Total serum immunoglobulin levels in patients with RA after multiple B-cell depletion cycles based on rituximab: relationship with B-cell kinetics

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Abstract

Objective. To investigate whether the incidence of secondary hypogammaglobulinaemia in patients with RA following rituximab was related to patterns of B-cell return and relapse.

Methods. CD19+ B-cell and serum immunoglobulin (sIg) determinations were done every 2 or 3 months in 137 consecutive patients treated with one or more courses of rituximab-based B-cell depletion therapy. The pattern of B-cell return, either concordant or discordant with relapse, was also recorded.

Results. There were 119 responders. Before treatment, three patients had low IgM and four had low IgG. After the first cycle, low IgM or IgG was present in 9.2% (11/119) and 11.8% (14/119) of the patients, respectively, increasing to 38.8% (8/18) and 22.2% (4/18) after five cycles. The mean percent maximum sIg decrease/cycle was relatively constant. The CD19+ B-cell count at repopulation was not correlated with immunoglobulin (Ig) levels after each cycle. Patients discordant for B-cell return and relapse developed significantly lower serum IgM and more low IgM episodes than concordant patients (P < 0.05).

Conclusion. Patients with lower baseline sIg levels tended to develop persistent IgM and IgG hypogammaglobulinaemia, resulting from an accumulation of incremental decreases after repeat cycles. This was not due to lower numbers of returning B cells in those developing low sIgs. The association of low IgM in patients with a discordant pattern of relapse suggests that underlying defects in B cells relating to survival and maturation into Ig-secreting cells, as well as attrition of IgG plasma cells may be contributing to low sIg levels in some patients.

Key words: rheumatoid arthritis, rituximab, immunoglobulins, CD19+ B cells, B-cell depletion.

Introduction

In patients with RA, the rationale of B-cell depletion therapy (BCDT) based on rituximab was to eliminate autoreactive B-cell clones, as precursors of autoantibody secreting cells, while minimizing the period of impact on normal B cells and production of protective antibodies [1]. When adequate levels of depletion are attained in the circulation, clinical benefit can last for months, or in some cases years [2, 3]. Approximately half of the patients relapse at B-cell return or within 3 months (concordant relapse), while in others clinical relapse can be delayed for up to a further 2.5 years after B-cell return (discordant relapse) [4]. In both situations, further BCDT cycles or other treatments are needed to restore clinical response. The long gap between repopulation and relapse in some patients suggests that re-engagement of pathogenic pathways may rely on the accumulation or expansion of pathogenic effector cells or is actively suppressed.

The initial theoretical basis for using B-cell depletion in patients with RA was to eliminate autoreactive B cells, and therefore their autoantibody-producing daughter plasma cells. Autoantibody containing pathogenic immune complexes and the cycle of B-cell self-perpetuation would thus be reduced as a consequence [1]. However, others have concluded that the therapeutic success of rituximab was the result of removing B cells more directly involved in synovitis through, among other mechanisms, production...
of cytokines and through antigen presentation to effector T cells (reviewed in [5]). Hiepe et al. [6] have also suggested that disease memory may reside in autoreactive plasma cells.

Reconstitution of CD27+ memory B cells following rituximab is often slow. In some studies, an increased proportion of memory B cells at repopulation has been related to shorter clinical responses [3, 7], and the number of residual memory B cells after rituximab has also been related to earlier relapse [7, 8]. However, relapse was not consistently related to the total number of circulating memory B cells [3, 9]. The persistence of plasma cells in joints has also been related to a poorer clinical response at 16 weeks [10–12]. Decreases in autoantibody levels may occur solely as a result of a general decrease in circulating immunoglobulin (Ig) levels, but we and others have described selective decreases in autoantibodies, in particular RFs, in peripheral blood and suggest that these are produced proportionately more by relatively short-lived plasma cells [4, 9]. Decreases in ACPAs [10] have been correlated with clinical improvement and increases in autoantibodies (RF) with clinical relapse [13].

Although decreases in total Ig levels following single cycles of BCDT based on rituximab are reported to be modest, there is evidence for progressive decreases with repeated cycles [4, 14]. Descriptive data from safety profiles obtained from clinical trials showed that the proportion of patients with low IgM at 6 months post-rituximab increased in each additional course, from 10% of patients after the first cycle up to 40% following five cycles. The proportion of patients with low IgG by course remained relatively stable, at 3–6% [14]. The percentage of patients with low IgA did not differ from baseline, at <1% for any number of courses.

As part of ongoing studies of the mechanisms of relapse following BCDT, we have previously reported differences in expression of key B-cell differentiation receptors [B-cell activating factor (BAFF)-binding receptors] between patients relapsing concurrent or discordant with B-cell return [15, 16]. As these receptors also play a major role in the determination of whether particular B-cell clones will proceed to mature into short- or long-lived plasma cells, we have investigated whether the development of hypogammaglobulinaemia in patients with RA responding to one or more cycles of rituximab therapy was related to the pattern of clinical response. We also determined whether there was any relationship between the ability to retain Ig levels within the normal range and the number of repopulating B cells or baseline Ig levels.

Methods

A total of 137 consecutive patients with active RA who fulfilled ACR criteria [17] were included in the study, of whom 119 (86.9%) were responders according to the European League Against Rheumatism (EULAR)/DAS-28 definition of response [18]. The time of follow-up per patient from first treatment ranged from 6 months to 10 years. The patients were all attending the Department of Rheumatology at University College London Hospitals and receiving repeated cycles of BCDT, based on rituximab (non-fixed re-treatment based on clinical need at variable intervals not shorter than 6 months). BCDT consisted of two infusions of 1 g rituximab 1–2 weeks apart, each preceded by 100 mg i.v. methylprednisolone, as previously described [4]. The study was approved by the Hospital Ethics Committee (the ethics committee was the Joint UCL/UCLH Committees on the Ethics of Human Research, Committee Alpha; REC reference number 08/H0715/18), and all patients gave informed consent before entering the study. The median age was 53 (range 36–86) years and the median disease duration was 21 (range 8–50) years. Eight patients were RF negative. Patients had failed a median of three DMARDs (range 1–6) and 107/137 patients had previously failed TNF inhibitor therapy [median 1.5 (range 0–3)]. Those who had not received TNF inhibitor therapy had a formal contraindication. All patients were active before starting BCDT based on rituximab (DAS-28 ≥ 5.1).

Blood samples were obtained at baseline and every 2–3 months. Absolute CD19+ B-cell count, CRP and Ig levels were collected prospectively. As previously, the normal range for CD19+ B cells used by the local pathology laboratory was 0.03–0.40 × 10^9/l. Levels <0.005 × 10^9/l CD19+ B cells were defined as undetectable [2]. Depletion of B cells in the peripheral blood was deemed to have occurred when CD19+ B cells were undetectable. B-cell return (repopulation) was defined as when CD19+ B cells were again detectable in the circulation (≥0.005 × 10^9/l). Ig levels were available for all cycles. Although we were using a protocol for data collection, some CD19 measurements were not available (<10%).

B-cell kinetics, including time to repopulation (absolute circulating CD19+ B cell count ≥0.005 × 10^9/l) and to clinical relapse, as defined by (i) any return or increase of signs and symptoms caused by inflammation owing to RA with or without (ii) a rise in CRP, were documented. For each clinical relapse, patients were classified as concordant (relapse ≤3 months after repopulation in peripheral blood) or discordant (relapse >3 months after repopulation in peripheral blood) [3, 4].

Ig levels were measured by nephelometry, with normal ranges defined as IgG, 7.0–16.0 g/l; IgM, 0.4–2.3 g/l; and IgA, 0.7–4.0 g/l. Baseline levels for each cycle as well as minimum levels (between treatment with rituximab and relapse) were collected within each cycle and maximum decreases per cycle were calculated.

Descriptive studies for categorical and quantitative variables were first performed. Non-parametric tests and multiple linear regression for detecting confounding factors were performed a posteriori using the SPSS package for Windows.

Results

Incidence of hypogammaglobulinaemia in patients with RA after repeated BCDT cycles

We analysed data from 119 of 137 responding patients receiving one cycle, 85 patients two cycles, 48 patients three cycles, 30 patients four cycles and 18 patients five
Four patients had low levels of IgG before treatment (<7 g/l) (Fig. 1a). The percentage of patients with low IgG increased from 11.8% (14/119) after the first cycle to 22.2% (4/18) after the fifth cycle (Fig. 1a). The greatest number of patients (10) developed low IgG for the first time after the first cycle (Fig. 1b). After the first cycle of rituximab, 14/119 (11.8%) had low IgG. In the 18 patients who had had four cycles, 2/18 (11%) had low IgG after the first cycle and 4/18 (22%) after the fourth cycle, thereby showing a true increase in the incidence of hypogammaglobulinaemia with repeat cycles. In the case of IgM, three patients had low IgM (<0.4 g/l) before treatment (Fig. 1a). Low IgM after the first cycle was noticed in 9.2% (11/119) of patients, increasing to 38.8% (8/18) after the fifth cycle (Fig. 1a). In contrast to patients developing low IgG, more patients developed low IgM (four compared with one and seven compared with none) after the third and fourth cycles, respectively (Fig. 1b). Two patients had low IgA before treatment, four (3.4%) patients developed low IgA after the first cycle, but no new patients developed low IgA after consecutive cycles (Fig. 1b).

The mean percent decrease after the first cycle was significantly greater for IgM than either IgA or IgG (P < 0.001) (Fig. 2). After the first cycle, the mean percent-ages of the maximum decrease per cycle were relatively constant for each of the three classes of Igs. Fig. 2 shows the spread but also the tendency through each cycle, with no significant differences between cycles (Kruskal–Wallis test; P > 0.05).

**Relationship of hypogammaglobulinaemia with baseline Ig levels**

Although more patients developed low IgG after the first cycle, the mean maximum percent decrease after the first cycle was significantly greater for IgM than either IgA or IgG (P < 0.001) (Fig. 2). Fig. 3 shows the mean (s.d.) at baseline and minimum values of each Ig class per cycle. Although IgA levels also suffered incremental decreases with successive cycles (Fig. 3c), serum levels of IgA were...
relatively high before treatment and therefore were less likely to drop below normal levels compared with IgM and IgG (Fig. 3a and b, respectively). Linear regression analysis (Supplementary Fig. S1, available as supplementary data at Rheumatology Online) did indeed show a correlation between baseline Ig levels and minimum levels reached in each cycle ($r^2 > 0.7$ for most cycles). However, analysis of baseline IgM and IgG in patients with minimum values for Igs either within or below the lower limit of the normal range in each cycle (Fig. 1c and d) indicated that although low starting levels were predictive of lower minimum levels achieved, considerable overlap was found. Therefore, starting Ig levels within the normal range could not reliably predict a propensity to development low IgG or IgM.

CD19 count at repopulation following repeated cycles of treatment: relationship with developing low levels of Igs

It could be argued that the development of low Ig levels after BCDT may be related to the absolute number of CD19+ B cells returning at repopulation. As can be seen for IgM and IgG in Fig. 4a and b, respectively, absolute CD19 counts at repopulation did not correlate with baseline IgM and IgG levels over four cycles of treatment. However, there was a significant tendency for lower numbers of returning B cells to be associated with the presence of low IgG levels by Cycle 4 ($P < 0.05$).

Other associations

Using multivariate regression study, age or concomitant disease-modifying drugs did not influence these results (data not shown). As the time to repopulation (2–25 months) and relapse (5–55 months) varied within the patient cohort, the relationship between IgM, IgG and IgA in terms of grams per litre and months to repopulation and to relapse were analysed using linear regression (data not shown). The only significant finding was a weak negative relationship between IgA levels and time to repopulation ($r^2 = 0.24$, $P = 0.007$).

Pattern of clinical response and relation with low Igs

After four cycles, most patients with low IgG levels also had low IgM (4/6 patients), but a total of 7 patients developed only low IgM (Fig. 1a and b). IgM levels dropped to $<0.1$ g/l in some patients after repeated cycles. In contrast with the majority of patients experiencing low IgG levels after repeated cycles, patients developing low IgM did not tend to increase IgM levels at repopulation (data not shown). However, IgG levels tended to increase once B cells returned to the circulation (data not shown). IgG levels only rarely decreased to $<5$ g/l. In this series, low Igs were not associated with a noticeable increase in infectious episodes and only two patients developed recurrent chest infections requiring antibiotics following
development of low IgG levels. This may partly reflect caution in re-treatment of patients with low IgG levels (four patients had delayed re-treatment due to IgG <5.5 g/l).

The levels of IgM and IgG following four cycles of treatment are shown for patients with concordant or discordant patterns of response (Fig. 5a and b). Patients who had a concordant pattern of repopulation/relapse tended to have higher median levels of IgM compared with those with a discordant pattern across all cycles, with significant differences after the second \((P = 0.02)\) and third cycles \((P = 0.03)\) (Fig. 5a). IgG levels were similar in both concordant and discordant patients over four cycles (Fig. 5b).

Of those patients who had low IgG after the first cycle, eight (57%) were concordant and six (43%) discordant (Fig. 5d). After consecutive cycles, there was a tendency towards a constant pattern of response (from 57% patients presenting with a concordant pattern after the first cycle to 50% after the fourth cycle; Fig. 5d).

From those patients developing low IgM after the first cycle, four (36%) of them were discordant and seven (64%) were concordant. After consecutive cycles, however, there was a tendency towards patients with low IgM being discordant (from 36% after the first cycle to 72% after the fourth cycle) rather than concordant for repopulation/relapse (Fig. 5c).

**Discussion**

In initial Phase IIa clinical trials of the treatment of patients with RA with rituximab, mean Ig levels stayed within the normal range [19]. Long-lasting hypogammaglobulinemia following treatment with rituximab (monotherapy or in combination with chemotherapy) has, however, been seen in patients with post-transplant Epstein–Barr virus-associated lymphoproliferative disorder (EBLPD) [20–23], autoimmune cytopenias [24], post-autologous bone marrow transplantation [25–27] or HIV-associated lymphoma [28].

In this study, we found similar frequencies of low IgM as reported in the clinical trials (38 vs 40%) but a greater incidence of the development of low IgG (6 vs 22%) after five cycles of rituximab, possibly due to small sample size [14]. Decreases in IgG levels below the normal range were seen chiefly after the first or second cycle. Progressive decreases with multiple cycles did appear to predominantly affect IgM levels. After three cycles we only observed new decreases of IgM, not IgG, below the normal range. Although many patients seemed to tolerate low Ig levels well, two patients developed recurrent chest infections in association with persistently low IgG levels, with the need to consider replacement IVIG treatment. Rituximab was discontinued in both patients. In general, the proportion of patients developing low Ig levels increased with repeated cycles, but the percentage maximum decrease per cycle was relatively constant in all classes. The accumulation of these incremental decreases in Ig levels explains why those patients starting with lower baseline Ig levels may be more prone to develop persistent hypogammaglobulinemia.

The absolute CD19+ B-cell count at repopulation was not correlated with whether the patient developed low Ig levels within the limits of sensitivity of the method used here. However, as we were not able to analyse the number of memory B cells remaining after adequate depletion as defined here \((<0.005 \times 10^9/l)\), it would be interesting to determine in future studies whether there was any correlation of residual memory B-cell numbers with resulting Ig levels. As the mean percent maximum decrease was relatively constant per cycle, this suggested that a similar proportion of B-cell precursors capable of becoming Ig-secreting plasma cells were removed to a similar extent with each repeat cycle. Much of the serum IgM is produced from short-lived plasma blasts and plasma cells, and the finding that decreases in IgM were more
common after repeat cycles suggests that naïve and/or pre-switch memory B cells (follicular B cells) were failing to differentiate into plasma cells. Additionally, marginal zone B cells (IgM⁺CD27⁺) may not be able to regenerate quickly after depletion, contributing to incremental decreases in IgM production as a result. As most serum IgG is produced from rituximab-resistant long-lived plasma cells [29], levels of IgG were more robust but also suffered smaller incremental drops over time. This may reflect a reduction in the number of class-switched B cells being generated, which are able to mature into long-lived plasma cells after each cycle, as well as time-dependent attrition of bone marrow IgG plasma cell populations. As polymeric IgA produced by intestinal mucosal plasma cells is rapidly cleared by the liver [30], serum IgA is mostly the monomeric form that is produced by bone marrow plasma cells. The retention of serum IgA levels largely within the normal range in our cohort of RA patients may therefore be due to an increased availability of survival niches, in bone marrow and inflammatory sites for instance, for rituximab-resistant IgA plasma cells outside the mucosa or the presence of expanded populations at baseline. B cells return after rituximab in a pattern mimicking ontogeny. Relapse following rituximab in patients with RA only occurs following B-cell return. Repopulating naïve B cells subsequently mature into marginal zone and memory B cells, at widely varying rates in individual patients. However, the absolute numbers of memory B cells generated do not always relate directly to relapse [3, 8], suggesting that the rate of selection and expansion of autoreactive B cells is a critical factor in restarting the inflammatory process. The ability of autoreactive B cells within the naïve population to survive may relate to altered censoring thresholds, such as those related to B-cell receptor (BCR) specificity, which allow their expansion, possibly at the expense of normal B cells [31]. Availability of the key B-cell survival cytokine (BAFF) is not limiting, as levels rise sharply following B-cell depletion, only falling to near normal levels at or some time after B-cell return [32]. There may, however, be a positive survival advantage for autoreactive B cells [33-35] through dysregulation of key B-cell receptors such as the BAFF-binding receptors (BBR), namely BAFF-R/transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI)/B-cell maturation (BCMA). In
support of this, we have described different patterns of dysregulation of BAFF-R and TACI expression in RA patients relapsing in a concordant or discordant pattern after rituximab [15, 16].

The relatively high incidence of persistently low IgM developing after rituximab in patients with RA and in other diseases [20–28] suggests that a proportion of these patients may have a defect affecting B-cell maturation into Ig-secreting cells. It is also possible that rituximab itself has an as yet unexplored effect on B-cell ontogeny. We suggest that investigation of the possible links between mechanisms underlying the development of hypogammaglobulinaemia and autoimmunity may reveal mechanisms underlying different clinical responses and patterns of relapse following rituximab.

**Rheumatology key messages**

- Percentage maximum slg decrease per cycle of BCDT was relatively constant in RA patients.
- RA patients relapsing >3 months after B-cell repopulation were most likely to develop low IgM.
- The number of repopulating B cells after BCDT in RA patients did not predict slg levels.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

**References**


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