Selective Inhibitors of T Cell Receptor Recognition of Antigen–MHC Complexes for Rheumatoid Arthritis

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S Supporting Information

ABSTRACT: Autoreactive T cells specific to human collagen type II have a crucial role in the development of rheumatoid arthritis (RA) in the context of MHC class II allele HLA-DRB1-*04. The protein–protein interactions between the T cell receptor (TCR) and the type II collagen bound to the allele MHC of class II may thus represent the target for the development of new drugs against RA. In this study, a structure-based pharmacophore model for potential small molecule inhibitors was developed from protein–protein interface structure. The 3D model obtained was used for a virtual screening workflow, which resulted in three hits for experimental follow up. Three compounds have been identified that interfere with the TCR/collagenII-MHCII (K_i values below 10 μ M) and open up new possibilities in the treatment of RA.

KEYWORDS: Protein–protein interactions, virtual screening, pharmacophore modeling, autoimmune diseases, antigen peptide

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory disease that affects 0.5–1% of the population and that leads to the functional impairment of multiple joints.¹ The etiology of the disease is unknown, but autoimmunity plays a dominant role in its pathogenesis. In fact, T cells and autoantibodies are present in the blood of RA patients, and a genetic predisposition to the disease has been associated with the HLA class II alleles DR that encodes for the so-called shared epitope (SE), a five amino acid sequence motif in residues 70–74 of the HLA-DR β chain including members of the *01- and *04-HLA-DRB1 allele groups.^{2,3} We have reported that several public T cells specific to the peptide 261–273 of collagen type II (huCollp261) are present in the blood at the onset of the disease, decrease during the clinical

remission, and increase again at the time of relapses.^{4,5} Since the etiology of RA is unknown, therapy has focused on the common pathogenic mechanism, i.e., inflammation.⁶ Usually RA therapy includes NSAIDs (nonsteroidal anti-inflammatory drugs), steroids, and so-called DMARDs (disease-modifying antirheumatic drugs), which in turn include conventional (for example, methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine) and biological (monoclonal antibodies blocking

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TNF- α , IL-1, IL-6, CTLA4, JAK-STAT, or cell subpopulations such as anti-CD20) response modifiers.^{7,8} Although current therapies are effective in decreasing the severity of the disease, they are nonselective and are associated with severe side-effects.⁹

In the past years, several attempts have been made to identify compounds capable of occupying the pocket of the HLA-DR4 molecule thus preventing TCR recognition, but these molecules lack of specificity, selectivity, and pharmacological feasibility.^{10,11} Consequently, there remains an ongoing need to find promising inhibitors of the ternary complex TCR-HLA-huCollp261 formation. Pharmacological inhibition of the interaction between the T cell receptor (TCR), located on the surface of T cells, and the HLA-huCollp261 complex may therefore offer a new option in the treatment of RA. Protein–protein interactions (PPIs) are of crucial importance in biological processes and therefore represent an attractive target for the design of novel therapeutics. Once considered “undruggable” with small chemical molecules, in the past decade PPIs emerged as a feasible option for drug discovery, and several inhibitors have reached clinical trials.^{12,13} A significant number of examples are reported in the field of neurological disorders,¹⁴ HIV disease,¹⁵ and oncology.^{16–18} In this latter therapeutic area in particular, several small molecule inhibitors of the p53-MDM2 interaction are available that entered clinical trials.^{19–22} To date, targeting PPIs remains a big challenge mainly for those interfaces exhibiting large surface areas and weak affinities.²³

In an effort to discover a compound able to directly bind the interaction surface of the major histocompatibility complex (MHC) of class II in complex with the huCollp261 peptide, thus sterically preventing the binding to the TCR, we used our previously reported three-dimensional model structure of the TCR/MHCII-huCollp261 interface²⁴ to generate a pharmacophore model for *in silico* screening. Three selective inhibitors of T cell receptor recognition of antigen-HLA complex in RA were identified by virtual screening and *in vitro* assays. The computational approaches presented herein could also be useful in general PPI inhibition strategies and open new possibilities in the treatment of RA.

In the first step of our rational approach (Figure 1) the model structure of TCR in complex with TCR/MHCII-huCollp261 was used to generate a suitable structure-based pharmacophore model.

Figure 1. Schematic representation of the virtual screening workflow.

The analysis of protein–protein contacts by LigandScout 3.1 enabled us to extract the chemical features mainly involved in the interactions between residues 97–105 of TCR V β domain and the collagen peptide bound to the HLA molecule. The pharmacophore hypothesis was composed of four features (one H-bond donor, two H-bond acceptors, one hydrophobic groups) and nine excluded volumes (Figure 2).

In particular, the H-bond donor reflects the OH group of TCR Ser101 that interacts with Gln70 of MHC shared epitope. The two H-bond acceptor features were occupied by the TCR Ala103 carbonyl oxygen atom and by Ser101 hydroxyl oxygen atom, which are hydrogen bonded to Gln70 of HLA-DR4 and Gln267 of huCollp261, respectively. The

Figure 2. Pharmacophore modeling using LigandScout from the model structure of TCR-CDR3 β (residues 97–105) in complex with HLA-DR4/huCollp261.¹⁵ (a) Ternary complex TCR/HLA-DR4/huCollp261 represented as ribbons (TCR in violet; HLA α and β chains in light blue and green, respectively; huCollp261 peptide in magenta) and CDR3 as stick models colored by atom type. The interacting region is marked as a yellow box. (b) Generated structure-based pharmacophore model consisting of one hydrophobic feature (yellow sphere), one H-bond donor (green arrow), and two H-bond acceptors (red arrow). For the sake of clarity, the nine excluded volumes are not shown. (c) Two-dimensional view of the ligand (residues 97–105 of CDR3) together with the assigned pharmacophore features.

hydrophobic sphere was occupied by the side-chain methyl group of TCR Thr98, which projects into a hydrophobic region generated by Ala61 of HLA-DR4.

The 3D pharmacophore model was then used as a query to screen two chemical libraries of commercially available compounds, namely, Asinex and Maybridge, in total 587 723 molecules downloaded from ZINC database. The *in silico* exploration of the chemical space was confined to the region of “drug-like” compounds based on the Ghose rules. After filtering by drug-like properties, the remaining 30 306 compounds were used for a virtual screening procedure based on combined pharmacophore-filtration and structure-based docking procedures. The filtered query results (152 compounds) by pharmacophore screening represented our focused library for docking to MHCII-huCollp261 target. In this last step of our *in silico* studies the AutoDock Vina docking protocol was applied using the MHCII-huCollp261 structure as in the ternary complex conformation. After running the docking process, the top-ranked energy hits were selected for subsequent biological evaluation (Table S1 and Figure S1).

In a preliminary experiment using annexin V-specific staining, we established that the first three top-ranked and selected compounds (Figure 3) did not induce cell death (necrosis or apoptosis) above the level observed in unstimulated cultured cells, for a concentration up to 10-fold higher than the maximum used in the inhibition experiment (data not shown).

The T cell proliferation test was performed on samples from HLA-DRB1 SE+ Early RA patients (ERA): DR4, DR7, and DR1 all have the SE and present the same peptides, and the response to human collagen peptide 261–273 restricted by

Figure 3. Molecular formula of 9-[4-[(3R)-3-phenyl-1-propylpyrrolidin-1-ium-1-yl]but-2-ynyl]fluoren-9-ol (I), (E)-3-(3,6-dichloro-9H-carbazol-9-yl)-N'-(4-hydroxy-benzylidene) propanidrazide (II), and N1,N5-bis(dibenzo[*b,d*]furan-3-yl) glutaramide (III).

these alleles use the same TCRs in the various patients. Three DR4+ ERA patients, one DR1+, and one DR7+ were compared to two patients negative for DR4, DR1, or DR7. The collagen-driven proliferation of individual public T cells was evaluated by immunoscope and was expressed as rate stimulation index (RSI) with a value of 1 corresponding to the absence of ag-driven expansion in the sample. Figure 4 shows

Figure 4. T cells proliferation after stimulation of huCollp261. The test is performed in the absence of antigen (ag−), in the presence of antigen (ag+), and in the presence of ag+ and of a candidate inhibitor compound (I, II, and III). Compound II is capable of strongly depressing (about 100%) the rate stimulation index (RSI) in the context of HLA-DR4 (blue symbols), HLA-DR1 (red), and HLA-DR7 (green). Compound I strongly decreases (about 100%) the RSI in the context of HLA-DR11 samples (black symbols), where compounds II and III are also important inhibitors, even if less effective (almost 20%).

the ability of (E)-3-(3,6-dichloro-9H-carbazol-9-yl)-N'-(4-hydroxy-benzylidene) propanidrazide (II) to block the proliferation of the T cells that respond to huCollp261 in the context of HLA-DR4, DR1, both sharing the SE, or DR7, and the ability to present the same collagen-derived epitope.

Compounds I and III also showed a significant ability to inhibit the proliferation of T cells specific for huCollp261 in the DR4+ context. Yet, these two other molecules tested (compounds I and III) showed a more modest efficacy in inhibiting T cell proliferation, in an experiment performed in *in vitro* test on T cells from a larger group of patients (Table S2). Their ability of inhibiting proliferation of the same T cells confirmed that compounds I, II, and III were inhibiting the interactions of the individual TCR/peptide/HLA complexes, despite their structural differences.

Three clones of T cells carrying huCollp261 specific TCRs (TRBV25-TRBJ2.2 with a length of 133 bases, TRBV19-TRBJ2.5 with a length of 101 bases and TRBV25-TRBJ2.2 with a length of 146 bases), obtained from a DR1+/DR4−

patient were analyzed in a dose/response proliferation test conducted in the absence of antigen (ag−), in the presence of huCollp261 (ag+), and in the presence of ag+ and of variable doses of each candidate inhibitor compound (I and II). The results were expressed as a percentage of inhibition of T cell expansion in the absence of inhibitor, at the indicated concentrations of the two inhibitors. As shown in Figure 4, compound I, i.e., 9-[4-[(3R)-3-phenyl-1-propylpyrrolidin-1-ium-1-yl]but-2-ynyl]fluoren-9-ol is able to strongly depress (about 100%) the relative stimulation index (RSI) of T cells in the DR1+ patient, in two cells out of three at a concentration 10 times lower than the compound II ((E)-3-(3,6-dichloro-9H-carbazol-9-yl)-N'-(4-hydroxy-benzylidene) propanidrazide).

These data demonstrate therefore that compound I is a specific inhibitor of the recognition of collagen I, more efficient in the context of DR1+ (Figure 5), with respect to compound

Figure 5. Results of a T cell proliferation test obtained from a patient DR1+, stimulated by the antigenic peptide 261–273 from human collagen (huCollp261). The test is performed in the absence of antigen (ag−) (not shown), in the presence of antigen (ag+) (not shown), and in the presence of ag+ and of two candidate inhibitors (compound II, black lines and circles; compound I, gray lines and triangles).

II that appears to be able to inhibit a larger repertoire of collagen specific T cells than compound II, in *in vitro* test performed on T cells from DR4+ patients. Taken together, these results showed that it was possible to inhibit (at least *in vitro*) the activation/proliferation of T cells specific for a self-antigen complexed with a self HLA molecule, not for subdominant epitope (Figure S2). No significant toxicity was observed (Figure S3).

Predicted binding mode of the three active molecules is shown in Figures 6 and S4. Our studies indicate that compounds I, II, and III exhibit a similar pattern of interaction with the target, focusing on the interactions between the aromatic moieties of the compounds and two Pro residues at the C-terminus.

Accordingly, the three molecules exhibit the same orientation with respect to the HLA-DR4/huCollp261 molecular target (Figure 6). Namely, the fluoren-9-ol moiety of compound I interacts with Pro269 and Pro273 of huCollp261 and with Gln70 of the shared epitope region of HLA β chain (Figure 6A) as well as the carbazolyl (Figure 6B) and the dibenzofuranyl (Figure 6C) moieties of compounds II and III, respectively. In the case of compound I, the

Figure 6. Predicted binding modes of compounds I, II, and III. Molecular interactions of I, II, and III are shown in the left (A), central (B), and right (C) panels, respectively. Hydrogen bonds, hydrophobic, and pi-ion interactions are represented as green, violet, and orange dashed lines. HLA-DR4/huCollp261 complex is represented as ribbons (HLA α and β chains in light blue and green, respectively; huCollp261 peptide in magenta).

ammonium head in the linker between the aromatic moieties points toward the solvent and does not interact with the HLA (Figure S5).

Rheumatoid arthritis (RA) has a relevant socio-economic impact and an unmet need in handling of this chronic and disabling disease is the management of side effects during all the current immunosuppressant and anti-inflammatory treatments. We present here data showing that the use of computational chemistry methods allowed the discovery of new therapeutic small molecules²⁵ specifically tailored for individual RA patients, able to selectively block only the immune response to the self-antigen, while leaving the rest of the immune system response intact and efficient and avoiding the comorbidity and side effects of immunosuppressant drugs.

This method leads to the identification of specific inhibitors of interaction between self-reactive T cells and the complex of HLA-II and human collagen type II (or an antigenic fragment thereof) of RA patients sharing the HLA-DRB1 SE haplotype, fostering the development of new therapeutics, in the perspective of personalized medicine.

EXPERIMENTAL PROCEDURES

Structure-Based Virtual Screening. The pharmacophore generation protocol in LigandScout 3.117 was used to generate a pharmacophore model from the protein–protein interactions revealed in the TCR/HLA-DR4/huCollp261 structure.²⁴ The program extracts and interprets the ligand and the macromolecular environment from the complex structure, then automatically creates and visualizes a pharmacophore model. A number of examples reporting LigandScout successful application has been published.^{26–29}

In LigandScout the macromolecule was represented by the HLA-DR4/huCollp261 structure, whereas the “ligand” comprised the TCR V β region spanning residues 97–105. The obtained pharmacophore model was used for virtual screening. Screening of compounds was performed against Asinex and Maybridge libraries (~600000 compounds) as implemented in ZINC.³⁰ The virtual library was first filtered for drug-like properties³¹ (MW between 160 and 500 Da, logP between –0.4 and 5.6, and number of hydrogen bond donors and acceptors less than 5 and 10, respectively). This resulted in 30 306 molecules that were converted into a multiconformer library consisting of 411 243 conformations using DiscoveryStudio 2.1 (Dassault Systemes). The pharmacophore screening yielded 152 compounds, which were further filtered by molecular docking on the HLA-DR4/huCollp261 using AutoDock Vina 1.0.³² Final ranking of the compounds was based on AutoDock scoring function.³³

Patients. Seven early rheumatoid arthritis (ERA) patients all satisfying the 2010 ACR classification criteria³⁴ were consecutively enrolled at the onset of the disease (for brevity their clinical,

demographic and laboratoristic characteristics are not shown). Samples of peripheral blood were collected from all the patients in order to perform the typing for HLA-DRB1 for testing for the presence of huCollp261 peptide response by immunoscope technique.³⁵

Cell Cultures, Immunoscope, and HLA-DRB1 Genotyping.

From seven ERA patients peripheral blood cells (PBM) were collected and cultured as described in our previous works^{4,5} in five different conditions: (i) unstimulated or in presence of: (ii) stimulation with huCollp261 (AGFKGEQPKGE) at a concentration of 20 mg/mL; in addition to huCollp261 the addition of three new compounds: (iii) compound I 9-[4-[(3R)-3-Phenyl-1-propylpyrrolidin-1-ium-1-yl]but-2-ynyl]fluoren-9-ol and its analogues, (iv) compound II ((E)-3-(3,6-dichloro-9H-carbazol-9-yl)-N'-(4-hydroxy-benzylidene) propanidrazide), (v) compound III (N1,N5-bis(dibenzo-[b,d]furan-3-yl) glutaramide). The presence of specific TCRs was assessed through immunoscope analysis as described.^{4,5} HLA-DRB1 genotyping was performed using the INNO-LiPA HLA-DRB1 plus kit (Fujirebio) and following manufacturer's instructions.

ASSOCIATED CONTENT

Supporting Information

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Additional experimental details (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): M.C.D.R., F.R., B.G., G.F., D.P., and C.N. are inventors on patents EP2831041 (B1), US9630954 (B2), and US9994524 (B2), which are exploited by Galsor S.r.l.

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