

The effect of infliximab on antiviral antibody profiles in patients with rheumatoid arthritis

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Received: 5 December 2008 / Accepted: 3 May 2009
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Abstract The duration of humoral immunity in patients treated with immunosuppressive drugs is poorly defined. The objective of the study was to investigate the effect of infliximab on the levels of antiviral antibodies against poliomyelitis, rubella and measles in rheumatoid arthritis (RA) patients. Fifty-two consecutive RA patients being treated with 3 mg/kg infliximab were prospectively studied. The antiviral antibody profiles for measles, rubella and three serotypes of poliomyelitis were tested on the day of the first infusion of infliximab and 6 months later. The study group comprised 36 women and 16 men (mean age 54 years, range 33–81) with a mean disease duration of 15 ± 9 years. Forty-two (81%) patients were being treated with methotrexate and 22 (42%) were receiving prednisone. All patients had baseline protective levels of antibodies against measles and the three strains of polio, while 48 (92%) patients had protective antibodies against rubella. No significant change in the levels of antiviral

antibodies was observed after 6 months of treatment with infliximab: from 3.67 at baseline to 3.87 IU/ml for measles, 169.50–197.0 IU/ml for rubella. No change was noticed for the geometric mean concentrations of antibodies against strains of poliomyelitis: 366–478 IU/ml for the Mahoney polio strain, 906–845 IU/ml for the MEF strain and 175–196 IU/ml for the Sauket strain. Patients with longstanding RA conserve long-term immunity to common viruses despite the use of immunosuppressive drugs. Levels of antiviral antibodies against measles, rubella and polio remain stable under treatment with infliximab.

Keywords Rheumatoid arthritis · Poliomyelitis · Rubella · Measles · Infliximab

Introduction

Infliximab, an IgG1 monoclonal chimeric antibody against tumor necrosis factor alpha (TNF α), is a novel treatment for patients with severe rheumatoid arthritis (RA). One of the gravest complications of TNF α blocker agents is serious and even fatal infection [1]. Most patients being treated with infliximab suffer from longstanding disease and have concomitant or past use of other immunosuppressive drugs.

Maintenance of long-term antibody response is critical for protective immunity. The mechanism, by which immunity is preserved, however, is unknown, even to common antigens, both in the general population and in immunocompromised patients [2]. The data on long-term immunity in patients with longstanding RA are scarce. This information may be crucial from an epidemiological point of view, especially in light of the recent reports of several outbreaks of viral diseases, such as measles [2]. Measles is rare in developed countries, but it still remains a serious threat through the importation of new

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cases or within the growing population of non-vaccinated people. Polio had been considered a devastating disease before the development and implementation of an effective vaccine, while rubella was the leading cause of birth defects in the developed countries until the 1970s when immunization programs were implemented [2].

The influence of long-term immunosuppressive treatment, including infliximab, on the duration of humoral immunity to viral antigens has not yet been studied. We now investigated the levels of antibodies against polio, rubella and measles in a large population of longstanding RA patients and studied the effect of infliximab on these antiviral antibodies.

Patients and methods

Patients

All 52 consecutive RA patients who were being treated in two rheumatology departments in two medical centers and who were candidates for treatment with infliximab were included in the current study. They all fulfilled the American Rheumatology Association criteria for RA [3]. These patients received infliximab at a dosage of 3 mg/kg according to the standard protocol. Serum was frozen on the day of the first infusion of infliximab and 6 months later. The antiviral antibody profiles of measles, rubella and three serotypes of poliomyelitis were performed.

Methods

Measles enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG)

The ELISA for the determination of specific measles IgG was conducted using the automated system Vidas RUB IgG assay (bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions. The multianalyte, Virotrol MuMZ (mumps, measles and varicella-zoster virus) (Blackhawk Biosystem, Inc. CA, USA), and an established local low positive IgG serum were included as internal quality control reagents in every ELISA run. The results were expressed qualitatively or by using indices (OD serum/OD cutoff = TV). The protective titer of anti-measles antibodies was defined as >0.7 IU/ml.

Rubella ELISA IgG

The ELISA for the determination of specific rubella IgG was conducted using the automated system Vidas Measles IgG assay (bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions.

The multianalyte, Virotrol ToRCH (toxoplasma, rubella, cytomegalovirus and herpes) (Blackhawk Biosystem, Inc. CA, USA), and an established local low positive IgG serum were included as internal quality control reagents in every ELISA run. The results were expressed quantitatively in international units (IU) as follows: <10 IU/ml = negative, 10–15 IU/ml = equivocal, and >15 IU/ml = positive. In Israel, especially during child-bearing age and pregnancy, an IgG titer of >31 IU/ml is considered protective.

Polio micro-neutralization assay

Blood samples were centrifuged and sera were kept at -20°C until tested. Sera were tested by a micro-neutralization assay (*) against the Mahoney, MEF and Sauket poliovirus strains. A fourfold dilution of the sera was prepared in tubes and distributed in duplicate in 96-well microtiter plates. Sera were diluted between 1:8 and 1:8,192. One-hundred TCID₅₀ (between 32 and 320) of poliovirus were added to each well. Mixtures were incubated at 36°C for 24 h after which 20,000 Hep-2 cells were added to each well. Following incubation at 36°C for 4 days, the cells were fixed, stained and examined. A positive control (composed of a pool of 20 positive sera) was also tested in each run. Titers were determined as the highest dilution of the serum that protected 50% of cultures against a challenge virus. A neutralizing antibody titer of $\geq 1:8$ was considered indicative of protection.

Statistics

Associations between the response to vaccination and patient group and drug use were examined by applying the χ^2 test. Comparisons between patients with positive reactions to vaccination and those who did not respond were examined by applying the Mann–Whitney *U* test according to baseline values of age, disease duration, drug use and presence of the rheumatoid factor (RF). Multiple regression analysis was used to assess the importance of the different variables relative to the humoral response. Continuous variables were compared between groups using *t* tests. Change in drug use was evaluated by the McNemar's test, change in the number of drugs by the Wilcoxon test, and change in drug dosage by paired *t* tests. Statistical analysis was carried out using the SAS system for Windows, release 8.02.

Results

Characteristics of patients

Fifty-two patients were recruited into the study and they included 36 women and 16 men. Their mean age was

54 years (range 33–81), and the mean disease duration for the group was 15 ± 9 years. Forty-two (80%) patients were being treated with methotrexate and 22 (42%) were on prednisone when infliximab was started. Thirty-seven (71%) were positive for rheumatoid factor. All 52 patients continued treatment with infliximab during the study period.

Effect of infliximab treatment on the level of antibodies against measles

The protective level of anti-measles antibodies was set as >0.7 IU/ml (Table 1). All patients had baseline protective levels of anti-measles antibodies. The mean baseline level of anti-measles-specific antibodies was 3.67 ± 1.31 IU/ml, and the titer did not change significantly after 6 months of treatment with infliximab (3.87 ± 1.31 IU/ml).

Effect of infliximab treatment on the level of anti-rubella antibodies

Forty-eight (92%) patients were seropositive for rubella antibodies. The mean level of anti-rubella antibodies was 169.50 ± 140.54 IU/ml before the treatment with infliximab and 197.0 ± 154.56 IU/ml after the treatment. A putative protective level of at least 15 IU/ml of anti-rubella antibodies was reached in 48 out of 52 (92%) patients.

Effect of infliximab treatment on the level of anti-poliomyelitis antibodies

The geometric mean concentration of anti-polio antibodies was stable before and after 6 months of treatment with infliximab: the respective values were 366–478 IU/ml for the Mahoney polio strain, 906–845 IU/ml for the MEF strain and 175–196 IU/ml for the Sauket strain, indicating a highly protective level of neutralizing antibodies. All patients achieved baseline protective levels of antibodies against the three strains of polio without exhibiting any significant change after treatment with infliximab.

Table 1 Effect of infliximab treatment on the geometric mean concentrations of antibodies against polio, measles and rubella

Antigen	Rheumatoid arthritis + infliximab		P value
	Before treatment	After 6 months	
Polio: Sauket (IU/ml)	175	196	0.119
Polio: MEF (IU/ml)	906	845	0.476
Polio: Mahoney (IU/ml)	366	478	0.127
Measles (IU/ml)	3.67 ± 1.31	3.9 ± 1.3	0.065
Rubella (IU/ml)	169.50 ± 140.54	197 ± 154.6	0.035

The influence of various selected variables on the level of antimicrobial antibodies

Multiple regression analysis showed no influence of age, sex, disease duration and RF presence on the humoral response before and after treatment with infliximab. There was no difference in the titer of antimicrobial antibodies against polio, measles and rubella among the patients treated with prednisone and/or methotrexate. The level of protective antibodies remained stable for them all after treatment with infliximab.

Discussion

Rheumatoid arthritis itself and the common use of immunosuppressive drugs may induce cellular immunodeficiency and increase host susceptibility to various viral, bacterial, fungal and parasitic infections [4]. Thus, both the underlying disease and the treatment for it may increase the individual's susceptibility to infection and may potentially decrease response to common vaccines or reduce the basic protective levels of common antibodies. With the growing use of aggressive therapies for RA, such as anti-tumor necrosis (anti-TNF) factor agents and rituximab, the risk of serious or even fatal infections has increased [4, 5], heightening the need for appropriate understanding of the immunosuppressive state in this setting.

Intensive chemotherapy in children with malignancies causes partial immune deficiency, including long-term impairment of humoral immunity. Cytotoxic therapy has been shown to induce a significant lowering of antibody levels against measles and poliomyelitis [6].

In our current study, we investigated the influence of infliximab on the levels of antiviral antibodies against polio, rubella and measles in patients with RA. Despite longstanding immunosuppressive treatment, it emerged as the protective baseline levels of antibodies against measles, and the three strains of polio in all patients and the protective antibodies against rubella in 92% of patients were unaffected by infliximab. This was demonstrated by the absence of any significant changes in the level of antiviral antibodies after 6 months of treatment.

These encouraging findings are concordant with previous studies on an adequate humoral response of infliximab RA-treated patients actively vaccinated against influenza.

We and others have reported that treatment with infliximab or other TNF α blockers does not significantly affect the humoral response to influenza vaccine [7, 8]. Although the response was lower than in non-infliximab-treated patients for one of the antigens, it was still adequate and probably protective [7]. Similarly, a prospective study by

van der Bijl et al. [9] found a diminished response in patients on anti-TNF agents compared with RA patients not on anti-TNF therapy and a group of healthy controls. The data on the efficacy of pneumococcal vaccine in RA patients are controversial. Elkayam et al. [10] found that a significant subset of patients on anti-TNF therapy did not respond as well as healthy controls. Kapetanovic et al. [11] and O'Dell et al. [12] found a lower response rate to pneumococcal vaccine among patients on methotrexate.

In contrast, Visvanathan et al. [13] did not detect any significant difference in pneumococcal polysaccharide vaccine response, when comparing RA patients on infliximab with methotrexate and patients on placebo with methotrexate. Finally, a randomized, double blind, placebo-controlled trial of 208 patients by Kaine et al. [14] reported no significant difference between response rates and antibody concentrations in RA patients on adalimumab and those who received placebo.

Maintenance of long-term antibody response is critical for protective immunity against many pathogens. Amanna et al. [2] performed a longitudinal analysis of antibody titer specific for eight viral antigens, including measles and rubella, in 45 healthy subjects for a period of up to 26 years. They also measured antigen-specific memory B-cells. Those authors found that the antiviral antibody response was remarkably stable, with half-lives ranging from an estimated 50 years for varicella-zoster to more than 200 years for other viruses, such as measles and mumps. B-cell memory was long-lived, but there was no significant correlation between peripheral memory B-cell numbers and antibody levels for the tested antigens [2]. The participants in their study were in good health, although some of them suffered from hypertension and asthma.

Immunosuppressive treatment may affect humoral immunity, resulting in more rapidly decreasing antibody response. Only a few studies assessed the effect of biologic agents on humoral response to viral antigens. RA patients treated with rituximab demonstrated stable levels of anti-tetanus toxoid and of antibodies to pneumococcal capsular polysaccharide [15], suggesting that biologics do not affect pre-existing levels of protective immunity.

In conclusion, our findings demonstrated that patients with longstanding RA conserved long-term immunity to common viruses despite the use of immunosuppressive drugs. The levels of antiviral antibodies against polio, measles and rubella remained stable among the patients in our cohort who were under treatment with infliximab. We are aware of the limitations of our study, which include a relatively small number of patients and a follow-up of only 6 months. Nevertheless, this is the first attempt to assess this important issue, and we propose that further prospective studies on response to vaccinations in RA patients

treated with biological therapies are warranted to validate our results.

Acknowledgments The study was supported by internal institutional funds.

Conflict of interest statement None.

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