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Original article

Immunogenicity of tocilizumab in patients with rheumatoid arthritis

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ABSTRACT

Objective: The immunogenicity of tocilizumab (TCZ) has been poorly studied. We assessed the immunogenicity of TCZ and serum TCZ trough levels in rheumatoid arthritis (RA) patients and the preexisting TCZ-specific CD4+ T cell repertoire in healthy controls.
Methods: Anti-drug antibodies (ADAs) to TCZ and serum TCZ trough levels in RA patients were assessed at different times by ELISA. Frequencies of naive anti-TCZ CD4+ precursors were studied in healthy controls.
Results: In total, 91 samples from 40 RA patients were analyzed: 21 patients within the first 6 months after treatment initiation and 19 during follow-up after a mean TCZ treatment duration of 21 ± 13 months. None of the 91 samples showed persistent ADAs to TCZ. Only 3 RA patients showed transient and low titers of anti-TCZ ADAs. Serum TCZ trough levels were associated with neither patient characteristics (gender, body mass index) nor disease activity and were identical for patients with and without co-treatment with methotrexate. Three of 9 healthy donors showed preexisting TZC-specific CD4+ T cells at a low level.
Conclusion: Serum TCZ trough levels were not affected by patient characteristics. The occurrence of ADAs to TCZ was a rare event. Because healthy donors show the same frequency of naive TCZ-specific and infliximab-specific CD4+ T cell precursors, the low prevalence of ADAs to TCZ might result from interleukin-6 blockade.

1. Introduction

Currently, 9 different biologic therapies are approved for the treatment of rheumatoid arthritis (RA): 7 inhibitors of pro-inflammatory cytokines (5 targeting tumor necrosis factor alpha [TNFα], one interleukin 1 [IL-1] and one IL-6) as well as T- and B-lymphocyte–targeting agents (abatacept and rituximab, respectively). All anti-TNFα biologic agents (infliximab [IFX], adalimumab, etanercept, golimumab and certolizumab) are implicated in the formation of anti-drug antibodies (ADAs) [1–6]. Nevertheless, ADAs to etanercept are absent or are detected at low levels [4,7,8]. Thus, the immunogenicity profiles of biologic agents can vary highly depending on their molecular design.

The ADA response, as for all immunoglobulin G (IgG) responses, is a T cell–dependent process and therefore relies on the activation of helper CD4+ T lymphocytes specific to the biological agents. CD4+ T lymphocytes are educated in the thymus, and the deletion of autoimmune T cells by self-peptides is one of the main

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mechanisms of tolerance. Thymic or central tolerance probably represents in part the origin of the reduction in immunogenicity provided by the humanization of therapeutic monoclonal antibodies. However, as illustrated by the large number of cases of ADAs to chimeric, humanized and fully human antibodies [5,6], humanization is not sufficient to confer a total lack of immunogenicity. Several reports indicate that the repertoire of naive CD4+ T lymphocytes resulting from thymic education and present at the time of the injection shapes the immunogenicity degree and the amplitude of the potential T cell response against biologic drugs [9,10]. Accordingly, in healthy donors, immunogenic therapeutic proteins [11] and therapeutic monoclonal antibodies [12] feature a pool of specific CD4+ T cells.

Several lines of evidence concerning anti-TNF agents demonstrate that ADA formation is associated with low serum drug trough levels [13], a mechanism underlying therapeutic failure and loss of response over time in RA [14,15]. As well, the concomitant use of methotrexate (MTX) is well known to greatly reduce the occurrence of ADAs [16,17], thus allowing for better treatment maintenance.

Tocilizumab (TCZ) is a humanized anti-IL-6 receptor (IL-6R) monoclonal antibody approved for RA patients with failure to respond to at least one disease-modifying anti-rheumatic drug (DMARD). TCZ is generated by grafting the complementary determining regions of a mouse anti-human IL-6R antibody onto a human IgG1κ. TCZ blocks IL-6–mediated signal transduction by inhibiting the binding of IL-6 to both membrane and soluble IL-6R [18]. The recommended TCZ dose in Europe is 8 mg/kg intravenously every 4 weeks. At this concentration, the TCZ half-life is about 13 days [19,20].

Several phase 3 clinical trials have demonstrated the TCZ efficacy for severe RA [21–23] both combined with MTX but also in monotherapy. Interestingly, Dougados et al. showed that the TCZ + MTX combination was not statistically superior to TCZ monotherapy in terms of Disease Activity Score in 28 joints (DAS28) remission, American College of Rheumatology (ACR) response or arrested progression of structural damage [24].

We have little data on the immunogenicity of TCZ. The ACT-RAY study reported 1.3% of neutralizing anti-TCZ ADAs during the first 52 weeks of follow-up (among 553 RA patients receiving TCZ) [25]. This percentage was much lower than that reported for TNF blockers [15]. For example, Bartelds et al. reported that about 28% of patients developed ADAs to adalimumab over 3 years, most (67%) in the first 6 months [14]. The possibility that TCZ could be less immunogenic than the other biologic agents used for RA could agree with comparable response profiles for TCZ used as monotherapy and TCZ used with MTX [24].

Other than data provided by the TCZ development company [25–28], prospective studies assessing ADAs to TCZ are lacking. We conducted a prospective observational study to examine immunogenicity to TCZ in 2 hospital departments of rheumatology in France. We also performed an in vitro analysis of peripheral blood from healthy subjects to search for a preexisting TCZ-specific CD4+ T cell repertoire.

2. Methods

2.1. Patients

TCZ-treated RA patients from 2 hospital departments of rheumatology in France were prospectively included. Patients were recruited between May 2010 and August 2013. The following clinical data were collected with use of a structured questionnaire: age, gender, body mass index (BMI) (kg/m^2), RA activity (DAS28-C-reactive protein [DAS28-CRP]), previous biologics used and causes of failure, co-treatment with DMARDs, duration of TCZ treatment, mean dosage and interval between 2 infusions. Serum CRP level was recorded.

2.2. Schedule of sample collection

One to 3 blood samples were taken from patients. Patients from the Montpellier department were recruited at the initiation of TCZ treatment and formed the “early TCZ” group. They were assessed just before initiation, month 0 (M0), and 3 serum samples were scheduled to be taken at M1, M3 and M6 after initiation. RA patients from the Paris Sud department were consecutively recruited during a longer follow-up after treatment; this group was named “ongoing TCZ”. Samples were taken from consecutive RA patients every 3 months for 6 months (samples 1, 2 and 3). All samples were taken before TCZ infusion and thus corresponded to serum TCZ trough levels. Both serum TCZ trough levels and ADAs to TCZ were quantified by use of a commercial “European Community”-marked ELISA kit (LISA Tracker, Theradiag, Croissy Beaubourg, France) in the context of the routine care of these RA patients. For TCZ dosage, human IL-6R is coated onto a polystyrene microtiter plate and anti-human IgG biotinylated antibodies and horseradish peroxidase-labelled streptavidin are used for detection. The threshold value of this ELISA for TCZ detection was 1 μg/mL (range: 1–50 μg/mL). Determination of the cut-off value was based on analysis of 150 sera obtained from healthy donors. The immunogenicity assay of the LISA Tracker kit is a bridging ELISA method, with TCZ coated onto a polystyrene microtiter plate. Biotinylated TCZ and horseradish peroxidase-labelled streptavidin are used for the detection. Anti-TCZ monoclonal antibody was used for standard curve determination. The threshold value for anti-TCZ ADAs was 5 ng/mL (range: 5–100 ng/mL).

2.3. T cell repertoire analyses

2.3.1. Generation of protein-specific CD4$^+$ T cell lines

Buffy-coats from healthy subjects were provided by the Établissement français du sang (EFS, Rungis, France). They were collected from anonymous donors after they gave their signed informed consent according to EFS guidelines. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque PLUS density gradient centrifugation (GE Healthcare, Little Chalfont, UK). HLA-DR genotypes were determined by use of the Gold SSP DRB1 typing kit (Invitrogen, Carlsbad, CA) after DNA extraction from PBMCs with use of the NucleoSpin Blood L Kit (Macherey Nagel, Düren, Deutschlandville, Germany). Monocyte-derived dendritic cells (MoDCs) were generated from plastic-adherent PBMCs by 5-d culture in AIM-V medium supplemented with 1000 U/mL human rGM-CSF (R&D Systems, Minneapolis, MN) and 1000 U/mL human recombinant IL-4 (R&D Systems). MoDCs were loaded with 1 μM protein and matured with 1 μg/mL lipopolysaccharide (LPS; Sigma, St. Louis, MO) overnight. After washings, loaded MoDCs were added to round-bottom microwells with 2×10^5 autologous CD4+ T lymphocytes in 200 μL Iscove’s modified Dulbecco medium (IMDM) supplemented with 10% human serum (Lonza, Levallois-Perret, France), 1000 U/mL recombinant human IL-6 (rh-IL-6; R&D Systems) and 10 ng/mL rh-IL-12 (R&D Systems). CD4 T lymphocytes were isolated from autologous PBMCs by positive selection with an anti-CD4 antibody coupled to magnetic beads, as recommended by the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of the obtained CD4+ T cell population was consistently > 95% as assessed by flow cytometry. A total of 36 wells per donor was seeded with the co-culture of MoDCs and CD4$^+$ T lymphocytes and incubated at 37 °C in 5% CO_2. The CD4$^+$ T lymphocytes were restimulated on days 7, 14, 21 and 28 with autologous MoDCs freshly loaded with protein and grown in complete IMDM supplemented with 20 U/mL IL-2 and 5 ng/mL IL-7 (R&D Systems). The