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Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility

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Specific HLA class II alleles are strongly associated with susceptibility to rheumatoid arthritis (RA); however, how HLA class II regulates susceptibility to RA has remained unclear. Recently, we found a unique function of HLA class II molecules: their ability to aberrantly transport cellular misfolded proteins to the cell surface without processing to peptides. Rheumatoid factor (RF) is an autoantibody that binds to denatured IgG or Fc fragments of IgG and is detected in 70–80% of RA patients but also in patients with other autoimmune diseases. Here, we report that intact IgG heavy chain (IgGH) is transported to the cell surface by HLA class II via association with the peptide-binding groove and that IgGH/HLA class II complexes are specifically recognized by autoantibodies in RF-positive sera from RA patients. In contrast, autoantibodies in RF-positive sera from non-RA individuals did not bind to IgGH/HLA class II complexes. Of note, a strong correlation between autoantibody binding to IgG complexed with certain HLA-DR alleles and the odds ratio for that allele’s association with RA was observed (r = 0.81; P = 4.6 × 10−7). Our findings suggest that IgGH complexed with certain HLA class II alleles is a target for autoantibodies in RA, which might explain why these HLA class II alleles confer susceptibility to RA.

autoimmune disease | major histocompatibility complex

Autoantibodies are produced in many autoimmune diseases and are frequently used in their diagnosis. Rheumatoid factor (RF) is an IgM autoantibody that binds to denatured IgG or Fc fragments of IgG and is detected in about 80% of RA patients but also in 5–10% of healthy individuals, as well as in other autoimmune diseases (1). However, the natural antigens that are recognized by RF are unknown, partly because such denatured IgG does not exist in physiological situations. Specific HLA class II alleles are strongly associated with susceptibility to many autoimmune diseases (2). Because peptide repertoires presented on HLA class II molecules reflect the peptide-binding groove without processing to peptides. We found that IgG heavy chain is transported to the cell surface by HLA class II molecules in association with the peptide-binding groove of HLA class II molecules (3, 4). Indeed, direct association between HLA class II molecules and misfolded HLA class I molecules was detected in HLA class II-expressing B-cell lines. Furthermore, the intact misfolded proteins transported to the cell surface by HLA class II molecules were efficiently stimulated antigen-specific B cells (5). Accordingly, we hypothesized that misfolded proteins aberrantly transported to the cell surface by HLA class II molecules might be targets for autoantibodies detected in autoimmune diseases.

Results

IgG Heavy Chain Alone Is Expressed on the Cell Surface in Association with MHC Class II Molecules. Because denatured IgG is a target for RF, we addressed whether intracellular misfolded IgG heavy chains (IgGHS) are transported to the cell surface by the RA-susceptible HLA-DR4 (HLA-DR4*01:01/DRB1*04:04) molecule. Five different secreted forms of IgG1 heavy chains cloned from human peripheral blood mononuclear cells (PBMCs) transfected into 293T cells were not detected on the cell surface (Fig. 1A) but were detected intracellularly in similar amounts (Fig. 1B). In

Significance

Cellular misfolded proteins are transported to the cell surface by MHC class II molecules via association with the peptide-binding groove without processing to peptides. We found that IgG heavy chain is transported to the cell surface by MHC class II molecules in association with the peptide-binding groove of HLA class II molecules. Furthermore, IgG heavy chain associated with MHC class II molecules is recognized by autoantibodies in rheumatoid arthritis patients. Autoantibody binding to IgG heavy chain complexed with different MHC class II alleles was strongly associated with rheumatoid arthritis susceptibility conferred by certain MHC class II alleles. These findings suggest that misfolded proteins complexed with MHC class II molecules could be targets for autoantibodies in autoimmune diseases, which might be involved in autoimmune disease susceptibility.


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Next, we analyzed involvement of the peptide-binding groove of HLA-DR4 molecules but only weakly to the membrane-bound form of IgG expressed on the cell surface by cotransfection with light chain, IgG, and HLA-DR. The level of expression of the membrane-bound form of IgG compared with the secreted form of IgG bound to HLA-DR was almost the same. The presence of light chain did not affect IgG cell surface transportation by HLA-DR and autoantibody binding to IgGH complexed with HLA-DR. Autoantibodies in the RF standard serum did not bind to the membrane-bound form of IgG even in the presence of HLA-DR molecules. To confirm that autoantibodies bind to the IgGH/HLA class II complexes, we used a human monoclonal RF antibody derived from an RA patient, RF61, which shows similar specificity to RF (10, 11). RF61 bound to the secreted form of IgGH complexed with HLA-DR molecules but not to the membrane-bound form of IgG.

Fig. 1. IgGH is transported to the cell surface by HLA-DR. (A) The secreted forms of IgGHs cloned from human PBMCs that have different V regions were cotransfected with HLA-DR4, DRB1*04:04 (HLA-DR4), and GFP. Cell surface IgG on GFP-expressing cells was detected with anti-human IgG Fc-specific Ab (red lines). Cells transfected with IgGH alone were stained as control (shaded histograms). (B) The secreted form of IgGH clones was cotransfected with GFP, and intracellular IgGH was detected by intracellular staining with anti-human IgG Fc-specific Ab (red lines). Cells transfected with GFP alone were stained as control (shaded histograms). MFIs of IgG complexed with HLA-DR or intracellular IgG are shown in the figure (C). Data are representative of at least three independent experiments. (C) HLA-DR4 (red lines), Cw3-pep-HLA-DR4 (blue lines), or control plasmids (black lines) were cotransfected with IgGH clone 1 and GFP. Expression of IgG and HLA-DR on GFP-positive cells is shown. (D) HLA-DR4, DR3, Cw3-pep-DR4, and IgGH clone 1 (IgGH) were transfected, HLA-DR was immunoprecipitated from cell lysates, and IgGH and HLA-DR in precipitates and whole-cell lysates were immunoblotted. (E) N-terminal Flag-tagged or C-terminal His-tagged IgGH was transfected with GFP in the presence (red lines) or absence (shaded histograms) of HLA-DR4. Cell surface IgG on GFP-positive cells was detected by anti-Flag or anti-His mAb, respectively. Data are representative of at least three independent experiments.

In contrast, the secreted form of IgGH were detectable on the cell surface in the presence of HLA-DR molecules, with the amount of IgG detected being different for each of the five different heavy chains (Fig. 1A). This suggests that the variable (V) region of IgG influences the association with HLA-DR molecules. Because the antibody light chain was not required for expression, the structure of the IgGH complexed with the HLA-DR molecule seems to be different from that of IgGH in normal antibody that is associated with light chain.

Next, we analyzed involvement of the peptide-binding groove of HLA class II molecules in the transport of IgGH to the cell surface. We generated HLA-DR4 covalently attached with an HLA-Cw3 peptide, one of the most common naturally bound peptides in the peptide-binding groove of HLA-DR4 (9). Wild-type HLA-DR4 transported IgGH to the cell surface but HLA-DR4 covalently attached with HLA-Cw3 peptide failed to induce cell surface expression of IgGH (Fig. 1C). Furthermore, full-length IgGH (50 kDa) was coprecipitated with HLA-DR4 but not with HLA-DR4 containing a covalently attached peptide (Fig. 1D). When IgGH with a His tag at the C terminus or a Flag tag at the N terminus was transfected with HLA-DR, cell surface IgGH was detected using anti-Flag or anti-His mAb (Fig. 1E). These data indicate that full-length IgGH, rather than degraded IgGH, is expressed on the cell surface in association with the peptide-binding groove of HLA-DR and that the V region of IgG influences the association with HLA-DR. To determine where HLA-DR associates with IgGH, we analyzed the association using pulse-chase experiments (Fig. 2). Endoglycosidase H (Endo H)-sensitive HLA-DR was coimmunoprecipitated with IgGH just after pulsing cells for 15 min with [35S]methionine and cysteine (0 h). Association of IgGH with HLA-DR was also detected after 2 and 4 h. These data indicate that IgGH associates with nascent HLA-DR molecules in the endoplasmic reticulum (ER).

Autoantibodies from RA Patients Bind to IgGH Complexed with MHC Class II Molecules. We analyzed binding of IgM autoantibodies in a RF standard serum to IgGH bound to HLA-DR4 molecules (Fig. 3A). Autoantibodies in the RF standard serum bound strongly to the secreted form of IgGH complexed with HLA-DR molecules but only weakly to the membrane-bound form of IgG expressed on the cell surface by cotransfection with light chain, IgG, and HLA-DR. The level of expression of the membrane-bound form of IgG compared with the secreted form of IgGH bound to HLA-DR was almost the same. The presence of light chain did not affect IgG cell surface transportation by HLA-DR and autoantibody binding to IgGH complexed with HLA-DR. Autoantibodies in the RF standard serum did not bind to the membrane-bound form of IgG even in the presence of HLA-DR molecules. To confirm that autoantibodies bind to the IgGH/HLA class II complexes, we used a human monoclonal RF antibody derived from an RA patient, RF61, which shows similar specificity to RF (10, 11). RF61 bound to the secreted form of IgGH complexed with HLA-DR molecules but not to the membrane-bound form of IgG.
IgGH complexed with HLA-DR is specifically recognized by autoantibodies in RA patients. (A) The IgGH, light chain (L), and GFP were cotransfected with (red) or without (blue) HLA-DR4. The membrane form of IgGH containing the same V region as IgGH clone 1 (mIgGH) was transfected with the light chain, IgL, IgG, and GFP with (red) or without (blue) HLA-DR. Cell surface expression of HLA-DR or IgG and binding of autoantibodies in the RF standard serum and RF61 mAb by GFP-positive cells are shown. (B) IgGH, HLA-DR4, and GFP were cotransfected. Binding of IgM autoantibodies in sera from RA patients and healthy controls to GFP-positive cells expressing IgGH/HLA-DR complexes is shown (red lines). The same cells were stained by APC-conjugated anti-human IgM Ab only as control (shaded histograms). RF activities (units per milliliter) of serum samples are shown in the figure. (C) RF titers of sera from 151 RA patients, 20 SLE patients, 117 APS patients, and 128 healthy controls were plotted against IgM autoantibodies in sera to IgGH complexed with HLA-DR4 (anti-IgGH/HLA-DR Ab titer). Samples with autoantibody titers under 400 were plotted in red and those between 400 and 2,000 were plotted in blue. Linear regression line (dashed line), the correlation coefficient (r), and P value of the regression line are shown in the plots of RA patients.

We analyzed serum samples from RA patients and healthy donors for binding to IgGH/HLA class II complexes. RF-positive sera (defined as sera that bind to IgG Fc fragments) from RA patients contained IgM autoantibodies against IgGH complexed with HLA-DR molecules (Fig. 3B). On the other hand, sera from healthy donors, including some RF-positive sera from healthy donors, did not contain autoantibodies against IgGH/HLA-DR complexes. When sera from 151 RA patients were analyzed, autoantibody titers against IgGH complexed with HLA-DR molecules were well correlated with their RF titers as determined by binding to human IgG Fc fragments (r = 0.85, P = 5.9 × 10^-13) (Fig. 3C). On the other hand, autoantibody titers against IgGH complexed with HLA-DR molecules were low in sera from systemic lupus erythematosus (SLE) patients, anti-phospholipid syndrome (APS) patients, and healthy donors, including individuals with high RF titers. These data suggest that RA patients possess autoantibodies to both Fc fragments of IgG and IgGH/HLA-DR complexes. IgGH/HLA-DR complexes, but not Fc fragments of IgG, seem to represent a specific target for autoantibodies in RA patients.

A Strong Correlation Between Autoantibody Binding to IgG Complexed with Each HLA-DR Allele and the Odds Ratio for That Allele’s Association with RA. We compared the efficiency of IgG transport by different HLA-DR molecules. Although RA-susceptible HLA-DR4 efficiently induced cell surface IgGH expression, cell surface IgG transport by RA-resistant HLA-DR3 (HLA-DR*01:01/HLA-DRB1*03:01) was quite inefficient, although there was no difference in the expression of these HLA-DR molecules on the cell surface (Fig. 4A). Moreover, IgG was coprecipitated with HLA-DR4 but not HLA-DR3 (Fig. 1D). Invariant chain (Ii), which blocks the peptide-binding groove of nascent MHC class II molecules and transports MHC class II to the endosomal components, partially inhibited transport of IgG to the cell surface by these HLA-DR molecules. However, HLA-DR4, not HLA-DR3, still efficiently transported IgGH to the cell surface (Fig. 4A). Autoantibodies from an RF-positive RA patient bound to the IgG complexed with HLA-DR4 but less efficiently with IgGH complexed with HLA-DR3. A more pronounced difference was observed in the autoantibody binding to IgGH between HLA-DR4 and HLA-DR3 in the presence of Ii. A cysteine-mutant misfolded Hen egg lysozyme (HEL) protein (8) was transported to the cell surface by HLA-DR3 better than HLA-DR4, indicating that the failure of HLA-DR3 to transport IgGH did not reflect a general inability to associate with all misfolded proteins (Fig. 4B). In addition, Ii blocked mutated HEL (mutHEL) transport by HLA-DR4 more efficiently than HLA-DR3, suggesting that the ability of Ii to block the
transport of misfolded proteins by HLA-DR is allele-specific (Fig. 4B). On the other hand, HLA-DM destabilizes the association of Ii-derived peptides with HLA class II molecules in endosomal compartments, which promotes HLA class II molecules to associate with peptides derived from endosomes (12). Therefore, there is a possibility that HLA-DM also destabilizes the association of IgGH with HLA-DR. However, HLA-DM did not affect the IgGH transported by HLA-DR4, although HLA-DM blocked cell surface expression of IgGH transported by HLA-DR3 (Fig. 4C). This suggests that HLA-DM affects the association of IgGH with the RA-susceptible HLA-DR protein in the ER less than Ii.

We further tested whether there is a correlation between binding of IgM autoantibodies from RA patients to IgGH complexed with different HLA-DRB1 alleles and the RA susceptibility conferred by certain HLA-DRB1 alleles in the presence of Ii and light chain, which represent physiological situations (Fig. 5A). Strikingly, a strong correlation was observed between the binding of autoantibodies from RA patients to IgGH complexed with each HLA-DR allele and the odds ratio for that allele’s association with RA ($r = 0.81; P = 4.6 \times 10^{-5}$). There was no correlation between misfolded HEL transport and the odds ratio for each of these HLA-DR alleles (Fig. 5B). These data suggest that autoantibody binding to IgGH complexed with certain HLA-DR molecules is involved in susceptibility to RA.

In Situ Association of IgGH with HLA-DR in Synovial Membrane from RA Patients. The presence of specific autoantibodies against IgGH complexed with HLA-DR suggests that HLA-DR/IgGH complexes exist in tissues of RA patients as a target antigen for the autoantibodies. To test this possibility, we analyzed the association of IgGH and HLA-DR using a proximity ligation assay (PLA) that detects protein–protein interactions closer than 40 nm (13). PLA signals between HLA-DR and IgGH were observed in synovial membrane from RA patients but not from osteoarthritis patients (Fig. 6). These data suggested that IgGH and HLA-DR complexes are present in synovial membrane in RA patients as a target for autoantibodies.

Discussion

Ii binds newly synthesized HLA class II molecules and prevents associations with other proteins (3, 14). However, the affinity of Ii-derived peptide (CLIP peptide) binding to HLA class II proteins is not always higher than other peptide antigens (15). Therefore, linear epitopes exposed in IgGH might associate with HLA class II molecules in the ER in place of Ii if they have a high affinity or high abundance. Indeed, in our study, the efficiency of the blockade of the binding of IgGH to HLA class II molecules by Ii varied according to the allele examined. Ii efficiently blocked transportation of IgGH by HLA-DR3, whereas a significant amount of IgGH was still transported to the cell surface by RA-susceptible HLA-DR4 and was recognized by autoantibodies from RA patients even in the presence of Ii and light chain. These are compatible with a prior report that an Ii-derived CLIP peptide has a higher affinity for HLA-DR3 than HLA-DR4 (16).

Binding of IgGH to the peptide-binding groove of HLA-DR suggested that linear epitopes revealed on unfolded IgGH are involved in the association with HLA-DR. Indeed, peptide fragments of IgGH, including V regions, are often eluted from HLA-DR (4, 17, 18). On the other hand, when extracellular protein antigens are endocytosed and unfolded in endosomes, these proteins form a large molecular complex with HLA class II molecules via the peptide-binding groove, followed by cleavage and/or trimming of the bound protein by endosomal proteinases (19, 20). Therefore, it is possible that misfolded IgGH transported to the cell surface by HLA class II molecules are also cleaved and/or trimmed to peptide fragments after endocytosis of IgGH/MHC class II complex. The MHC class II molecules that appear on the cell surface with the IgGH-derived peptides after endosomal processing might elicit antigen-specific T-cell responses to enhance autoantibody production. Indeed, IgG RF is produced in some RA patients, although most RF is IgM (1).

RF is frequently used in the diagnosis of RA, and a recent large-scale prospective cohort study indicated that healthy individuals with high RF have a 26-fold greater long-term risk of RA than RF-negative individuals (21). However, RF is not thought to be directly involved in pathogenicity of RA, partly because RF
is detected in other diseases that lack symptoms of arthritis (1). Most RFs from RA patients and non-RA individuals bind to the Fc portions of IgG1, IgG2, and IgG4. In contrast, RFs from non-RA individuals rarely bind to the Fc portion of IgG3 (less than 5%), whereas RFs from some RA patients bind to IgG3 (about 30%) (22–24). These observations suggested that epitopes recognized by RFs from RA patients are different from RFs of non-RA individuals (22–24). In our study, we found that autoantibodies against IgG1 complexed with HLA-DR are specifically detected in most RA patients but not in non-RA individuals. Therefore, RF epitopes on IgG1 complexed with HLA-DR seem to be different from those on the Fc portion of IgGs not complexed with HLA-DR.

At present, it is unclear whether autoantibodies against IgG/HLA-DR complexes are directly involved in pathogenesis of RA. However, a strong correlation between autoantibody binding to IgG/HLA-DR complex and RA susceptibility was observed. In addition, it is well recognized that autoantibodies against ubiquitously expressed self-antigens (for example, glucose-6-phosphate isomerase or type II collagen) can induce arthritis in mouse model systems (25, 26). Considering these observations, autoantibodies to IgG/HLA-DR complexes might also play a role in the pathogenesis of RA.

IgG-positive B cells in PBMCs, including those from RA patients, are not recognized by autoantibodies from RA patients, suggesting that normal peripheral IgG-positive B cells expressing HLA class II molecules do not display IgG/HLA-DR complexes. We have shown that IgG/HLA-DR complexes are detectable in synovial membrane from RA patients by using a PLA assay. Furthermore, an unfolded protein response is detected in plasma cells in the synovial membrane of RA patients (27). These observations suggested that abnormal B cells producing unusual IgG/HLA-DR complexes specifically differentiate in the synovial membrane of RA patients. The diffuse distribution of PLA signals in synovial membranes from RA patients suggests that abnormal B cells producing these IgG/HLA-DR complexes might have secreted these complexes. MHC class II molecules can be secreted from B cells or antigen-presenting cells as exosomes (28). Therefore, abnormal B cells might have secreted these IgG/HLA-DR complexes in synovial membranes as exosomes, which could be a target for autoantibodies in RA patients. Autoantibodies against citrullinated proteins are also detected in RA patients (29, 30). Because citrullination is known to cause protein misfolding (31), such proteins might be effectively transported to the cell surface by HLA class II molecules and might induce autoantibody production. Therefore, it is important to characterize abnormal B cell populations that can secrete IgG/HLA-DR complexes and to investigate in vivo situations in which misfolded or structurally altered proteins are complexed with HLA class II molecules. Further analyses of misfolded or structurally altered proteins complexed with HLA class II molecules might help us to better understand HLA class II-related autoimmune diseases.

Materials and Methods

Human Samples. The collection and use of human sera and synovial tissues were approved by the institutional review board (IRB) of Hokkaido University, Dohgo Spa Hospital, and Osaka University. Written informed consent was obtained from all participants according to the relevant guidelines of the IRB. Diagnoses of RA and SLE were based on the American College of Rheumatology classification revised criteria for RA (32) and the 1982 American College of Rheumatology classification revised criteria for classification of SLE (33), respectively. The diagnosis of APS was based on the preliminary classification criteria for definite APS (34). Some sera were purchased from American Type Culture Collection (ATCC) and GenWay Biotech. Some HLA-DR alleles were generated using QuickChange mutagenesis kits (Agilent Technologies) from HLA genes with similar sequences. All cDNA sequences for HLA were based on information contained in the Immunogenetics/HLA Database (www.ebi.ac.uk/imgt/hla/index.html).

HLA-Ab3-pep-HLA-DRB1*04:04 containing a covalently attached peptide was generated by adding the peptide sequence (Cv3: GSHMRMYVFYAVSRPGR) and linker (GGSGGS) between the signal sequence and extracellular domain of these MHC class II cDNA as previously described (35). A cDNA for the murHL in which two cysteine residues at positions 30 and 64 were replaced with alanine was generated as described (8). V regions of RF61 RF mAb were synthesized according to the published sequence (accession numbers: for heavy chain, X54437; for light chain, X54438). These V regions were cloned into pME185 expression vectors containing mouse secreted form of IgG1 constant region (accession no. L27437.1) or human λ constant region (accession no. X06876). Plasmids containing heavy and light chain genes for the Abs were cotransfected into 293T cells. The culture supernatants were collected 3 d later and were used for staining. Nucleotide sequences of all of the constructs were confirmed by DNA sequencing (ABI3130xl). IgGs were amplified from human PBMCs (36) with cDNA using sense primers for V regions (5’TGTTCTGTCGCGAGGTACCTGGAACGAGGAGG-3’ for clone 1 and 3, 5’GCCACCTCCAGGTGCAGCTGGTGCA-3’ for clone 2, 5’TGAGCCAGTGACGGCGCTGCTGAGGACGAGG-3’ for clone 5). IgG/HLA-DR complexes might also play a role in the pathogenesis of RA.

IgG-positive B cells in PBMCs, including those from RA patients, are not recognized by autoantibodies from RA patients, suggesting that normal peripheral IgG-positive B cells expressing HLA class II molecules do not display IgG/HLA-DR complexes. Further analyses of misfolded or structurally altered proteins complexed with HLA class II molecules might help us to better understand HLA class II-related autoimmune diseases.
Autoantibody-Binding Analysis to IgGH/HLA-DR Complex. Human secreted IgGH (clone 1) or membrane IgG1 containing the same V region as clone 1 was cotransfected with either HLA-DRα, Ig κ chain, Igα, Igλ, and GFP. The transfectants were mixed with 1:500 diluted RF standard serum (1,060 U/mL) or sera from RF-positive and -negative patients, followed by biotin-conjugated anti-human IgM Ab (Jackson Immunoresearch) and then APC-labeled streptavidin (eBioscience). RF61 mAb was premixed with APC-labeled anti-mouse IgFc Ab and used directly for the staining. Cell surface IgG was detected by APC-labeled anti-human IgG Fc Ab (Jackson Immunoresearch). IgG bound on the GFP-expressing cells were detected by APC-labeled anti-mouse IgG Fc Ab. Mean fluorescence intensities (MFIs) of autoantibody binding to HLA-DR- and IgGH-transfected cells were calculated by subtracting MFI of autoantibody binding to GFP-negative transfectants. Anti-IgGH/HLA-DR complex Ab titers were calculated based on IgM autoantibody binding to IgGH bound to HLA-DR by a standard RF serum of which the RF titer is known (1,060 U/mL).

Measuring RF Titers. RF titers were measured by a sandwich ELISA. Human IgG Fc fragment (Jackson Immunoresearch) was coated on 96-microwell plates (Costar), and IgM bound to the plates was detected by peroxidase-conjugated rabbit anti-human IgM Ab (Jackson Immunoresearch). Peroxidase activities were detected using OptEIA (BD Bioscience). RF titers were calculated by using the standard RF serum of which the RF titer is known (1,060 U/mL; GenWay Biotech).

Proximity Ligation Assay. A Duolink in situ PLA kit was used according to the manufacturer’s instructions (Olink Bioscience) for in situ proximity-ligation assays. Pan-embodied tissue sections from RA patients and osteoarthritis patients were treated with target retrieval solution (S1700; Dako) according to the manufacturer’s instructions to retrieve epitopes for Abs and were incubated with goat anti-human IgG Fc-specific Ab (Jackson Immunoresearch) together with anti–HLA-DR mAb (TAL 185; Dako), and Cy3 PLA signals were developed using anti-mouse MINUS and anti-goat PLUS PLA probes. Nuclei were stained with DAPI fluorescence dye. The assayed tissue sections were analyzed by Axioplan 2 fluorescence microscopy.

Statistics. To assess the significance of the correlation, Pearson’s product-moment correlation coefficient was used, and the correlation coefficient (r) and P value of the linear regression line were calculated. Odds ratios for the association between different HLA-DRB1 alleles and RA, which were obtained from a recently reported large-scale genetic study (7), were log-transformed to normalize the distribution. P values of <0.05 were regarded as statistically significant.

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