

# What Can We Learn from Treatment-Induced Changes in Rheumatoid Factor and Anti-Citrullinated Peptide Antibodies?



Basic and clinical research initiatives on the 2 major autoantibody systems in rheumatoid arthritis (RA), rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA), have moved in parallel in recent years<sup>1-3</sup>. Indeed, recent works have disclosed some of the mechanisms underlying the genesis, maintenance, and role of the humoral autoimmune response in RA, identifying defective B cell tolerance checkpoints<sup>4</sup> and dissecting the interactions among environment, genes, and adaptive immunity<sup>3</sup>. On the other hand, monitoring the autoimmune response in RA through its most accessible marker, i.e., serum autoantibodies, has gained growing interest as RF and ACPA are recognized as powerful predictive, diagnostic, and prognostic tools in RA.

Several studies in recent years have focused on changes in RF and ACPA levels during different treatment strategies, especially with biological agents such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) inhibitors, summarized in Table 1<sup>5</sup>, and B cell targeted therapies<sup>6</sup>. These studies are welcome for a number of reasons. First, the identification of different pretreatment values and/or different rates of decline of RF and ACPA might offer accessible biomarkers of clinical response. Second, treatment-related changes of serum autoantibodies might provide insights into the specific immunoregulatory activity of a single drug or class of drugs. Third, monitoring the serum autoimmune response in RA might shed new light on mechanisms underlying the generation and maintenance of autoreactive B cells. Last, assuming that autoantibodies play a pathogenetic role in RA, treatment-induced seroconversion could be regarded as one of the goals for true remission or cure.

The article by Bos and colleagues<sup>7</sup> in this issue of *The Journal* provides a significant step forward in understanding the serum autoimmune response after effective treatment in RA: the authors analyze a large cohort of patients homogeneously treated with adalimumab. The results emerging from their study are: (1) decrease from baseline values is

much greater for IgM RF with respect to ACPA (31% vs 8%); (2) seroconversion (from positive to negative) is unusual for ACPA, while 17% of patients become negative for IgM RF; and (3) decreased antibody levels are associated with clinical response for both IgM RF and ACPA, and with decreased acute-phase reactants for IgM RF.

A drop in IgM RF levels has been nearly unanimously reported with TNF- $\alpha$  inhibitors (Table 1) as well as with other biological and conventional disease modifying antirheumatic drugs (DMARD)<sup>6,8,9</sup>. Such decrease was shown to be stable over time<sup>10</sup>, and a more conspicuous decline was associated with good clinical response<sup>11</sup>. IgA and IgG RF isotypes are also strongly reduced by conventional DMARD and TNF- $\alpha$  inhibitors, although this decrease does not appear to be related to clinical response<sup>8,11</sup>. Changes in ACPA have been less consistent (Table 1), possibly due to different assays for ACPA measurement, different disease duration, different study periods, and different criteria of analysis (inclusion of all patients vs positive patients only). Data provided by Bos and colleagues<sup>7</sup> indicate that, although a significant drop of IgG ACPA can be observed in patients achieving clinical response, ACPA levels are much less affected than RF by TNF- $\alpha$  inhibitors, as also reported with rituximab<sup>6</sup> and conventional DMARD<sup>9</sup>. It remains to be determined whether the reduction in ACPA levels is as stable as that found for IgM RF<sup>10</sup>.

A direct application of these results in clinical practice appears at present to be just fascinating. Indeed, no data support a better performance of autoantibody measurement with respect to acute-phase reactants and clinical assessment in monitoring response to therapy. Until the temporal relationship is specifically dissected, it remains undetermined whether IgM RF levels are a consequence of inflammation (thus being a redundant marker) rather than a cause. Further, it is still unclear whether pretreatment levels of different autoantibodies and/or isotypes may be able to predict

---

See Differential response of RF and ACPA during adalimumab treatment in patients with RA, page 1972

Table 1. Characteristics of longitudinal studies on RF and ACPA levels during RA treatment with TNF- $\alpha$  inhibitors.

Study	Treatment	Patients, n	Followup, weeks	IgM	RF IgG	IgA	ACPA (IgG)
Bobbio-Pallavicini <sup>10</sup>	Infliximab/MTX	30	30; 78	50% decrease (sustained at 78 wks)	—	—	Small decrease (not sustained at 78 wks)
Nissinen <sup>19</sup>	Infliximab/DMARD	25	2	Decrease	—	—	No effect
Alessandri <sup>20</sup>	Infliximab/DMARD	43	24	20% decrease (responders)	—	—	15% decrease (responders)
Caramaschi <sup>21</sup>	Infliximab/MTX	27	22	50% decrease	—	—	No effect
De Rycke <sup>22</sup>	Infliximab/MTX	62	30	50% decrease	—	—	No effect
Yazdani-Biuki <sup>23</sup>	Etanercept/DMARD	12	36	No effect	~40% increase	~35% increase	No effect
Braun-Moscovici <sup>24</sup>	Infliximab/DMARD	30	14	No effect	—	—	Decrease (responders)
Chen <sup>25</sup>	Etanercept/DMARD	90	12	35% decrease (responders)	—	—	30% decrease (responders)
Atzeni <sup>26</sup>	Adalimumab/MTX	57	48	40% decrease (responders)	—	—	30% decrease (responders)
Ahmed <sup>27</sup>	Infliximab/DMARD	33	30; 54	Decrease (sustained at 54 wks)	No effect	Decrease (not sustained at 54 wks)	Decrease (not sustained at 54 wks)
Bobbio-Pallavicini <sup>11</sup>	Infliximab/MTX, etanercept/MTX, adalimumab/DMARD	132	54	25% decrease (responders)	20% decrease (responders and nonresponders)	10% decrease (responders and nonresponders)	No effect
Vis <sup>28</sup>	Infliximab/MTX	62	46	65% decrease	—	—	25% decrease

ACPA: anti-citrullinated peptide antibodies; DMARD: disease modifying antirheumatic drugs; MTX: methotrexate; RA: rheumatoid arthritis; RF: rheumatoid factor; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; TLR: Toll-like receptors.

different response rates to different therapies<sup>5</sup>. Additional studies are warranted on this topic.

Translating these results into basic research is even more speculative. Indeed, the mechanisms that underlie the ability of TNF- $\alpha$  inhibitors to decrease RA-specific autoantibodies are far from fully elucidated, although several pathways have recently been explored<sup>12</sup>. These include restoration of the regulatory T cell pool and function, inhibition of dendritic cell maturation, inhibition of interleukin 6 and B cell activating factor synthesis and Toll-like receptor (TLR) expression, and, more recently, direct interference with the B cell compartment through disruption of germinal centers<sup>13</sup>. It is likely that such mechanisms, at least in part, are not class-specific but are shared by most of the immunomodulatory drugs in RA, as conventional DMARD and B cell targeted therapies have shown similar patterns of reduction of RF and ACPA<sup>6,8,9</sup>.

More intriguingly, the marked qualitative and quantitative differential responses of RF and ACPA during antirheumatic treatment raises the important question of whether and how the 2 autoantibody systems are differently regulated. Although little is known about IgM ACPA fluctuations, such different behavior is not fully attributable to a different clearance between the IgG isotype of ACPA and the IgM isotype of the RF, since marked reduc-

tion of all RF isotypes has been described after both anti-TNF- $\alpha$ <sup>11</sup> and rituximab treatment<sup>6</sup>. Again, it is likely that several mechanisms account for the diversity of the RF and the ACPA systems. Although only speculative, these might include: (1) different roles of innate and adaptive immunity, as suggested by the ability of TLR to activate RF+ B cells<sup>14</sup> and, in contrast, the strong association of ACPA with major histocompatibility complex-class II susceptibility loci and the requirement of T cell help<sup>3</sup>; (2) a different contribution of various antibody-secreting cells, such as plasmablasts, short-lived and long-lived plasma cells<sup>15</sup>, characterized by different lifespans, different environmental niches, and different responses to therapies; (3) different sites of production, which include spleen, bone marrow, lymph nodes, and the synovial tissue itself, as well as other ectopic lymphoid sites<sup>16-18</sup>, possibly characterized by different accessibility and different ability to host B cell responses and support plasma cell survival.

We cannot predict whether monitoring RF and ACPA levels during therapies will ever enter routine clinical practice. However, we encourage further research as the understanding of treatment-induced changes of autoantibody levels provides a framework for dissecting the pathophysiological bases of the B cell autoimmune response in RA.

**FRANCESCA BOBBIO-PALLAVICINI, MD,**  
 Chair and Division of Rheumatology;  
**ROBERTO CAPORALI, MD,**  
 Associate Professor,  
 Chair and Division of Rheumatology;  
**SERENA BUGATTI, MD,**  
 Chair and Division of Rheumatology;  
**CARLOMAURIZIO MONTECUCCO, MD,**  
 Full Professor, Director,  
 Chair and Division of Rheumatology,  
 University of Pavia, Fondazione IRCCS Policlinico San  
 Matteo, Pavia, Italy

Address reprint requests to Prof. C. Montecucco, University of Pavia,  
 Fondazione IRCCS Policlinico San Matteo, Piazzale Golgi 2, 27100  
 Pavia, Italy. E-mail: montecucco@smatteo.pv.it

## REFERENCES

- Westwood OM, Nelson PN, Hay FC. Rheumatoid factors: what's new? *Rheumatology Oxford* 2006;45:379-85.
- Bugatti S, Codullo V, Caporali R, Montecucco C. B cells in rheumatoid arthritis. *Autoimmun Rev* 2007;6:482-7.
- Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu Rev Immunol* 2008;26:651-75.
- Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J Exp Med* 2005;201:1659-67.
- Bobbio-Pallavicini F, Caporali R, Alpini C, Moratti R, Montecucco C. Predictive value of antibodies to citrullinated peptides and rheumatoid factors in anti-TNF-alpha treated patients. *Ann NY Acad Sci* 2007;1109:287-95.
- Cambridge G, Leandro MJ, Edwards JC, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum* 2003;48:2146-54.
- Bos WH, Bartelds GM, Wolbink GJ, et al. Differential response of the rheumatoid factor and anti-citrullinated protein antibodies during adalimumab treatment in patients with rheumatoid arthritis. *J Rheumatol* 2008;35:1972-7.
- Alarcon GS, Schrohenloher RE, Bartolucci AA, Ward JR, Williams HJ, Koopman WJ. Suppression of rheumatoid factor production by methotrexate in patients with rheumatoid arthritis. Evidence for differential influences of therapy and clinical status on IgM and IgA rheumatoid factor expression. *Arthritis Rheum* 1990;33:1156-61.
- Mikuls TR, O'Dell JR, Stoner JA, et al. Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. *Arthritis Rheum* 2004;50:3776-82.
- Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. *Arthritis Res Ther* 2004;6:R264-72.
- Bobbio-Pallavicini F, Caporali R, Alpini C, et al. High IgA rheumatoid factor levels are associated with poor clinical response to tumour necrosis factor alpha inhibitors in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:302-7.
- Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008;117:244-79.
- Anolik JH, Ravikummar R, Barnard J, et al. Cutting edge: anti-tumor necrosis factor therapy in rheumatoid arthritis inhibits memory B lymphocytes via effects on lymphoid germinal centers and follicular dendritic cell networks. *J Immunol* 2008;180:688-92.
- Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002;416:603-7.
- William J, Euler C, Shlomchik MJ. Short-lived plasmablasts dominate the early spontaneous rheumatoid factor response: differentiation pathways, hypermutating cell types, and affinity maturation outside the germinal center. *J Immunol* 2005;174:6879-87.
- Reparon-Schuijt CC, van Esch WJ, van Kooten C, et al. Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. *Arthritis Rheum* 2001;44:41-7.
- Bugatti S, Caporali R, Manzo A, Vitolo B, Pitzalis C, Montecucco C. Involvement of subchondral bone marrow in rheumatoid arthritis: lymphoid neogenesis and in situ relationship to subchondral bone marrow osteoclast recruitment. *Arthritis Rheum* 2005;52:3448-59.
- Rangel-Moreno J, Hartson L, Navarro C, Gaxiola M, Selman M, Randall TD. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. *J Clin Invest* 2006;116:3183-94.
- Nissinen R, Leirisalo-Repo M, Peltomaa R, Palosuo T, Vaarala O. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. *Ann Rheum Dis* 2004;3:681-7.
- Alessandri C, Bombardieri M, Papa N, et al. Cytokine and chemokine receptor profile of peripheral blood mononuclear cells during treatment with infliximab in patients with active rheumatoid arthritis. *Ann Rheum Dis* 2004;63:1218-21.
- Caramaschi P, Biasi D, Tonolli E, et al. Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment. *Rheumatol Int* 2005;26:58-62.
- De Rycke L, Verhelst X, Kruithof E, et al. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005;64:299-302.
- Yazdani-Biuki B, Stadlmaier E, Mulabecirovic A, et al. Blockade of tumour necrosis factor (alpha) significantly alters the serum level of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;64:1224-6.
- Braun-Moscovici Y, Markovits D, Zinder O, et al. Anti-cyclic citrullinated protein antibodies as a predictor of response to anti-tumor necrosis factor-alpha therapy in patients with rheumatoid arthritis. *J Rheumatol* 2006;33:497-500.
- Chen HA, Lin KC, Chen CH, et al. The effect of etanercept on anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis. *Ann Rheum Dis* 2006;65:35-9.
- Atzeni F, Sarzi-Puttini P, Dell'Acqua D, et al. Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titer reduction: a one-year prospective study. *Arthritis Res Ther* 2006;8:R3.
- Ahmed MM, Mubashir E, Wolf RE, et al. Impact of treatment with infliximab on anticyclic citrullinated peptide antibody and rheumatoid factor in patients with rheumatoid arthritis. *South Med J* 2006;99:1209-15.
- Vis M, Bos WH, Wolbink G, et al. IgM-rheumatoid factor, anti-cyclic citrullinated peptide, and anti-citrullinated human fibrinogen antibodies decrease during treatment with the tumor necrosis factor blocker infliximab in patients with rheumatoid arthritis. *J Rheumatol* 2008;35:425-8.