Evolution of autoreactive B and T cells in pemphigus patients with Rituximab or corticosteroid regimen treatment

Maud MAHO-VAILLANT¹, Corinne PERALS³, Marie-Laure GOLINSKI¹, Claire MIGNARD¹, Vivien HEBERT¹, Gaëtan RIOU², Manuelle VIGUIER⁴, Olivier BOYER², Sébastien CALBO², Nicolas FAZILLEAU³ and Pascal JOLY¹

¹Department of Dermatology, Inserm U1234, Rouen University Hospital, France.

²Inserm Unit 1234, Normandy University, Rouen, France. ³Inserm U1043, CNRS UMR5282, Toulouse, France.

⁴Department of Dermatology, University Hospital Center of Reims, France.

AIM OF STUDY

Pemphigus is a B-cell-mediated autoimmune disease affecting skin and mucous membranes. Pathogenic autoantibodies (Abs) are directed against desmogleins (DSG-1/DSG-3), which are keratinocyte adhesion proteins. We recently showed that first line treatment with Rituximab (RTX) was more effective than standard oral corticosteroid (CS). Compared to a standard regimen of CS, RTX associated to short term prednisone regimen allowed almost 89% of patients to achieve remission off therapy at month 24. To understand the immunological mechanisms that mediate the long-lasting clinical remission after RTX treatment relative to CS regimen, we characterized their impacts on autoreactive B and T follicular helper cells (TFH) in the blood of patients with pemphigus.

METHODS

The frequency and phenotype of B DSG specific B cells and non specific B cells were studied by flow cytometry using recombinant DSG tagged. The number of DSG⁺ IgG Abs Secreting Cell (ASC) were detected by ELISPOT after stimulated with R848 and rhIL-2 for 72h. The frequency and phenotype of TFH and DSG-3 specific circulating TFH were determined by flow cytometry using DRB1*0402 tetramers loaded with previously identified immunodominant peptide of DSG3.

RESULTS

Evolution of DSG-1 and DSG-3 specific B cells in pemphigus patients after RTX treatment

- DSG-specific B cells were still detected after B cell recovery at the M36 evaluation in remitted patients from the RTX group

RTX: Evolution of phenotype of B cells and DSG-1 and DSG-3 specific B cells

- At baseline, DSG-specific B cells were enriched class switched IgG memory B cells
- Balance between naïve and memory B cells was modified after RTX
- DSG-specific B cells' phenotypes which reappeared were impacted by RTX
- Their frequency of class switched memory B cells was very low relative to baseline at M36

RTX: Evolution of IgG ASC anti-DSG-1 and DSG-3

- DSG-1 and DSG-3 ASC were no more detectable at M36 in patients in complete remission
- DSG-3 ASC were still detected in the only patient with persistent mucosal pemphigus after RTX treatment

Evolution of DSG-1 and DSG-3 specific B cells in pemphigus patients after CS treatment

- DSG-specific B cells were still detected at the M12 evaluation in patients from the CS group

CS: Evolution of phenotype of B cells and DSG-1 and DSG-3 specific B cells

- DSG-specific B cells' phenotypes were not impacted by CS at M12 relative to baseline

CS: Evolution of IgG ASC anti-DSG-1 and DSG-3

- DSG ASC were still detected in patients in remission and those with persistent disease activity after CS therapy

Evolution of TFH and DSG-3 specific TFH cells in pemphigus patients according to treatment regimen

Evolution of TFH cells and serum IL-21 level in pemphigus patients according to treatment regimen

Kruskal-Wallis test versus baseline (*p<0.05; **p<0.01; ***p<0.001) in patients RTX group (below) and CS group (above)

- The frequency of TFH cells was reduced by 50% in parallel with B-depletion after RTX treatment and was increased to the initial value after B cells returned
- No variation of TFH cell frequency was observed during the course of patients in CS group
- Serum IL-21 level was decreased persistently in patients treated with RTX

DSG3 specific TFH cells in pemphigus patients with the HLA-DRB1*0402 allele according to treatment regimen

- DSG3 specific TFH were detected after treatment in both patients from the CS and the RTX group
- The frequency of DSG3 specific TFH was very low in patients from the RTX group relative the CS group
- A lower frequency of TFH1 phenotype was observed in DSG3 specific TFH cells relative to non-autoreactive TFH cells
- Higher frequency of TFH2 phenotype was observed in DSG3 specific TFH cells from patients treated with RTX
- TFH1 / TFH2+TFH17 ratio was modified between non autoreactive TFH and DSG3 specific TFH cells

CONCLUSION

The response to RTX in pemphigus involves two mechanisms: i) a sustained depletion of IgG switched memory autoreactive B cells leading to the disappearance of DSG ASC, and ii) the decrease of DSG-3 specific circulating TFH cells, which is likely involved in the blockage of B cell maturation and the delayed reappearance of DSG memory B cells. In contrast, the high rate of relapses in patients from the CS group can be explained by the persistent high frequency of IgG switched memory autoreactive B cells and DSG ASC are certainly responsible for the re-increase of anti DSG antibodies observed at the M24 and M36 evaluations.