fig. 4.

Epitopes in the F3 region

Of interest, 87.5% of the 32 anti-CD69 autoantibody-positive samples recognized the F3 fusion protein and 71.4% of these recognized only the F3 protein. We further investigated the epitopes in the F3 region. Specifically, we prepared four truncated fusion proteins, F3a, F3b, F3c, and F3d by manipulating pMAL-CD69F3 (Fig. 1a). We tested the reactivity of the serum samples which had reacted with the F3 fragment by Western blotting, to each truncated fusion protein by Western blotting. As summarized in Table II, all 22 serum samples reacted to F3d, and 20 serum samples recognized only F3d. Representative results are shown in Fig. 5b. From these data, the dominant autoepitope in the CD69 molecule is located within the F3d fragment, which is only 16 aa long. A homology search revealed that a 7-aa stretch in F3d has homology with low-density LRP2 (Megalgin or gp330) of humans and rats, as shown in Fig. 6. To determine whether this homologous region is an antigenic determinant, we prepared an MBP fusion protein of LRP2 (LRP2H) which contained aa 532–547 (aa 535–541 of LRP2 homologous to F3d of CD69), and tested the reactivity of the 28 serum samples which recognized the F3 protein, to LRP2H. The ELISA study showed that all of the serum samples that had positively reacted to CD69F3d recognized the LRP2H fusion protein (data not shown). To confirm the cross-reactivity between CD69 and LRP2, we investigated the reactivity of the serum samples to CD69 or LRP2H by ELISA, using fusion proteins of the CD69,
LRP2H, F3d, and F3c as inhibitors. Adsorption of the patient serum with each of the CD69, LRP2H, and F3d recombinant proteins equally reduced its reactivity to the CD69 fusion protein in a dose-dependent manner. In contrast, adsorption with F3c that does not contain the homologous region, showed no inhibitory effect (representative cases are shown in Fig. 7a). Adsorption of patient serum with the CD69, LRP2H, and F3d recombinant proteins similarly reduced its reactivity to the LRP2H fusion protein; however, adsorption with F3c did not (Fig. 7b). These results indicate that the same autoantibodies reacted to the homologous amino acid sequence of CD69 F54 and LRP2H.

**Binding activity of the anti-CD69 autoantibody to native CD69 molecules**

We showed in this study the existence of autoantibodies to the rCD69 produced in *E. coli*; however, it remains to be solved whether they bind to the native form of CD69 on the lymphocytes. Therefore, we investigated it by flow cytometry using the three serum samples of SLE17, RA16, and RA35, which had relatively high Ab titers to rCD69. Specifically, we used Jurkat cells, which were CD69-negative in the untreated condition, but expressed CD69 by stimulation with PMA (Fig. 8a). As a representative case is shown in Fig. 8b, the anti-CD69 autoantibody-positive serum samples by ELISA and Western blotting were found to bind to the PMA-stimulated Jurkat cells more strongly than to untreated cells even though the shift of the mean fluorescence intensity (MFI) was slight. This indicates the possibility that the anti-CD69 autoantibodies bound to the native form of CD69 on the Jurkat cells. To exclude the possibility that autoantibodies to other cell surface molecules whose expression could be induced by the PMA stimulation bound to the Jurkat cells, we measured the shift of MFI by adsorbing the anti-CD69 autoantibodies from the tested serum samples. As shown in Fig. 8c, the MFI shift caused by the anti-CD69 autoantibody-positive serum samples were markedly reduced by removing the CD69 autoantibodies from the identical serum samples. This evidenced that the anti-CD69 autoantibodies reacted with the native form of CD69 molecules expressed on the lymphocytes.

**Laboratory parameters of RA patients whose sera do and do not contain anti-CD69 autoantibodies**

Because anti-CD69 autoantibodies were detected most frequently in the sera of patients with RA, we compared the laboratory parameters of the anti-CD69 autoantibody-positive and -negative RA patients (Table III). The serum level of RFs and erythrocyte sedimentation rate (ESR) of the anti-CD69 autoantibody-positive patients were significantly higher than the respective value of the anti-CD69 autoantibody-negative patients (RFs, 303 ± 100 vs 71 ± 25, p < 0.05; ESR, 48 ± 7 vs 28 ± 3, p < 0.05). However, the peripheral lymphocyte count, white blood cell count, platelet count, C-reactive protein, and serum levels of IgG, IgA, and IgM of the anti-CD69 autoantibody-positive and -negative RA patients, did not significantly differ (Table III).

**Effects of RFs on the assays for anti-CD69 Ab**

In addition, to exclude the possibility that RFs affected the measurement of the anti-CD69 autoantibodies, we checked the titers of anti-CD69 autoantibody in serum sample with both RFs and anti-CD69 autoantibodies after removing RFs. Similar to representative case RA44 shown in Fig. 9, removal of RF did not alter the Ab titers to CD69. Together with the fact that some serum samples with anti-CD69 autoantibodies did not contain RF (data not shown), we conclude that RF did not affect our ELISA for the anti-CD69 Abs.

**Discussion**

To our knowledge, this is the first study that shows that autoantibodies to CD69 are generated in patients with systemic autoimmune diseases. Further, we demonstrated that most of the anti-CD69 autoantibody-positive serum samples recognized only one distinct epitope located at the C terminus of the CD69 molecule, and that the autoantibodies to this epitope cross-reacted to a homologous region of LRP2, which is an autoantigen in Heymann nephritis in rats.

Many epitope mapping studies of nuclear Ags have provided evidence that most antinuclear Abs are generated by an Ag-driven mechanism (38). In the case of the anti-CD69 Ab, 7 of the 32 anti-CD69 autoantibody-positive serum samples recognized multiple epitopes. This indicates an Ag-driven mechanism in which CD69-specific T cells help B cells specific for each of the epitopes. However, a majority of the serum samples solely recognized the F3d region, which is only 16 aa residues long. This finding indicates the possibility that CD69 is recognized by cross-reaction of Abs originally directed to other molecules. Accordingly, we found a homologous amino acid stretch of EKNLYW, aa 535–540 of LRP2, which matched EKNLYW, aa 187–192, of CD69. The inhibition assay demonstrated that the short region (aa 532–547) of LRP2 inhibited serum reactivity to F3d, and that F3d inhibited serum reactivity to LRP2H. This strongly suggests that EKNLYW in CD69 and EKRLYW in LRP2 are recognized by the same autoantibody. From this fact, we can speculate that autoantibodies to LRP2 were initially generated, and that autoantibodies directed to the EKRLYW in LRP2 recognized EKNLYW in CD69 by cross-reaction. In this scenario, B cells specific for the homologous epitope present the CD69 molecule to T cells to be activated. This is supported by the reports that activated B cells are effective APCs for their specific Ag (39), and that autoepitopes actually spread by cross-reactive Ag presentation (40). This may lead to the Ag-driven reaction in the above seven patients whose sera recognized multiple epitopes on the CD69 molecule. However, because of the same reason, the autoimmunity to CD69 can provoke the autoimmunity to LRP2. Further, it also possible that the autoimmunity to CD69 and that to LRP2 started independently and that the cross-reaction found in this study was detected by chance. Investigation of anti-LRP2 autoantibodies and epitope mapping of LRP2 would be needed to clarify this point. However, we thus far could not study epitopes of LRP2 in more detail, because the LRP2 molecule is too large (over 500 kDa in molecular mass) to map epitopes in this study.

CD69 expression is induced very early after lymphocytes are activated, and CD69-positive cells have been detected in lymphoid areas. Further, in vitro exposure of CD69-positive cells to anti-CD69 mAbs induced intracellular signaling. Thus, CD69 would be involved in the ongoing activation process of lymphocytes (41–45). In this context, we evidenced that the anti-CD69 autoantibodies were able to bind to the native CD69 on the lymphocytes even though the binding was weak. Thus, the anti-CD69 autoantibodies may alter some function of lymphocytes in patients with autoimmune disease. We found significant differences in the serum levels of RF and ESR between the anti-CD69 autoantibody-positive and -negative RA patients. Because the RF level is associated with the severity of RA (46) and because ESR is an actual marker of inflammation, the presence of anti-CD69 autoantibodies is thought to be associated with severe RA. Together with the reports that showed the synovial T cells of RA patients express a high level of CD69 (26, 27), the anti-CD69 autoantibodies may provide activation-related signaling through CD69 to synovial T cells and thus...
may exacerbate synovial inflammation in RA. Further studies are needed to explore this possibility.

We demonstrated the existence of anti-CD69 autoantibodies in the sera of patients with autoimmune diseases such as RA. As mentioned above, only one dominant epitope was detected, which was homologous to aa 535–540 of LRP2. Thus, the anti-CD69 autoantibody could be a part of the anti-LRP2 autoantibody. LRP2 is a well-known autoantigen that causes experimental glomerulonephritis in rats (Heymann nephritis), in which immune complexes of LRP2 and anti-LRP2 autoantibodies are deposited in the glomeruli (47). In our study, no tested patients with the anti-CD69/ LRP2 autoantibodies were associated with glomerulonephritis. Possible explanations follow. LRP2 is a huge molecule (~600 kDa) and thus would have multiple epitopes. Therefore, nephritogenic epitopes may be located on the different parts of LRP2. Alternatively, anti-LRP2 autoantibodies may not have nephritogenic potential in humans. Further studies on autoantibodies to various regions of LRP2 would be needed. From the functional aspects, LRP2 is a broad range receptor for various ligands including 

References


