Induction of Autoantibodies During Prolonged Treatment with Infliximab

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ABSTRACT. ***Objective.*** To determine the frequency and correlates of autoantibody formation in patients with rheumatic diseases treated with infliximab in a routine clinical setting.

Methods. All patients receiving at least 5 infusions of infliximab, and with anticipated continuation, were prospectively evaluated for the development of the following antibodies: antinuclear antibody (ANA), anti-DNA, anti-Sm, anti-RNP, anti-SSA and anti-SSB. Correlates with pharmacologic treatments, response to infliximab, and adverse events were assessed.

Results. Seventy-six percent of 42 patients receiving prolonged treatment with infliximab developed new autoantibodies, and these persisted in 57%. The most common new autoantibody was ANA in 45%, followed by anti-DNA in 33%, anti-Sm in 31%, and anti-RNP in 29%. New autoantibody formation was associated with both a greater number of infusions ($p = 0.015$) and a higher total dose of infliximab infused ($p = 0.047$). No other treatment, disease characteristic, or loss of efficacy to infliximab discriminated between those developing antibodies compared to those without new antibody formation. No patient developed clinical signs of a new connective tissue disease.

Conclusion. Autoantibody formation is seen commonly in patients receiving prolonged treatment with infliximab. Concomitant immunosuppressive treatments did not preclude the formation of antibodies. The clinical significance of antibody formation remains to be determined. (J Rheumatol 2003;30:2557–62)

Key Indexing Terms:
INFLIXIMAB AUTOANTIBODIES

The advent of the biologic agents to treat inflammatory arthritis has dramatically changed the management of patients in the practice of rheumatology. The past 3 years have seen increasing use of these agents for treatment of various inflammatory arthritic diseases, including rheumatoid arthritis (RA), inflammatory spondyloarthritis (SpA), and psoriatic arthritis (PsA)[1-7]. The clinical response has been remarkable and has resulted in increasing use of these agents in mainstream medicine[8-10]. Nevertheless, the longterm outcome of treatment with these biological agents, including continued efficacy and adverse reactions, is currently unknown.

Infliximab, a monoclonal antibody to tumor necrosis factor-α (TNF-α), binds to both circulating and cell-bound TNF-α and is one of several biological agents currently used in clinical practice[8-10]. In early reports of clinical trials with infliximab, the development of autoantibodies was noted in a minority of patients[11]. The extent of autoantibody development and the association with treatment efficacy and adverse effects have not been studied. We report on the autoantibody profiles in 42 patients with inflammatory rheumatic diseases who received at least 5 infusions of infliximab and were anticipated to continue treatment with infliximab.

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MATERIALS AND METHODS

All patients with rheumatic diseases referred for treatment with infliximab to the Rheumatology Clinic of the McGill University Health Centre at the Montreal General Hospital were followed by a standard clinical protocol. Patients receiving at least 5 infusions of infliximab for inflammatory arthritis were selected for this study. The methods of recruitment and followup have been described[9]. Disease and demographic information were recorded at the first visit. At each subsequent visit, an assessment of response to treatment, record of any adverse events, and routine blood testing were performed.

All blood for antibody testing was drawn on the same day as the infusion of infliximab, but prior to the infusion. Antibody measurements were performed in the clinical immunology and clinical chemistry laboratories of the Montreal General Hospital of McGill University Health Centre. Measurements included: antinuclear antibody (ANA) by the fluorescent antinuclear antibody assay using HEp-2 cells (Shield Diagnostics, Dundee, UK); anti-DNA by the commercial Farr assay (Ortho Diagnostics, Cardiff, UK); anti-Sm, anti-RNP, anti-SSA, and anti-SSB by ELISA (Inova Diagnostics, San Diego, CA, USA); and rheumatoid factor (RF) by immunoturbidimetric assay (K-Assay Rheumatoid Factor, Kamiya Biomedical Company, Seattle, WA, USA). ANA measurements were considered to be positive if the titer was at least 1:40. Titers of antibodies against Sm, RNP, SSA, and SSB were recorded as weakly positive (20–39 units), moderately positive (40–80 units), or significantly positive (> 80 units).

Both the total dose of infliximab administered and the infusion number

after which the first new antibody appeared were recorded. An antibody was defined as persistent if at least 2 measurements of that antibody were recorded to be positive.

Statistical analysis between and within groups was performed by chi-square and Fisher's exact test. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Patient description. Fifty-four patients with various rheumatic diseases were treated with infliximab during the study period. Forty-two have received at least 5 infusions of infliximab and fulfilled criteria for inclusion in the study. Thirty-five were previously described[9] in a clinical audit of treatment with infliximab. Twenty-four of the 42 patients receiving at least 5 infusions of infliximab were taking steroids and 35 were taking disease modifying anti-rheumatic drug (DMARD) therapy. Of the 12 patients who had received fewer than 5 infusions, 10 were receiving background DMARD, and the remaining 2 had either failed or developed side effects to multiple DMARD including methotrexate (MTX). A description of patient demographic and disease related information is given in Table 1. Patients received a total of 511 infusions of infliximab with a mean dose per patient of 3750 mg (SD ± 2500, range 1000–10,600). The mean number of infusions per patient was 12 (SD ± 5). Of the 20 patients who were ANA positive at baseline, one patient was also positive for anti-DNA, and one was positive for both anti-SSA and anti-RNP. No patient fulfilled criteria for a diagnosis of systemic lupus erythematosus (SLE) at the start of infliximab infusions.

New autoantibody formation. Seventy-six percent of patients developed a new autoantibody. The autoantibody profile for the 42 patients is shown in Table 2. Of the 20 patients with an initial positive ANA, there was an increase in titer of ANA in 4, and fluctuation between positive and negative in 2 more. At the end of the study 18 of the 20 with an initial positive ANA were still positive, and 15 of the 19 who developed a new ANA remained positive for ANA. Thus a positive ANA was recorded for 33 patients at the end of the study period. Of the patients developing new antibodies, ANA was the most prevalent (45%), followed by anti-DNA (33%), anti-Sm (31%), anti-RNP (29%), RF (10%), and anti-SSA (7%). No patient developed anti-SSB antibody. The first antibody to appear was ANA in 18 patients, anti-DNA in 9, anti-RNP in 4, and one each for RF, anti-SSA, and anti-Sm. A single patient simultaneously developed ANA, anti-Sm, and anti-RNP. Twenty patients (48%) developed more than one new autoantibody over the course of their treatment. Persisting new antibodies were recorded as follows: ANA in 15 (36%), anti-DNA in 8 (19%), anti-Sm in 6 (14%), anti-RNP in 4 (10%), and RF in one (2%) patient.

Autoantibodies first appeared after a mean of 5.7 (SD ± 4.3) infusions, with a range of one to 19 infusions. The mean dose of infliximab at the time of autoantibody formation was 1500 mg (SD ± 1400, range 200–6100 mg). Figure 1 shows the time course of new autoantibody development for ANA, anti-DNA, anti-Sm, and anti-RNP with the number of infusions. ANA was observed earlier than any other autoantibody, with the majority of patients exhibiting new ANA between infusions one and 5. Anti-DNA was also observed early and occurred between infusions 3 and 11. In contrast, anti-Sm was observed only between infusions 7 and 16, and the same was true for the majority of patients with new anti-RNP. Four patients developed a new RF (one patient at each of infusions number 7, 8, 10, or 11). Three patients became positive for new anti-SSA (one patient at each of infusions number 4, 5, and 19). Figure 2 depicts the percentage of patients within each disease group that developed a new autoantibody. Overall, the percentage of patients developing new autoantibodies was higher in the SpA and PsA groups than in the RA and juvenile RA groups. The maximal antibody titers of anti-Sm, anti-RNP, anti-SSA, and anti-DNA for each patient who developed a new autoantibody are shown in Figure 3. For anti-Sm, anti-RNP, and anti-SSA, most patients showed lower levels of antibodies. In contrast, anti-DNA antibody titers showed a broader range of 21–88%.

The group of patients developing new autoantibodies had received a significantly higher total number of infusions of infliximab ($p = 0.015$) as well as a greater total dose of infliximab ($p = 0.047$). No other disease or treatment variables, including concomitant DMARD or steroid therapy, loss of effect of treatment, and side effects, discriminated between those who developed new autoantibodies and those without autoantibody formation. Twenty-six of the 32 patients developing new autoantibodies were receiving DMARD therapy. Twenty-one were receiving MTX and 5

Table 1. Demographic and disease related information for 42 patients with rheumatic diseases treated with at least 5 infusions of infliximab.

Characteristic	Patients, n (%)
Age, yrs	53 ± 13
Female	37 (88)
Rheumatic disease	
Rheumatoid arthritis	33 (79)
Spondyloarthritis	5 (12)
JRA	2 (5)
Psoriatic arthritis (peripheral)	2 (5)
Current treatments	
Steroids	24 (57)
DMARD	35 (83)
Methotrexate	28 (67)
Hydroxychloroquine	13 (31)
Sulfasalazine	4 (10)
Azathioprine	4 (10)
Cyclosporine	3 (7)
DMARD, 2 or more	15 (36)
Autoantibodies at baseline	
ANA	20 (48)
RF	22 (52)

Table 2. New autoantibodies in 42 patients with rheumatic disease treated with infliximab.

Autoantibody	All Patients (% of total) n = 42	Patients Taking DMARD (%) n = 35	Patients Not Taking DMARD (%) n = 7
New autoantibody	32 (76)	26 (72)	6 (86)
ANA	19 (45)	13 (37)	4 (57)
Anti-DNA	14 (33)	10 (29)	4 (57)
Anti-Sm	13 (31)	10 (29)	2 (29)
Anti-RNP	12 (29)	9 (26)	2 (29)
RF	4 (10)	2 (6)	1 (14)
Anti-SSA	3 (7)	1 (3)	2 (29)
Anti-SSB	0 (0)	0 (0)	0 (0)
Two or more new autoantibodies	20 (48)	16 (46)	4 (57)

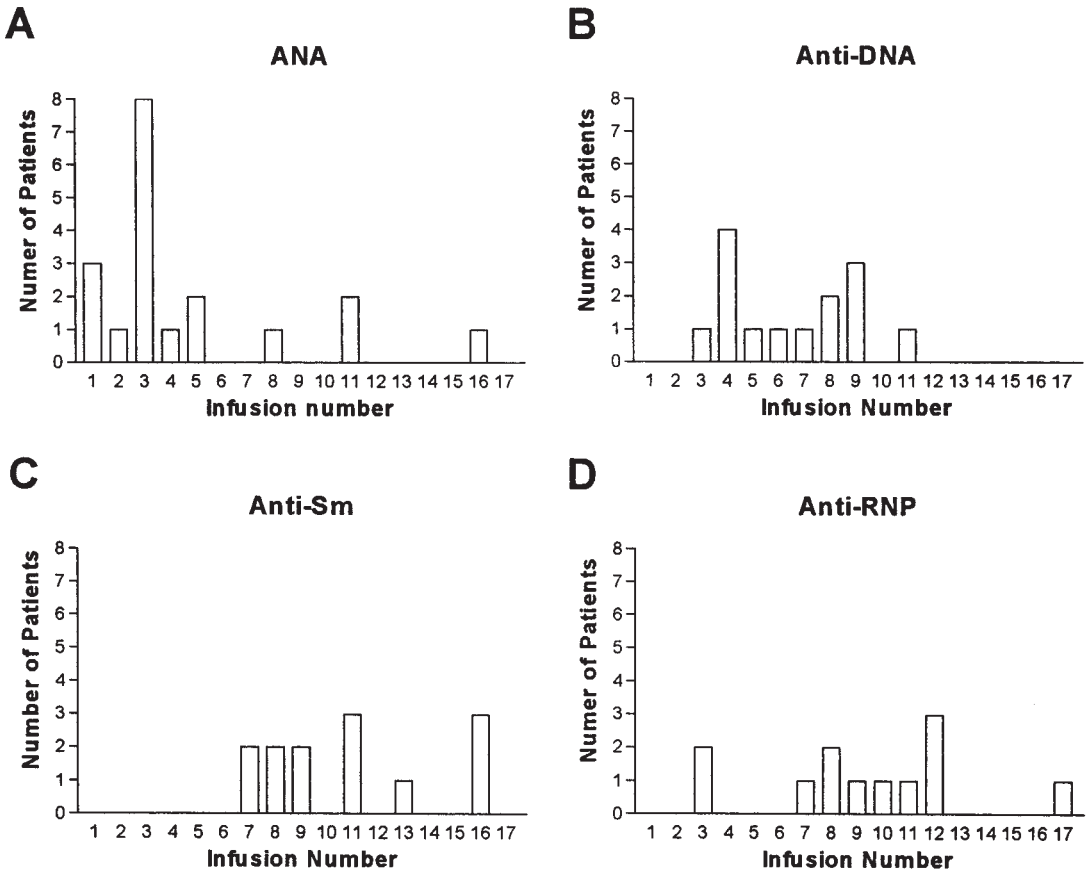

Figure 1. The numbers of patients treated with infliximab who developed new autoantibodies are shown, along with the infusion number after which these autoantibodies were observed.

not taking MTX were receiving some other DMARD, including hydroxychloroquine in 2, azathioprine in 2, and cyclophosphamide in one. Four of the 6 taking no DMARD and developing autoantibodies were receiving corticosteroid therapy. Nine of the 10 patients who did not develop new autoantibody formation were undergoing DMARD treatment as follows: 7 MTX, 5 hydroxychloroquine, and one sulfasalazine. Four of the 7 patients receiving no DMARD treatment experienced infusion reactions, and treatment with infliximab was discontinued in one due to the infusion reaction. Treatment with infliximab was discontinued after at least 5 infusions in 16 (38%) patients, due to inadequate response in 9 (21%), and side effects in 7 (17%). Of note, no patient developed clinical manifestations of SLE.

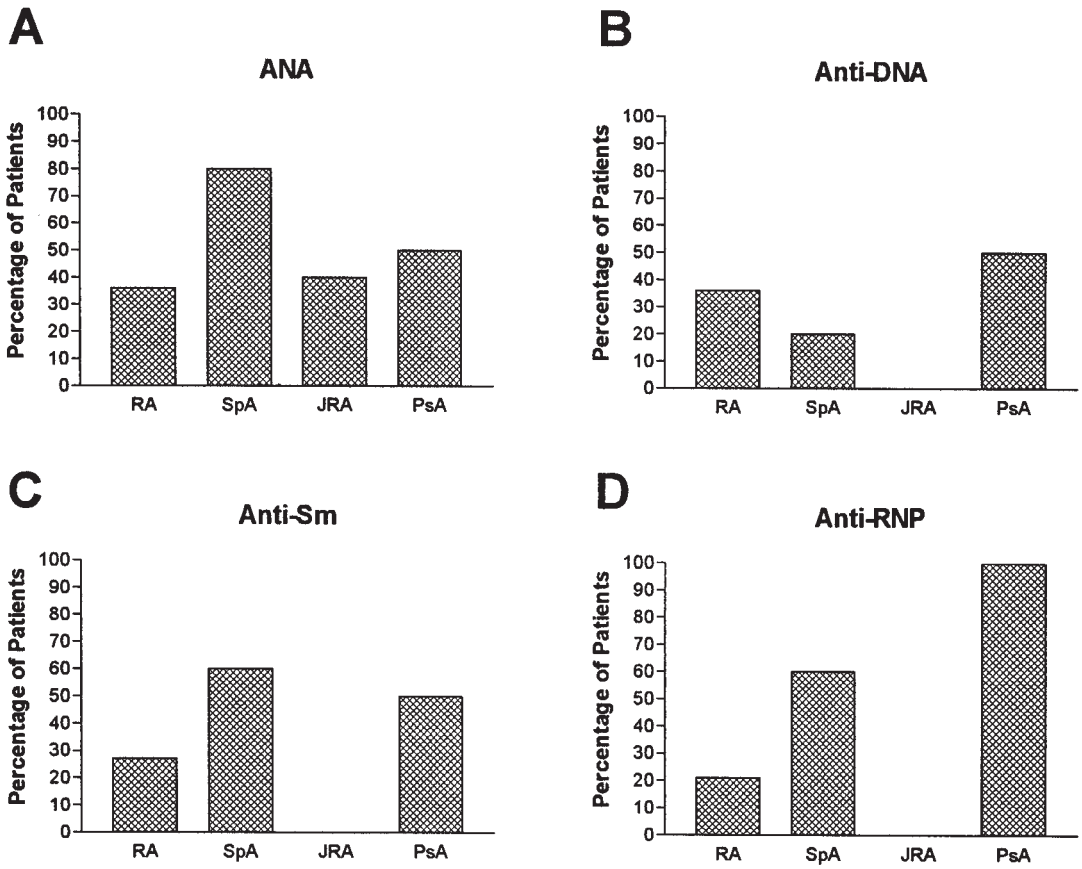

Figure 2. Percentage of patients within each disease group developing new autoantibodies. RA: rheumatoid arthritis, SpA: spondyloarthropathy, JRA: juvenile RA, PsA: psoriatic arthritis.

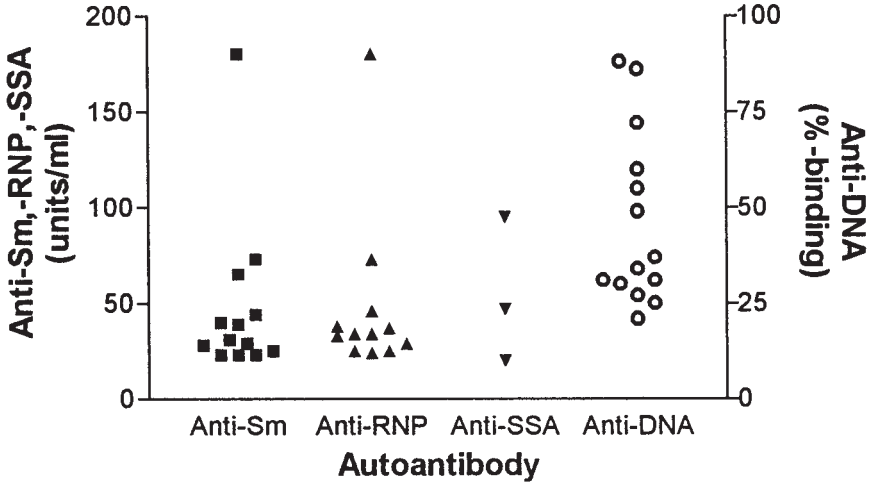

Figure 3. Maximal antibody titers for each patient developing a new autoantibody. Each point indicates the maximal titer for an individual patient with a new autoantibody. Anti-Sm, anti-RNP, and anti-SSA are shown in units/ml; anti-DNA is shown as percentage binding.

DISCUSSION

In this prospective observational study, we evaluated the prevalence of new autoantibody formation in patients receiving at least 5 infusions of infliximab for the treatment of various rheumatic diseases. We observed the appearance of a new autoantibody in over 75% of the patients. The first autoantibodies appeared after about 4 infusions of infliximab, often remained positive, and were often multiple. ANA was the most commonly observed autoantibody, but anti-DNA and anti-Sm, both autoantibodies that are generally associated with SLE, were observed in over 25% of the patients. Although ANA can encompass many autoantibody specificities (including anti-DNA, anti-Sm, anti-RNP, and anti-SSA), we discuss each autoantibody specificity as an independent measure to ensure that all new positive autoantibody results were taken into account. ANA and anti-DNA were observed as early as the first and third infusion, respectively, while anti-Sm and anti-RNP antibodies appeared to develop later (after the seventh infusion).

The reason for autoantibody formation during treatment with infliximab is not fully understood. It is possible that the presence of cytoplasmic and nuclear debris in the circulation, as a result of cell apoptosis, may be triggering autoantibody production[12]. Apoptosis may occur when infliximab attaches to cell-bound TNF-α[13]. Conversely, the reduction of TNF-α induced by infliximab may create an imbalance toward autoreactivity, resulting in a generalized immunological response. It is possible that an even wider spectrum of antibody formation may occur than was measured in this study. Our data suggest that there is a particular sequence in which the different autoantibody specificities arise after infusion with infliximab. This is consistent with the concept of epitope spreading, which has been proposed to explain how autoantibodies produced during the course of an autoimmune disease often bind to multiple B cell epitopes expressed by a single antigen or antigens clustered within a particular molecular or cellular structure[14]. The clinical significance of autoantibody formation, including its consequences regarding efficacy of treatment or development of other adverse events, is still not fully understood.

The occasional appearance of autoantibodies was first noted in early clinical trials of infliximab in RA[11]. In the initial report by Charles and colleagues[11], patients receiving up to 5 infusions of infliximab demonstrated a new ANA in 24% and anti-DNA antibodies in 14%. More recent reports note both a greater frequency and a broader range of autoantibody formation in patients receiving more prolonged treatment with infliximab[15-18]. In a study of combination leflunomide and infliximab, 100% of patients became ANA positive by 60 weeks[18]. The appearance of autoantibodies seems to occur after more prolonged treatments with infliximab, with ANA usually the first autoantibody to emerge. Two recent reports documented the development of anticardiolipin antibody (aCL) in association with infliximab treatment, with one patient developing new onset angina[15,16]. We did not routinely measure aCL at the outset of our study, but recently documented the presence of aCL-IgM in 62% of 14 patients tested. Consistent with the present study, the expression of a true lupus-like syndrome has only rarely been reported[2,11,15-19]. It is not known whether the autoantibodies observed are simply a normal response to an abnormal load of cellular antigens due to apoptosis and/or necrosis, or whether these autoantibodies predate the onset of a specific disease process such as SLE. In the absence of expression of disease, it appears that the induced autoantibodies are not pathogenic, at least in the first years of treatment.

The only clinical parameter that discriminated between patients forming autoantibodies and those not forming autoantibodies was the total number of infusions of infliximab and the total dose of infliximab administered. Concomitant treatment with MTX, other immunosuppressives, or corticosteroids did not preclude the development of autoantibodies. Although 25% of patients developed allergic-type phenomena associated with the infusions of infliximab, we did not observe a relationship between autoantibody formation and these reactions. There was also no association between the loss of efficacy of infliximab on the arthritic process and the development of autoantibodies.

This small preliminary study has limitations, which we acknowledge. First, autoantibody testing was done as part of usual clinical practice. For this reason, we did not request a full autoantibody profile including aCL, antithyroid antibodies, and antihistone antibodies. Second, the patients represent our usual clinical practice and reflect patients with severe disease not adequately responsive to usual DMARD treatment. Third, our patients had a variety of rheumatologic diseases and were thus not a homogeneous group. All patients receiving infliximab at our center for inflammatory arthritis were eligible for inclusion in this study, regardless of disease or concomitant treatment.

We observed a high rate of new and persistent autoantibody formation in patients with rheumatic disease receiving prolonged treatment with infliximab. Concomitant treatments, continued response to infliximab treatment, and the development of allergic reactions did not correlate with the appearance of new autoantibodies. Although no patient, to date, has manifested disease characteristics that might be related to autoantibody formation, longterm followup and meticulous continuing care of patients undergoing prolonged treatment with infliximab is indicated.

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