Poor serological responses upon influenza vaccination in patients with rheumatoid arthritis treated with rituximab

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Annual influenza vaccination is recommended for immunocompromised patients.[1 2] Rituximab, an anti-CD20 monoclonal antibody, recently approved by American and European authorities for the treatment of rheumatoid arthritis (RA) patients failing tumour necrosis factor (TNF)-blocking agents, diminishes the number of circulating B cells for a period of six to nine months after infusion.[3 4] Circulating plasma cells and immunoglobulin levels are not affected by this therapy. Based on data in lymphoma patients, the package insert states that patients should not be vaccinated from one month before the administration of rituximab until six months after.[5 6] In those studies the underlying lymphoma and treatment with chemotherapy contributed to the diminished immunological response. The effect of rituximab on the outcome of influenza vaccination in RA is not known.

We examined the humoral response upon influenza vaccination in four RA patients (three women, age range 55–61 years) treated with rituximab (1000 mg intravenously on days 0 and 14) combined with methotrexate (5–20 mg a week) and additional prednisone (5 mg) in one patient. Nineteen RA patients treated with TNF-blocking agents with or without disease-modifying antirheumatic drugs (79% women, mean age 56 years, range 40–71), and 20 healthy individuals (50% women, mean age 45 years, range 19–77) acted as controls. The three groups were well matched with respect to sex, age, prevaccination titres and previous influenza vaccination. Both patient groups had high disease activity scores (mean DAS28 3.47 and 4.44 for RA patients treated with rituximab and anti-TNF, respectively, $p = 0.088$, analysis of variance). Participants were vaccinated intramuscularly with a trivalent subunit vaccine (0.5 ml Influvac 2005–2006; Solvay, Weesp, the Netherlands). Haemagglutination-inhibition titres were measured just before vaccination and 28 days later, as described before.[7 8] Absolute lymphocyte counts were analysed using TruCOUNT tubes by flowcytometry. B cells were completely depleted ($<1\times10^6$ cells/l) in all four patients from day 28 to day 84 after the first rituximab infusion. The vaccine was administered shortly after day 84, with only marginal B-cell reconstitution at the time of vaccination (median B-cell count $<10\times10^6$ cells/l). As a result of low B-cell and patient numbers no trends could be determined in B-cell subsets.

Even though only four RA patients treated with rituximab were evaluated, we found significantly lower postvaccination titres (fig. 1) and protection rates (the proportion of a group with a titre ⩾40) in comparison with both control groups for all three antigens. These findings could not be explained by differences in disease activity. One other study reported a significantly lower response rate for only one out of three antigens in RA patients treated with rituximab.[9] The comparability with our results is limited because responses were poor in all groups and no information was provided on the dose of rituximab and number of B cells at the time of vaccination.

The present study shows that influenza vaccination, although not completely ineffective, will probably not protect rituximab-treated RA patients sufficiently against influenza infection. Larger studies are warranted to confirm our findings.

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Abbreviations: RA, Rheumatoid arthritis; TNF, tumour necrosis factor

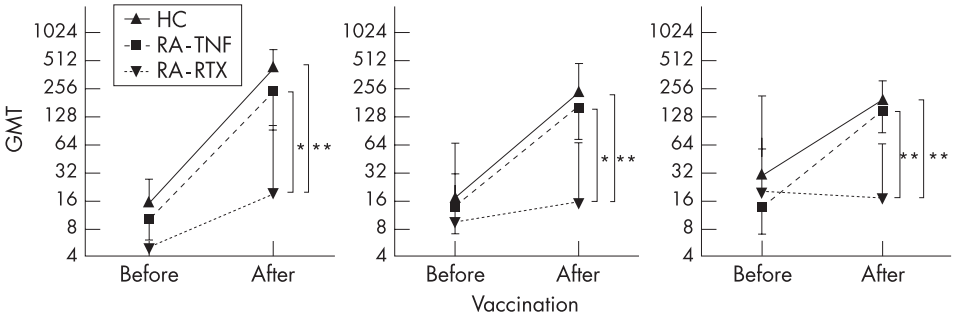

Figure 1 Pre and postvaccination serum geometric mean titres (GMT), with 95% confidence intervals, against influenza A/H3N2, A/H1N1 and influenza B for a group of patients with rheumatoid arthritis (RA) treated with rituximab (RA–RTX; n = 4), compared with RA patients treated with anti-tumour necrosis factor (TNF; RA–TNF; n = 19) and healthy controls (HC; n = 20). *p⩽0.02; **p⩽0.001.

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Changes in serum levels of glucosamine and sulphate after ingestion of glucosamine sulphate with and without simultaneous ingestion of glucose

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Glucosamine as its chloride or sulphate salt is now in widespread over-the-counter use for the treatment of osteoarthritis, ostensibly by stimulating or stabilizing cartilage chondroitin sulphate. Clinical trials have, however, provided equivocal results concerning its effectiveness,[1–3] and measurements of serum[4] and plasma[5] glucosamine levels after ingestion indicate that circulating glucosamine levels are probably too low to have any direct effect on cartilage.

Suggestions have been made that the sulphate of glucosamine sulphate might have a positive clinical effect on cartilage as a result of an increase in circulating levels after its ingestion.[6 7] This may be particularly pertinent because we previously described[8] a 9.3% mean decrease of sulphate levels after three hours of fasting by 14 experimental subjects, with a doubling of this mean decrease to 18.9% by the concomitant ingestion of 75 g glucose. In addition, we have described an effect of glucosamine sulphate ingestion by these subjects on glucose and insulin levels when 75 g glucose was ingested.[9] Results were in contrast to other investigations[10] that have indicated little or no effect when glucose was ingested the day after the ingestion of glucosamine rather than ingestion at the same time.

We have now examined the reciprocal effects of glucose ingestion on serum sulphate and glucosamine levels during a three hour period after an overnight fast. Methods, materials, subjects, study protocol, serum collection and analyses are detailed in previous publications.[4 8 9]

Sulphate levels during three hours after overnight fasting with no additional ingestion, with 0 time ingestion of 75 g glucose, 0 time ingestion of 1500 g glucosamine sulphate, and 0 time ingestion of 75 g glucose plus 1500 g glucosamine sulphate are shown in fig 1A. The ingestion of glucosamine sulphate without glucose restored mean sulphate levels to slightly above baseline at each time interval, in contrast to the decreases with fasting when no glucosamine sulphate was ingested as previously reported.[8] The simultaneous ingestion of glucose and glucosamine sulphate compared with glucose alone resulted in restoration to somewhat less than baseline for the first two hours and to slightly higher afterwards. The total amount of sulphate ingested (325 mg equal to 3.25 mmol) is consistent with the incremental amounts that were found in serum.

Mean glucosamine levels (fig 1B) demonstrated a delay in appearance and were higher and still climbing when glucose was ingested along with glucosamine sulphate, probably reaching still higher levels if serum had been taken at later times. Two subjects had later samples obtained, however, showing a drop towards baseline at five hours and baseline at eight hours. The delay in the increase in glucosamine levels when ingested with glucose rather than without glucose is

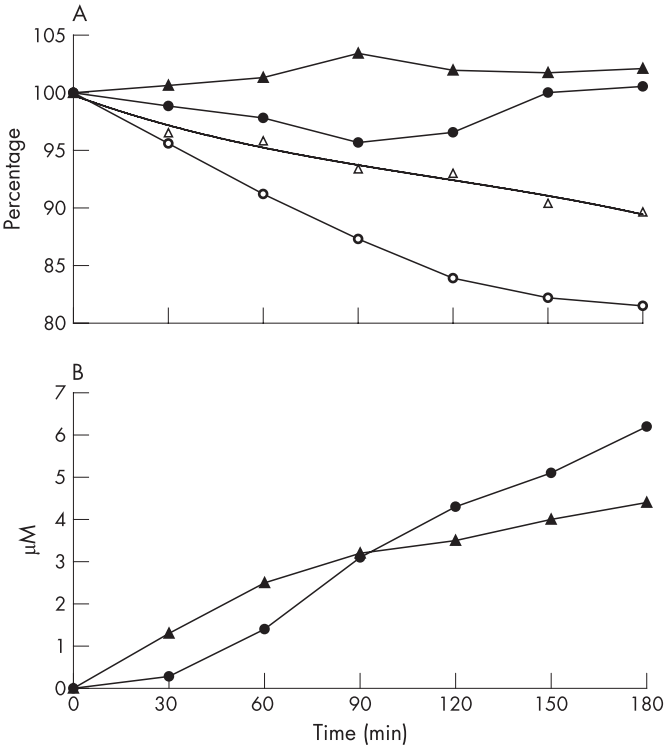

Figure 1 Effects of ingested glucose on serum sulphate and glucosamine levels. (A) Mean serum sulphate levels at timed intervals after ingestion of glucosamine sulphate with glucose (●——●), without glucose (▲——▲), and for comparison, without glucosamine sulphate with glucose (○——○), without glucose (△——△). (B) Mean serum glucosamine levels at timed intervals after ingestion of glucosamine sulphate with glucose (?——?), without glucose (▲——▲).