Kinetic study of B cell-depletion with a novel mAb anti-mouse CD20, clone SA271G2

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Introduction

CD20 is a member of the membrane-spanning 4A family (MS4A), involved in the transmembrane calcium-influx, activation and proliferation of B cells. CD20 is first expressed on Pre-B cells and its expression is sustained throughout the different stages of the B cell differentiation, with the exception of plasma cells. CD20 is also expressed by many malignant cells from B cell origin. Anti-human CD20 mAbs have been used in the treatment of B cell lymphomas, and in the treatment of some autoimmune diseases with a B cell involvement such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus.

Here we describe a novel anti-mouse CD20 antibody (clone SA271G2, rat IgG2b, κ), useful for in vivo depletion of B cells. A single intravenous dose of antibody (0.25mg) depleted more than 95% of B cells from peripheral blood, spleen and lymph nodes, without affecting $CD20^-$ cell types; while in bone marrow, the pre-pro, pro- and pre-B cell subsets are not noticeably affected, a significant reduction of the stage of immature B cells population was observed. This B cell depletion was sustained for over 20 days; then a gradual return of the B cell population was observed, and was fully recovered around day 50. During this time, no noticeable effect of this antibody on $CD20^-$ cells was observed.

Materials and Methods

B cell depletion: C57BL/6 mice were injected i.v. with 250 µg of Ultra-LEAF™ purified mAb SA271G2 or Ultra-LEAF™ purified rat IgG2b, κ isotype control; both groups were bled at the indicated time points to follow the kinetic of B cell depletion. To study the presence of B cells in different compartments, the mice were euthanized with CO_2 at day 7 and spleen, lymph nodes, bone marrow and the peritoneal cavity lavage were collected and stained with the indicated antibodies.

Flow cytometry analysis: After staining, the cells were acquired with an LSRFortessa™ flow cytometer (BD Biosciences), and the data analyzed using FlowJo™ software (Tree Star Inc). Data shown is representative of 4 mice per group.

Immunofluorescence staining: Frozen spleen sections were fixed with 4% PFA for 10 minutes at room temperature and blocked with 5% FBS plus 5% rat serum for 1 hour at minutes at room temperature; then the sections were stained overnight at 4ºC with the indicated antibodies.

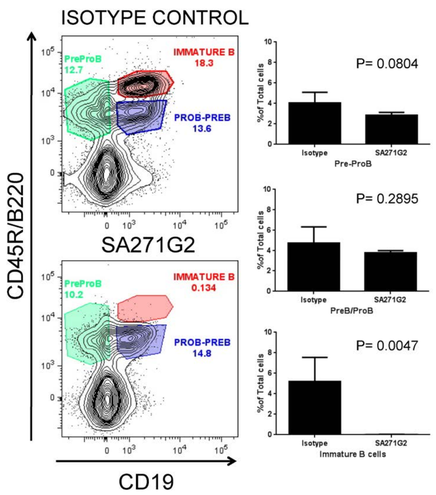

Figure 1. *In vivo* administration of mAb SA271G2 depletes the immature stage of bone marrow B cells. Bone marrow cells from SA271G2 treated-mice or the isotype-treated control group were stained to identify the different B cell subsets. Only the immature B cells ($CD19^{hi}$ $B220^{hi}$) were depleted, but not pre-pro, pro or pre-B cells. Data shown was gated in the lymphoid population.

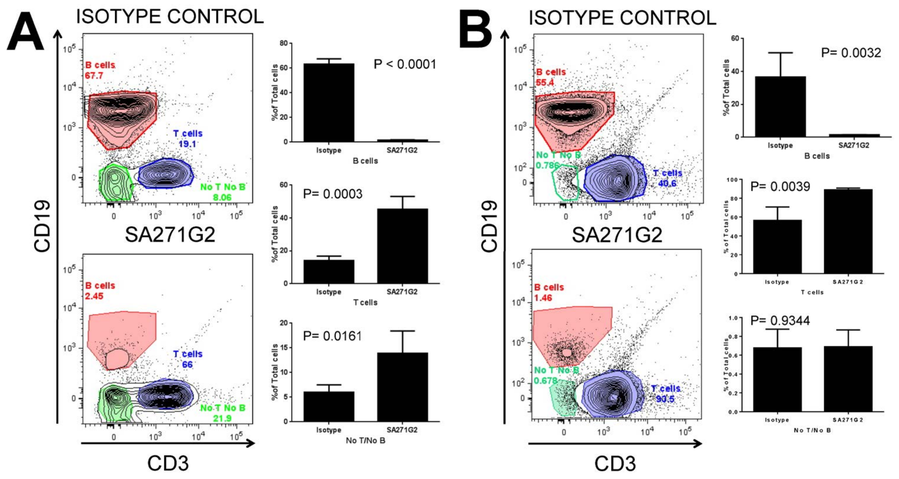

Figure 2. mAb SA271G2 depleted B cells from spleen and lymph nodes. After treatment with SA271G2 the percentage of B cells from spleen (A) and lymph nodes (B) were reduced; in consequence, the frequency of the other cell types increased.

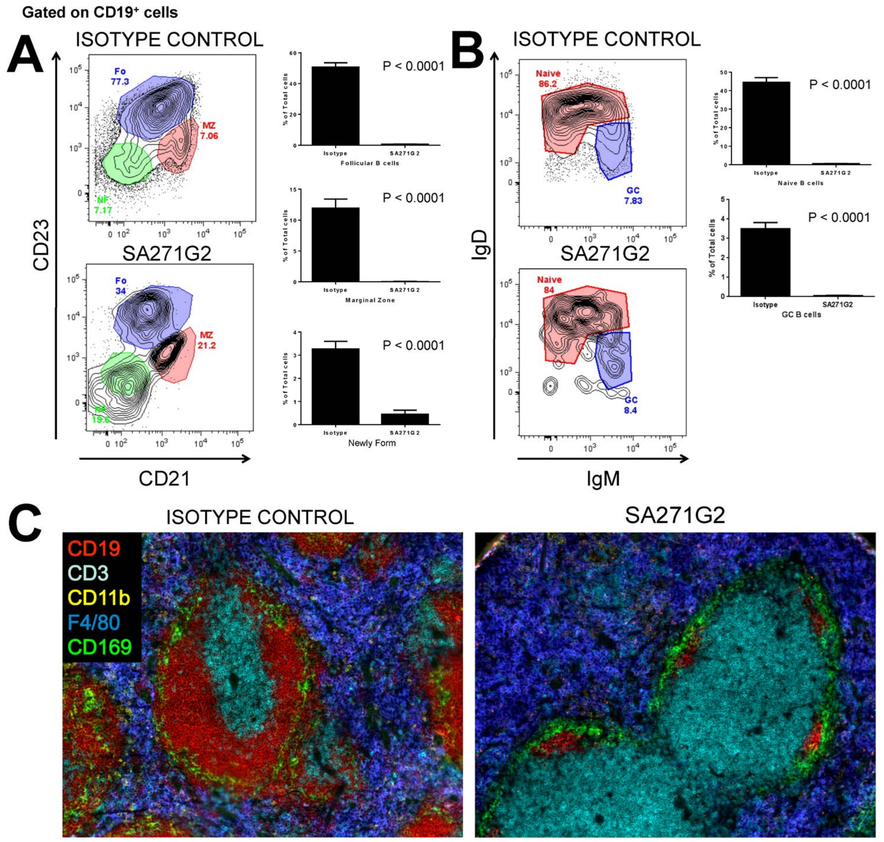

Figure 3. mAb SA271G2 depleted the different B cell subsets from spleen. mAb SA271G2 efficiently depleted newly formed (NF), marginal zone (MZ) and follicular (Fo) B cells from spleen (A). Within the follicle, mAb SA271G2 depleted both naïve and germinal center B cells (B). Immunofluorescence staining of spleen frozen sections showed that T cells, the different subsets of macrophages, and the overall architecture of the spleen were not affected by the treatment of mAb SA271G2. Interestingly, some B cells remained in focal zones of the spleen; whether those cells are new arrivals from bone marrow or in the process of differentiating into plasma cells needs to be determined (C).

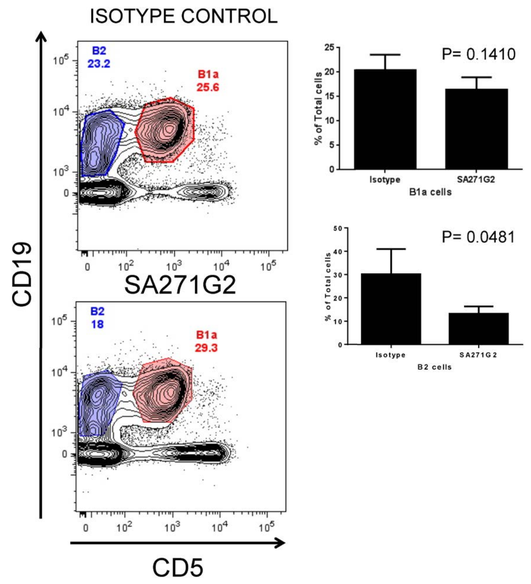

Figure 4. Treatment with mAb SA271G2 does not affect B cells from the peritoneal cavity. Cells from peritoneal cavity lavage were stained with CD5 and CD19 to differentiate between B1 and B2 cells. No significant difference was detected between the treated animals and the control group.

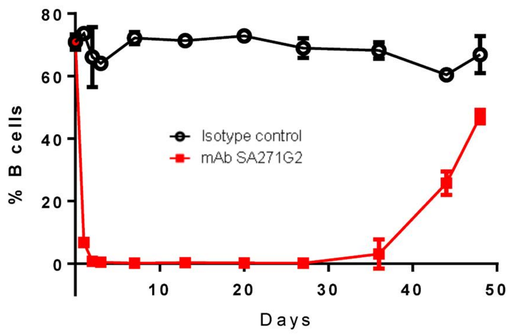

Figure 5. Kinetics of B cell depletion after treatment with mAb SA271G2. 24 hours after injection, a significant drop in the percentage of B cells in peripheral blood was observed (6.8% vs 74%), with an almost complete depletion of B cells at 48h (0.7% vs 66%). The percentage of B cells remained under 1% in the treated group until the 4th week, and a gradual but sustained increase on the B cell numbers was observed until full recovery around day 50.

Conclusions

- A single i.v. dose of mAb SA271G2 depleted the immature B cell subset from bone marrow and the B cells from peripheral blood, spleen and lymph nodes.
- mAb SA271G2 only depleted the $CD20^+$ cells, and no effect on T cells, macrophages or other cell types was observed. Moreover, the architecture of the spleen was conserved despite the almost complete absence of B cells.
- The B cell depletion induced by mAb SA271G2 lasted around 4 weeks, and it was fully reversible.
- mAb SA271G2 is a novel tool to study the role of B cells in the different mechanisms of the immune response.
- mAb SA271G2 eliminates the need to use genetically modified mice when animals deficient in B cells are needed.

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