

Adverse reactions in patients with B-cell lymphomas during combined treatment with rituximab: In vitro evaluation of rituximab hypersensitivity by basophil activation test

To the Editor: In patients treated with rituximab, adverse reactions (ADR) occur and should be distinguished from potentially life-threatening hypersensitivity reactions (HSR) [1]. In studying ADR in which a hypersensitive mechanism is suspected, the basophil activation test (BAT) seems to be an interesting method. BAT is based on the evaluation of CD63 or CD203 [2,3]. CD63, a surface molecule (glycoprotein-53), is the only marker expressed de novo with a high density on activated basophils, and its up-regulation has been shown to correlate well with histamine release after stimulation with N-formylmethionyl-leucyl-phenylalanine, anti-IgE antibody, or allergens [4,5]. The expression can be detected efficiently by flow cytometry. We report our experience with the ADR following rituximab therapy in a group of 18 patients (M:8; F:10, age range: 36–85) affected by lymphoproliferative diseases, according to the WHO classification. Patients received a standard dose of rituximab (375 mg/m²) combined with cyclophosphamide, vincristine, and prednisone or cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. On average, each patient received 4.5 infusions of rituximab (median, the number of infusions range between 2 and 8). Before rituximab infusion, a premedication using diphenhydramine (10 mg), dexamethasone (8 mg intravenous (IV)), and ranitidine (100 mg IV) was given to the patients. The following major ADR were reported in five patients: urticaria, hypotension, angioedema, and bronchospasms. The reactions required medical intervention and discontinuation of the infusion. Within one year from the reactions, the BAT was performed on all patients. To assess

the rituximab challenge, different concentrations, spanning several log scales, and mimicking in vivo concentrations, 0.25 and 0.50 µg/µL (i.e., 250 and 500 µg/mL) were evaluated. Two other higher concentrations 1.00 and 2.00 µg/µL, which do not represent therapeutic concentrations, were used to verify if these high concentrations are still able to elicit maximal stimulation. The test was then carried out in 18 healthy subjects to see if rituximab was able to stimulate the basophils of normal individuals. This was done to exclude the presence of nonspecific stimulatory components such as preservatives or the ability of rituximab to elicit nonspecific activation.

At two doses corresponding to “in vivo” concentrations of rituximab, mean ± standard deviation (SD) percentage of CD63+ basophils was higher in patients with ADR compared to those without, (6.75 ± 3.79 vs. 1.92 ± 1.16 , $P < 0.001$; 6.86 ± 5.60 vs. 2.20 ± 1.33 , $P < 0.05$, respectively). At 1.00 and 2.00 µg/µL, there were differences for all pairwise comparisons ($P < 0.05$; Fig. 1). No significant differences were obtained in patients without reactions and in the healthy subjects. The dose finding in our study showed that the maximal basophil reactivity is obtained using two concentrations of rituximab, 0.25 and 0.50 µg/µL, that represent those to be considered “optimal,” as they discriminate between patients and control individuals, eliciting maximal stimulation.

Further studies with a larger number of patients are required, but from the data so far available it would seem that the CD63 BAT could be very useful in evaluating patients suspected of being hypersensitive to rituximab, mainly in the absence of other diagnostic tests.

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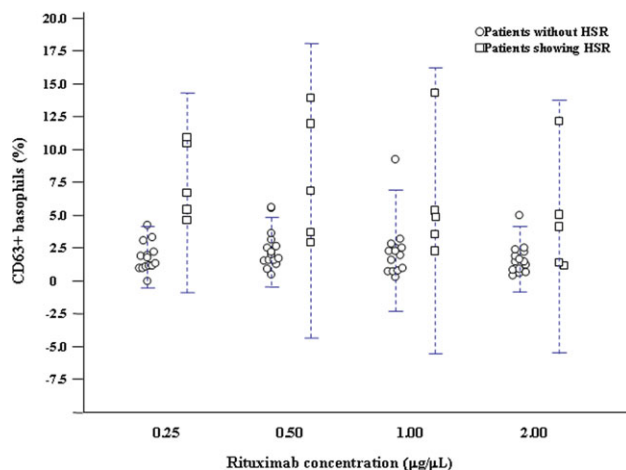


Fig. 1. Percentages of basophils CD63+ in patients without ADR and those with a hypersensitivity to rituximab. Results were obtained with therapeutic-like concentrations, that is, at 0.25 and 0.50 µg/µL ($P < 0.001$ and $P < 0.05$) and at higher concentrations that do not represent therapeutic doses of rituximab, that is, 1.00 and 2.00 µg/µL ($P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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