Cathepsin S inhibition abrogates immune complex glomerulonephritis because cathepsin S is essential for MHC class II - mediated CD4 T cell and B cell priming

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Abstract

Genetic studies consistently suggest the most common forms of immune complex glomerulonephritis (IC-GN) develop from polymorphisms in HLA genes, implying a role for MHC class II-mediated priming of (auto-)antibody production. The cysteine protease cathepsin S degrades the invariant peptide chain during MHC II assembly with antigenic peptide in antigen-presenting cells, therefore, we hypothesized that cathepsin S inhibition would be therapeutic in IC-GN. We developed a highly specific small molecule, orally available, cathepsin S antagonist, RO5461111, with suitable pharmacodynamic and pharmacokinetic properties that effciently suppressed antigen-specific T cell and B cell priming in-vitro and in-vivo. When given to MRL-Fas(lpr) mice with lupus nephritis-like IC-GN, RO5461111 significantly reduced the activation of spleen dendritic cells and the subsequent expansion and activation of CD4 T cells and CD4/CD8 double negative 'autoreactive' T cells. Cathepsin S inhibition impaired the spatial organization of germinal centers, suppressed follicular B cell maturation to plasma cells, and Ig class switch. This reversed hyergamma-globulinemia and significantly suppressed the plasma levels of numerous IgG (but not IgM) autoantibodies below baseline, including anti-dsDNA. This effect was associated with less glomerular IgG deposits, which protected kidneys from IC-GN. Together, cathepsin S promotes IC-GN by driving MHC II-mediated T and B cell priming, germinal center formation, and B cell maturation towards plasma cells. These afferent immune pathways can be specifically reversed with the cathepsin S antagonist RO5461111, which prevents IC-GN progression even when given after disease onset. This novel therapeutic strategy could correct a common pathomechanism of several IC-GNs.

Cathepsin S mRNA expression

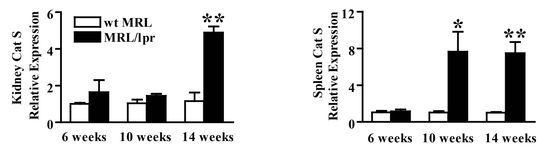

Figure 1. Cathepsin S mRNA expression levels in the kidney and spleen were determined in wildtype MRL and in MRL-Fas(lpr) mice by qPCR.

Cathepsin S in-situ hybridization and immunohistochemistry

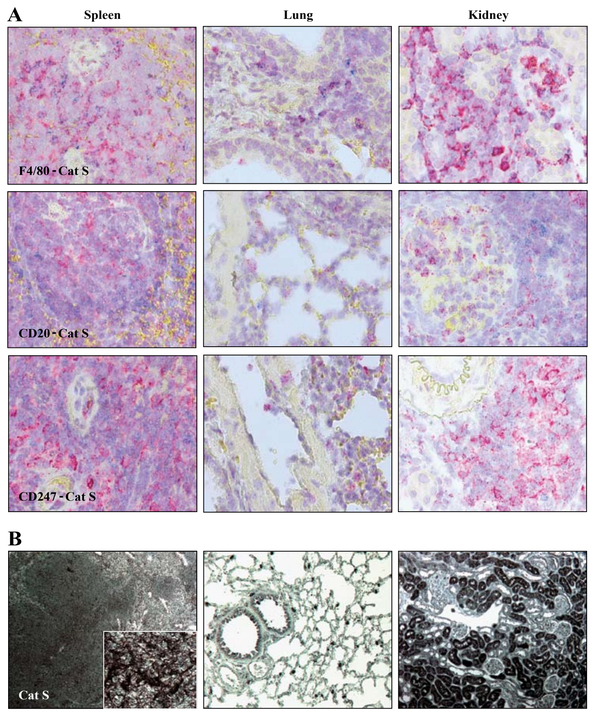

Figure 2. Spleen, lung and kidney sections were prepared for in-situ hybridization and Cat S immunostaining (B). Cat S mRNA expression is indicated by red colour. Co-staining by either F4/80 (myeloid dendritic cells), CD20 (B cells), or CD247 (T cells) is shown in blue colour, respectively. Representative images are shown here for all organs at original magnifications of 100x, 200x or 400x (insert).

Biochemical and pharmacological profile of R05461111

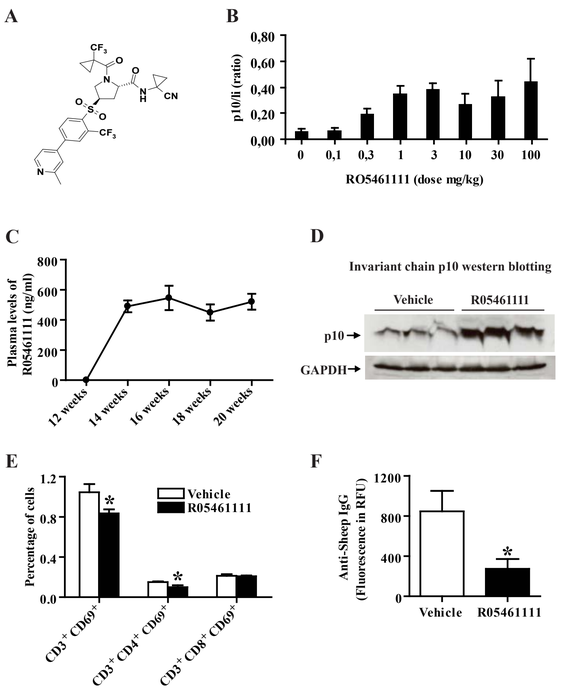

Figure 3. (A) Chemical structure of R05461111. (B) Upregulation of invariant chain p10 levels in the spleen tissue was estimated by western blotting, upon single dose feeding of mice with different doses of R05461111 as indicated. (C) Pharmacokinetics of R05461111 in female MRL-Fas(lpr) mice. (D) Invariant chain p10 levels were estimated from spleen tissue using western blotting. (E) C57BL/6 mice were fed R05461111 during the immunization with sheep IgG. Spleen suspension flow cytometry analysis of the percentage of total CD3+CD69+ T cells, CD3+CD4+CD69+ T cells and CD3+CD8+CD69+ T cells. (F) Anti-sheep IgG antibodies were estimated by ELISA.

Plasma cytokine ELISA

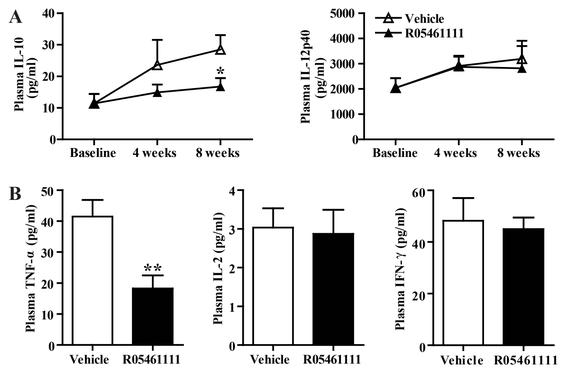

Figure 4. (A) Plasma protein levels of IL-10 and IL-12p40 were estimated by ELISA. (B) Plasma protein levels of IL-2, IFN-γ and TNF-α were estimated by ELISA.

Spleen qPCR and flow cytometry

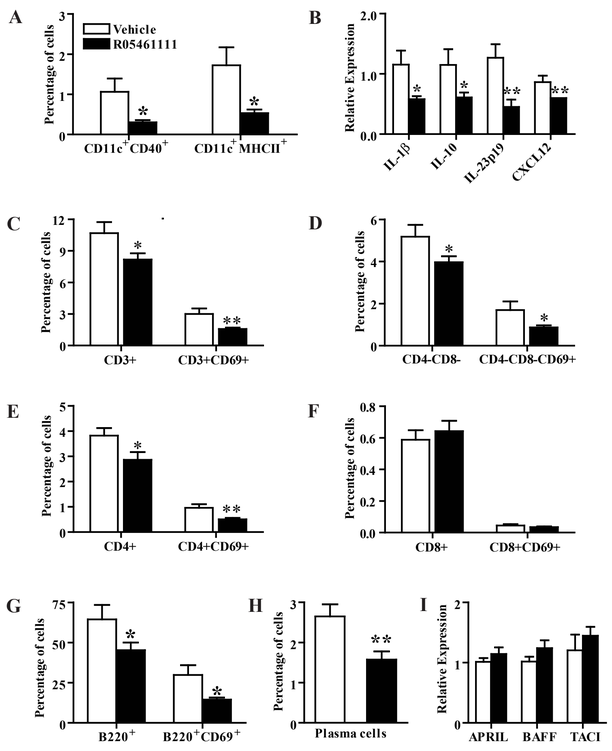

Figure 5. (A) FACS analysis of total numbers of CD11c positive cells that were also positive for the activation markers MHCII or CD40. (B) Splenic mRNA expression levels of IL-1β, IL-10, IL-23p19 and CXCL12 were determined by qPCR. FACS analysis of the percentage of total CD3+ and CD3+CD69+ T cells (C), percentage of total double negative and CD69+ double negative T cells (D), percentage of total CD4+ and CD4+CD69+ T cells (E), percentage of total CD8+ and CD8+CD69+ T cells (F). (G) FACS analysis of the percentage of total spleen B220+ and B220+CD69+ cells. (H) FACS analysis of the percentage of total spleen plasma cells. (I) Splenic mRNA expression levels of APRIL, BAFF/BlyS and TACI.

R05461111 affects germinal centers in MRL-Fas(lpr) mice.

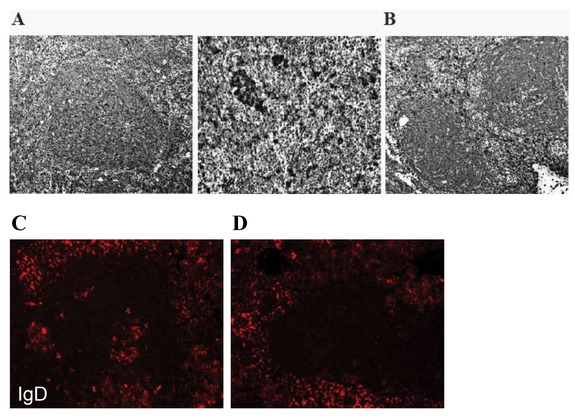

Figure 6. Peanut agglutinin (PNA) and IgD immunostaining was performed in spleens of both groups to identify follicular B cells in germinal centers of lymph follicles. (A/C) Vehicle, (B/D) R05461111.

R05461111 reduces IgG antibodies in MRL-Fas(lpr) mice

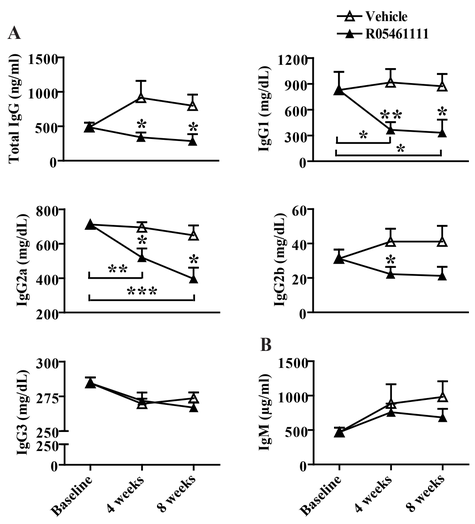

Figure 7. (A) Plasma levels of total IgG and its isotypes IgG1, IgG2a, IgG2b and IgG3 were determined by ELISA. (B) Plasma levels of IgM antibodies were determined by ELISA.

R05461111 reduces autoantibodies in MRL-Fas(lpr) mice

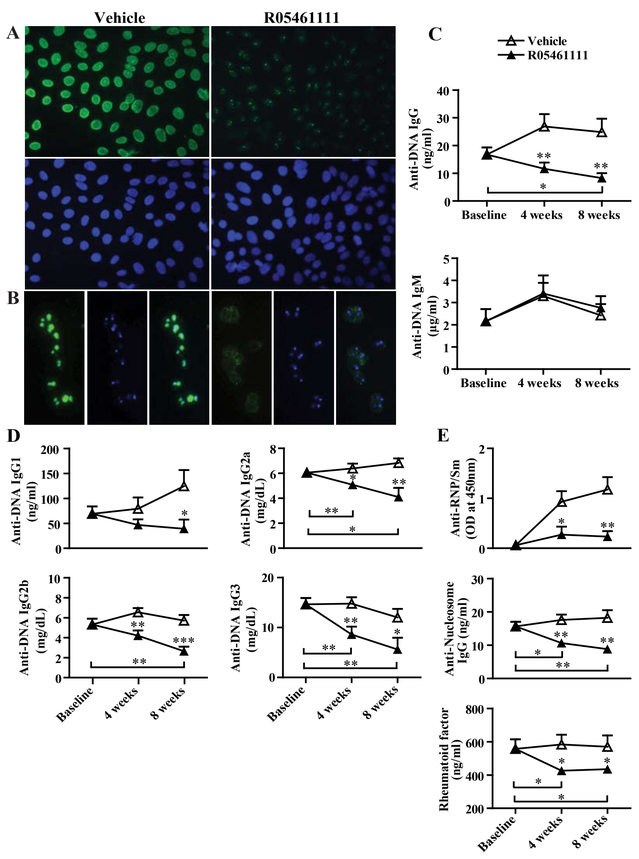

Figure 8. (A) ANA staining. (B) *Crithidia luciliae* staining. (C) Plasma levels of anti-dsDNA IgG and IgM antibodies (D) Plasma levels of IgG isotypes such as IgG1, IgG2a, IgG2b and IgG3 against ds-DNA. (E) Plasma levels of anti-RNP/Sm, anti-nucleosome antibodies and rheumatoid factor.

R05461111 suppresses renal pathology in MRL-Fas(lpr) mice

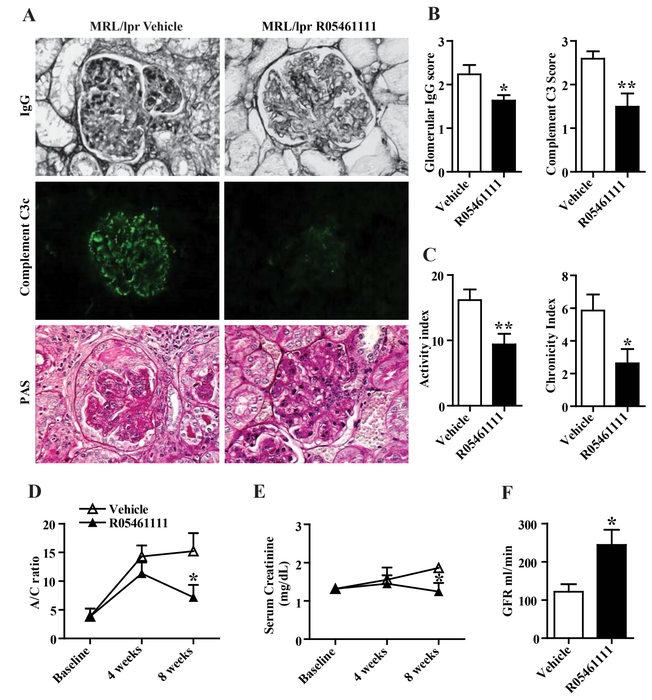

Figure 9. (A) Renal sections were stained for IgG and complement C3c. Renal sections were stained periodic acid Schiff (PAS). (B) Glomerular IgG and complement C3c deposition was compared between vehicle- and inhibitor-treated groups. (C) The lupus nephritis disease activity index (score ranging from 0 to 24), and the lupus nephritis chronicity index (score ranging from 0 to 12) were determined as markers of kidney damage in lupus nephritis. Renal functional parameters like proteinuria (D), plasma creatinine (E) and glomerular filtration rate (F) were determined from 15 mice in the each treatment group at 20 weeks of age.

R05461111 improves lung disease in MRL-Fas(lpr) mice

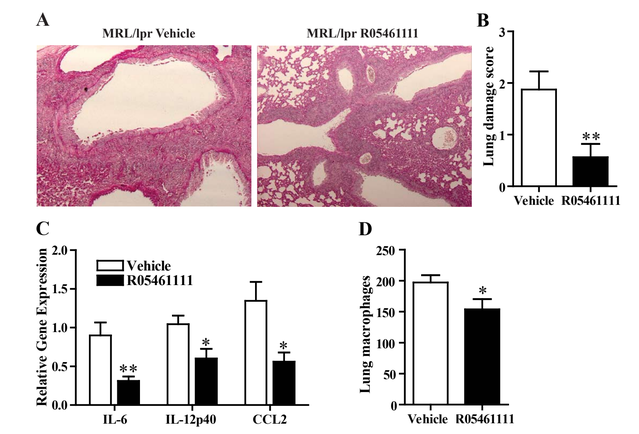

Figure 10. (A) Lung sections were stained with Periodic acid Schiff. (B) Lung damage score. (C) Lung mRNA expression levels of IL-6, IL-12p40 and CCL2 were determined by qPCR. (D) Quantification of lung macrophages.

CONCLUSIONS

Cat S is a non-redundant mediator of autoimmune IC-GN because it is required for the assembly of MHC class II molecules with autoantigenic peptides. Interfering with this process disturbs germinal center formation, i.e. the expansion and activation of CD4 T cells, as well as the activation and maturation of autoreactive B cells and subsequent production of high affinity IgG autoantibodies. Vice versa, therapeutic Cat S inhibition reverts the aberrant autoimmune response and protects from progressive IC-GN.