Role of antibody-secreting cells as early biomarkers of immune response to influenza vaccination in rheumatoid arthritis patients treated with anti-TNF agents

Evaluation of: Kobie JJ, Zheng B, Bryk P *et al.* Decreased influenza-specific B cell responses in rheumatoid arthritis patients treated with anti-tumor necrosis factor. *Arthritis Res. Ther.* 13, R209 (2011). Specific anti-influenza antibodies, early (days 5–7) effector and late (1–6 month) memory B cells have been found to be reduced in immunized rheumatoid arthritis (RA) patients undergoing treatment with TNF-α blocking agents compared with nontreated RA patients and healthy controls. On a per-patient basis, a direct correlation has also been demonstrated between the short-term antibody-secreting effector cells and the 1-month memory B cells, and influenza-specific antibody titer. This study has demonstrated for the first time the reduced specific B-cell and antibody response to influenza vaccine antigens in RA patients undergoing treatment with TNF-α-blocking agents and vaccinated against influenza. This methodological approach, which may allow the identification of early biomarkers, should be followed in order to improve knowledge of immune responses in selected categories of immunosuppressed patients and to modulate immune stimulation intensity and conditions to the level of defective immune response.

KEYWORDS: antibody response ■ biomarkers ■ effector B cells ■ influenza vaccine ■ memory B cells ■ rheumatoid arthritis ■ TNF-α antagonists

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TNF-α-blocking agents have been demonstrated to be effective in rheumatoid arthritis (RA) management by reducing disease progression and preventing joint destruction; however, this treatment is sometimes associated with adverse events, such as induction of infections, which can be severe [1]. Preventive vaccinations are, therefore, very useful in this context and, in particular, influenza vaccination may play a crucial role by preventing influenza, thus reducing morbidity and mortality.

Only in the last few years, however, have studies on influenza vaccination in RA patients undergoing treatment with TNF-α blockers been performed on a cumulative population sample of little more than 550 patients [2–4]. Vaccination safety has been demonstrated, whereas data are very poor on vaccine efficacy in preventing disease. Immunogenicity, although generally fulfilling the European Committee for Proprietary Medicinal Products (CPMP) parameters for protection, was normally lower than in healthy controls (HC). Among these studies, only a minority have addressed the important topic of lymphocyte subpopulation monitoring.

Published in *Arthritis Research and Therapy*, a group of scientists at the University of Rochester Medical Center (NY, USA) demonstrated that RA patients treated with anti-TNF-α drugs and vaccinated against influenza throughout four consecutive seasons showed a titer of influenza-specific antibodies that were generally protective, but lower than HC and untreated RA patients [5]. Moreover, such a reduced antibody response was parallel with the lowered specific B-cell response. In particular, early (postvaccination days 5–7) effector and late (1–6-month) memory B cells were reduced. On a per-patient basis, the authors could also demonstrate a direct correlation between the short-term antibody-secreting effector cells and the 1-month memory B cells and influenza-specific antibody titer.

B cells & TNF-α

The peripheral blood B-cell compartment is composed of mature naive and memory B cells, which can be distinguished on the basis of the expression of CD19, CD27, IgM and IgD, the former being $CD19^+CD27^-$ and the latter $CD19^+CD27^+$. Memory B cells can be further subdivided into IgM memory ($IgM^{++}IgD^+$) and switched memory B cells (IgM^-IgD^-) [6]. Following immunization and/or infection, mature naive and memory B cells proliferate in the germinal centers and differentiate into plasmablasts ($CD19^+IgD^-CD27^{++}CD38^{++}$), which may move to target sites or bone marrow, where they develop into long-lived plasma cells [7].

Such a physiologic trend may directly and indirectly, through monocyte-activated $CD4^+$ T cells, be heavily influenced by TNF-α. RA

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patients treated with TNF-α antagonists display, in fact, a scarcity of follicular dendritic cell network and germinal center reactions, accompanied by a reduction in total memory B cells, with an increase in mature and transitional, compared with controls [8].

Findings from the study

The group from the University of Rochester Medical Center demonstrated that TNF-α-blocking agents are able to impair the antibody response to influenza vaccination in RA patients. In particular, they observed a reduced geometric mean titer in patients treated with TNF inhibitors compared with HC. In detail, these patients had a seroconversion rate to H1 and B influenza vaccine antigens in significantly lower proportion compared with untreated RA patients and to H3 influenza vaccine antigen compared with HC. Moreover, the authors performed a trivalent inactivated influenza vaccine (TIV) IgG EliSpot from a patient subset to determine if anti-TNF therapy affects the influenza-specific memory B-cell response. They observed that the frequency of RA patients treated with TNF inhibitors who had a fourfold or greater increase in TIV-specific memory B cells at 1 month postvaccination, stimulated with IL-2 and CpG, was significantly lower than the frequencies in both HC and untreated patients. Effector B-cell response to influenza vaccination, evaluated as antibody-secreting cells (ASC) and plasmablast populations, which are strongly correlated with them, has also been studied at the short-term postvaccine period (days 5–7 and days 8–10) by Elispot and cytometry, respectively. Treated patients had a significantly decreased induction of $IgD^{-}CD27^{++}CD38^{++}$ plasmablasts at days 5–7 compared with both HC and untreated patients. Moreover, treated patients had significantly decreased TIV IgG ASC compared with untreated patients. These results indicate that effector B-cell response to influenza vaccine is impaired in RA patients treated with anti-TNF-α. Overall, a significant positive correlation of the IgG TIV-specific ASC measured at days 5–7 postvaccination with the frequency of IgG TIV-specific memory B cells, and with the serum hemagglutination-inhibition titer at 1 month was observed.

Impact on understanding of B-cell response to influenza vaccine in the course of anti-TNF-α therapy

The study by Kobie *et al.* represents a step forward in the brief history of the study of influenza vaccine immunogenicity in anti-TNF-α-treated RA patients, by proposing an elegant and useful methodological approach to explore the integrated specific B cell and antibody response. The majority of studies have demonstrated a sufficiently protective, although generally reduced, antibody response. Analyzing antibody-secreting B cells may allow the identification of early biomarkers of immune response, thus leading to the early identification of poor responder patients in order to recommend either adjuvanted vaccines, which, although used cumulatively in no more than 20% of these patients [2], have been tolerated even in active autoimmune diseases [3], or boosters. Moreover, such guided treatment may contribute to improved vaccine efficacy, thus avoiding further disaffection from an already poorly implemented vaccination practice in this vulnerable patient population [9]. The topic is considered relevant by the European League Against Rheumatism (EULAR), which has recently recommended that influenza vaccination should be strongly considered in these patients, and can be administered during TNF-α-blocking therapy [10].

Biologics (monoclonal antibodies and fusion proteins) represent a brand new approach to immunosuppressive therapy, where the recognition of a restricted immunological target is often accompanied by an increased infection risk [1]. As in the case of TNF-α inhibitors, this is likely due to immunosuppression, thus confirming the central role of TNF-α in the immune system. Vaccination of RA patients under biologics, thus represents not only good clinical practice, but also a model of specific immune response [11], which may be modulated according to the patient's needs. Influenza is also a very intriguing model, considering both its high variability and yearly recurrence. However, cohort studies have demonstrated broad crossreactivity of antibody response, probably due to the relative homology of the different drifted viral strains [12]. In the case of shifted pandemic strains, where the cohort effect of yearly vaccination is less operating, the possibility of an early identification of the newly recruited ASC may be particularly useful, as an accurate expression of the actual B-cell response capability in immunosuppressed populations. Moreover, if the memory B-cell reduction is confirmed for other vaccines, containing different, structurally stable and less diffuse T-cell-dependent antigens, for which effective response should be long term, advice on the need to improve vaccine efficacy and to monitor specific antibody levels over time becomes even more stringent.

The activation/expansion of B-cell subsets is, however, under multiple regulatory controls, including T-cell subsets. Thus, it is desirable to contemporaneously monitor specific antibody secretion together with the joint T and B effector and memory subpopulations following influenza vaccine stimulation in anti-TNF-α-treated RA patients. However, such a complete study has never been carried out in this context, and only single lymphocyte subsets, inside either the T or B compartment, have been analyzed very recently.

Regarding T-cell subpopulations, a significant Treg cell increase has been observed in RA patients undergoing treatment with TNF-α-blocking agents, 1 month after influenza vaccination, compared with baseline values, followed by a decrease at 6 months, in direct, significant correlation with the specific anti-H1 and -H3 antibodies. This suggests a regulatory influence on the antibody response, which maintains a protective level also at 6 months [13]. A postinfluenza-vaccine Treg increase has also been reported in an animal model, by monitoring specific Treg cells, and Kobie *et al.* have hypothesized that the observed reduced B-cell response may also depend on the anti-TNF-α-induced Treg increase, as previously described in patients responding to infliximab [14,15].

Post-vaccination cellular behavior and its relationship with TNF-α have also been analyzed in different populations. Liu *et al.*, in fact, demonstrated an impaired influenza-specific $CD8^+$ T-cell response in older healthy adults correlated with reduced TNF-α production by influenza-infected dendritic cells [16]. This may have important implications in understanding the consequences of anti-TNF-α treatment in relation to the defense against infectious agents. If age-related decreased TNF-α production impairs influenza-specific $CD8^+$ T-cell response in older adults, in fact, anti-TNF-α treatment, often performed in older RA patients, may further reduce the immune response. Another important point is the cumulative effect of immunosuppressive therapy. The frequent association of corticosteroids, able to reduce cytokine (including TNF-α) production [17], with anti-TNF-α biologic therapy, may have a greater synergistic, proinfective effect than biologic therapy alone, as recently demonstrated [18]. Anti-TNF-α therapy is only able to inactivate TNF-α, which is produced in high quantity in RA patients, without any effect on TNF-α synthesis, which is instead inhibited by corticosteroids. It would be of interest in the paper by Kobie *et al.* to look at the nearly 30% RA patients treated with corticosteroids and TNF-α blockers, in order to evaluate whether such a minority may influence the cumulative lowered immune response.

Conclusion

The Kobie *et al.* study has demonstrated, for the first time in RA patients treated with TNF-α-blocking agents and vaccinated against influenza, the correlated reduced specific B-cell and antibody response to influenza vaccine antigens. Although easily predicted, such a result has never been demonstrated before in this context. Moreover this study has a relevant theoretical and practical impact, in the possibility to identifying early, accurate biomarkers of immune response, which would be useful to suggest appropriate vaccination recommendations in this vulnerable patient population. Fulfilling CPMP serological criteria for protection, in fact, may make the adoption of boosters or adjuvanted vaccines redundant, which instead become necessary only if there is the demonstration of a borderline response, which may further be reduced in presence of comorbidities, older age or association with other immunosuppressive agents, thus becoming unprotective. The authors warn correctly of the need to immunize these patients with the best vaccine type and/or modality, which is a suitable reminder of the possibility to modulate the immune response in selected patient categories. Moreover, the invitation to repeat the vaccination annually is welcome and fits what has been demonstrated on the progressively annual growth of the specific antibody response to influenza vaccine antigens, probably due to the largely overlapping antigens among the different flu strains, responsible for the induction of broadly cross-neutralizing antibodies [12,13].

Future perspective

The joint specific T- and B-lymphocyte subsets together with antibody production should be explored in large and standardized populations of RA patients vaccinated against influenza and treated with TNF-α-blocking agents.

Moreover, the influence of single TNF-α inhibitors, other biologics, non-biological immunosuppressive drugs, other vaccines and repeated or adjuvanted vaccines should also be systematically explored in sufficiently large populations and in standard and comparable situations.

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Executive summary

Findings from the study

- Influenza-specific serum antibodies were reduced in rheumatoid arthritis patients treated with anti-TNF-α.
- Early (postvaccination days 5–7) effector and late (1–6 month) memory B-cell responses were reduced.
- Direct correlation between influenza-specific early (days 5–7) antibody-secreting effector cells, 1-month memory B cells and antibody titer.

Impact on understanding of anti-TNF-α & immune response to influenza vaccine

- Improvement of knowledge of specific B-cell immune response in rheumatoid arthritis patients treated with TNF-α inhibitors and immunized against influenza.
- Identification of early biomarkers of reduced immune response.

Conclusion

- Possibility of modulating the immune stimulation intensity and conditions to the level of defective immune response in selected categories of patients.

Future perspective

- Evaluation of joint specific T- and B-lymphocyte subsets together with antibody production in large and standardized populations of rheumatoid arthritis patients treated with TNF-α blocking agents and vaccinated against influenza.
- Systematic studies of influence of single TNF-α inhibitors, other biologics, nonbiological immunosuppressive drugs, other vaccines, repeated or adjuvanted vaccines in sufficiently large populations and in standard and comparable situations.

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