Article

The potential of IgG to induce murine and human thymic maturation of IL-10+ B cells (B10) revealed in a pilot study

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Figure S1

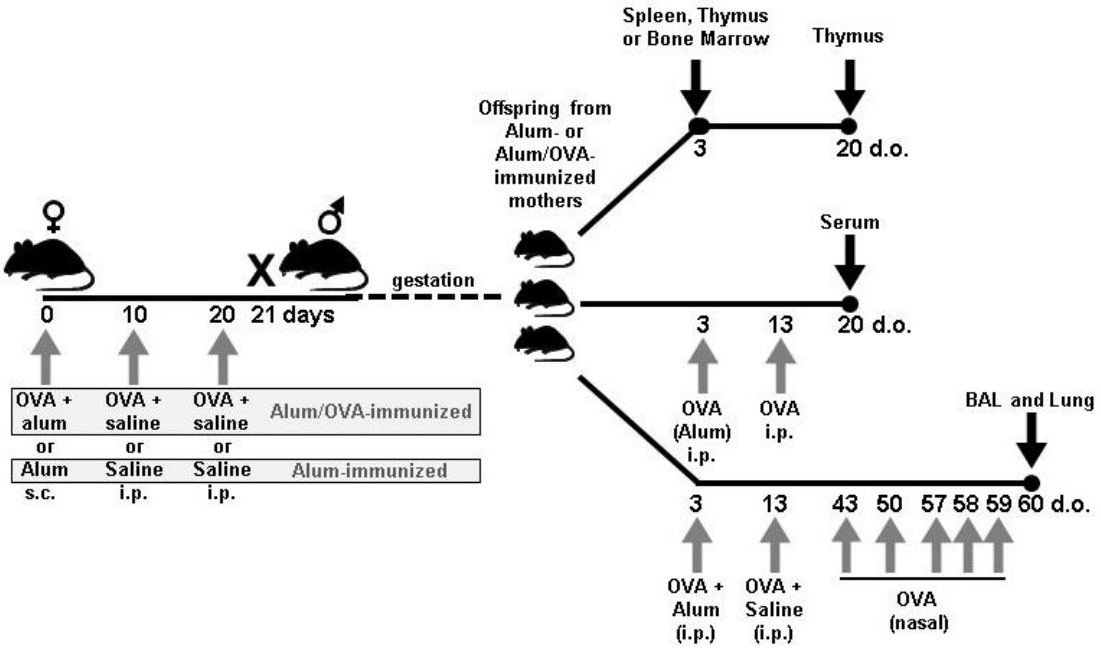

Figure S1. Schematic representation of the maternal immunization, offspring evaluation, offspring immunization and murine lung inflammation protocols. OVA= Ovalbumim; s.c.= subcutaneously; i;.p.= intraperitoneally; d.o.= days old; BAL= bronchoalveolar fluid.

Figure S2

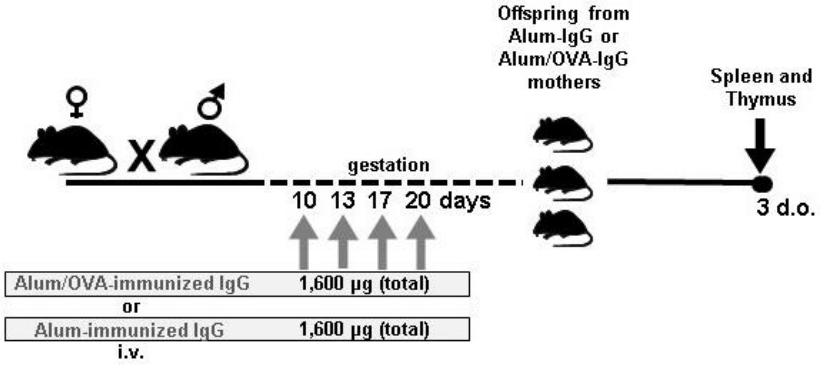

Figure S2. Schematic representation of the passive *in vivo* transfer of purified IgG protocol. OVA= Ovalbumim; i.v.= intravenously; d.o.= days old.

Figure S3

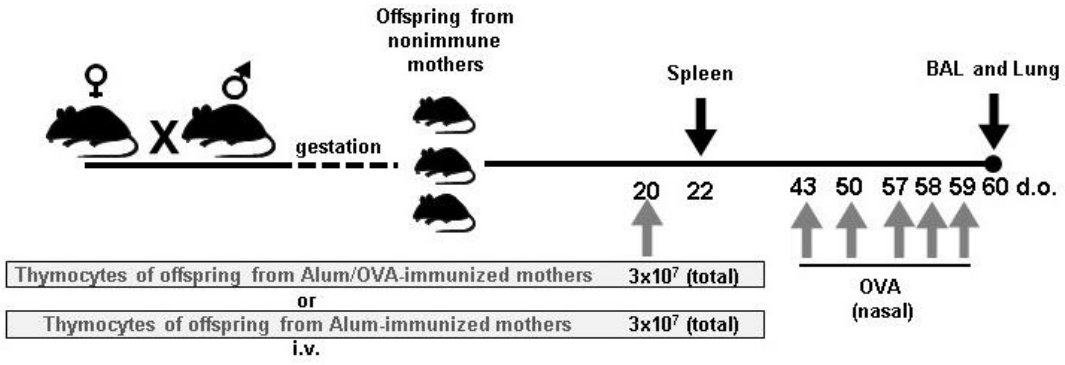

Figure S3. Schematic representation of the Passive in vivo transfer of thymocytes protocols. OVA= Ovalbumim; d.o.= days old; BAL= bronchoalveolar fluid.

Figure S4

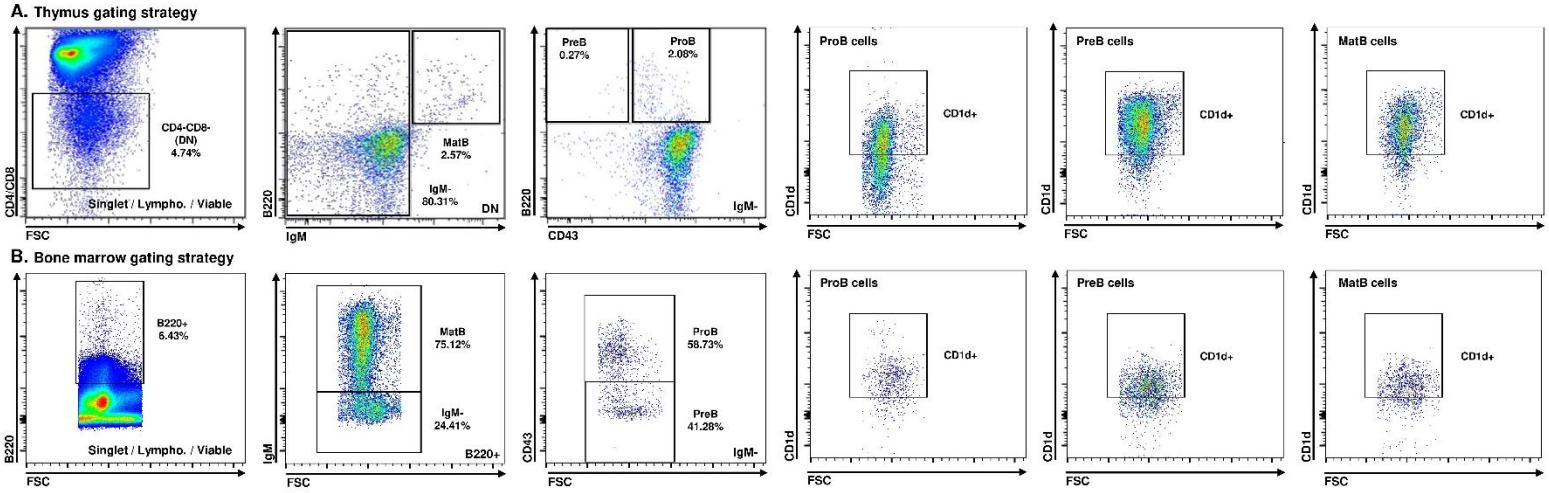

Figure S4. Identification of B cell precursors and CD1d expression evaluation in offspring bone marrow and thymic samples. Offspring thymocytes and bone marrow from both groups were evaluated at 3 d.o.. Each sample was acquired using a single-cell gate (determined by SSC-A/SSC-H parameters), a viable-cell gate (determined by LIVE/DEAD staining) and a lymphocyte gate (determined by relative SSC-A/FSC-A). For the thymus (A), double-negative (DN) cells (CD4-CD8-) were gated as B220+IgM+ to determine mature B cells (MatB), IgM- cells were gated, and the expression of CD43 was evaluated to determine ProB cells (CD43+) or PreB cells (CD43-). For bone marrow (B), B220+-gated cells were evaluated, IgM+ cells were gated to determine mature B cells (MatB), IgM- cells were gated, and the expression of CD43 was evaluated to determine ProB cells (CD43+) or PreB cells (CD43-).

Figure S5

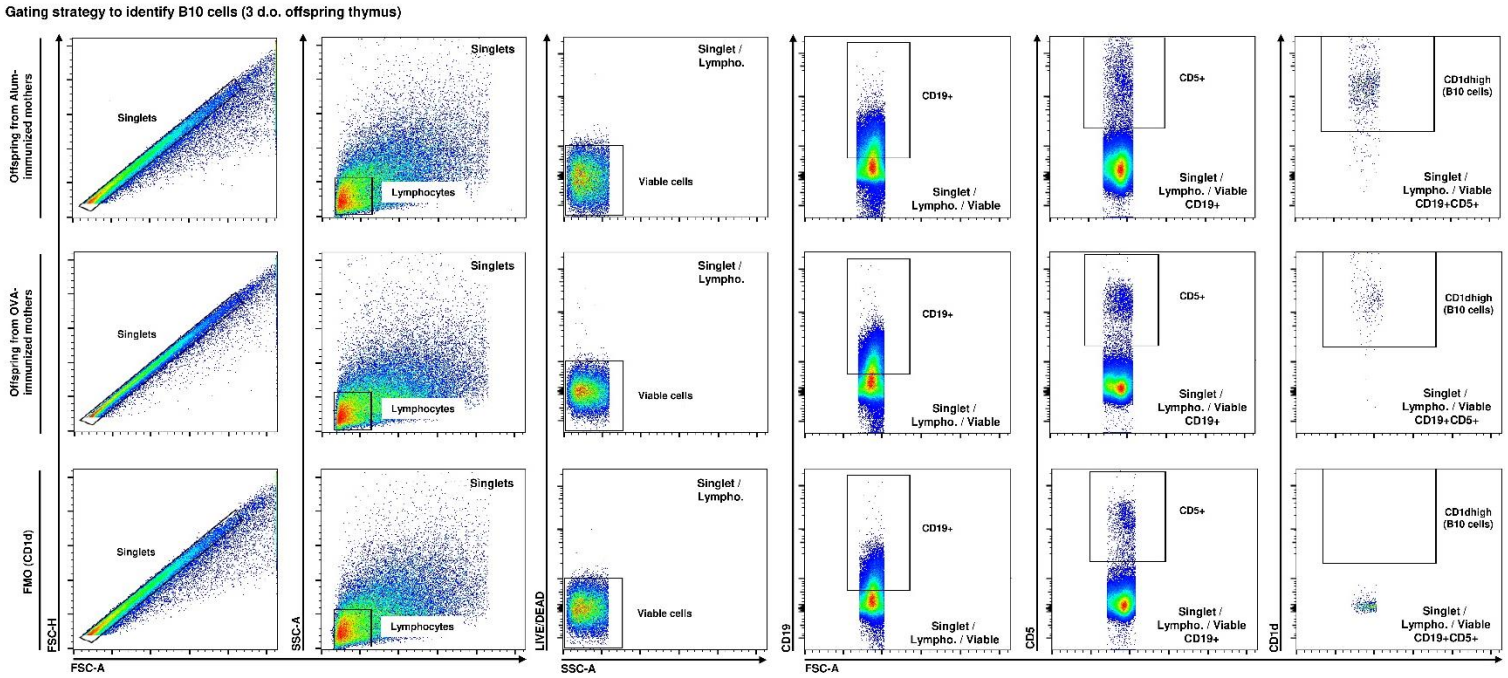

Figure S5. Gating strategy to identify 3 d.o. offspring thymus B10 cells. Each sample was acquired using a single-cell (Singlet) gate (determined by SSC-A/SSC-H parameters), a lymphocyte gate (determined by relative SSC-A/FSC-A). and a viable-cell gate (determined by LIVE/DEAD staining). Upper and middle panels illustrate the gating strategy to identify B10 cells on each group (Alum-immunized or OVA-immunized offspring). CD19+ cells were gated to determine B cells, and CD5 expression was evaluated on CD19+ cells. The expression of CD1d was evaluated on CD19+CD5+ cells, and CD1d^{high} cells were gated (as determined by FMO – lower panels) to identify B10 cells (Singlet+Lymphocytes+LIVE/DEAD-CD19+CD5+CD1d^{high}).

Figure S6

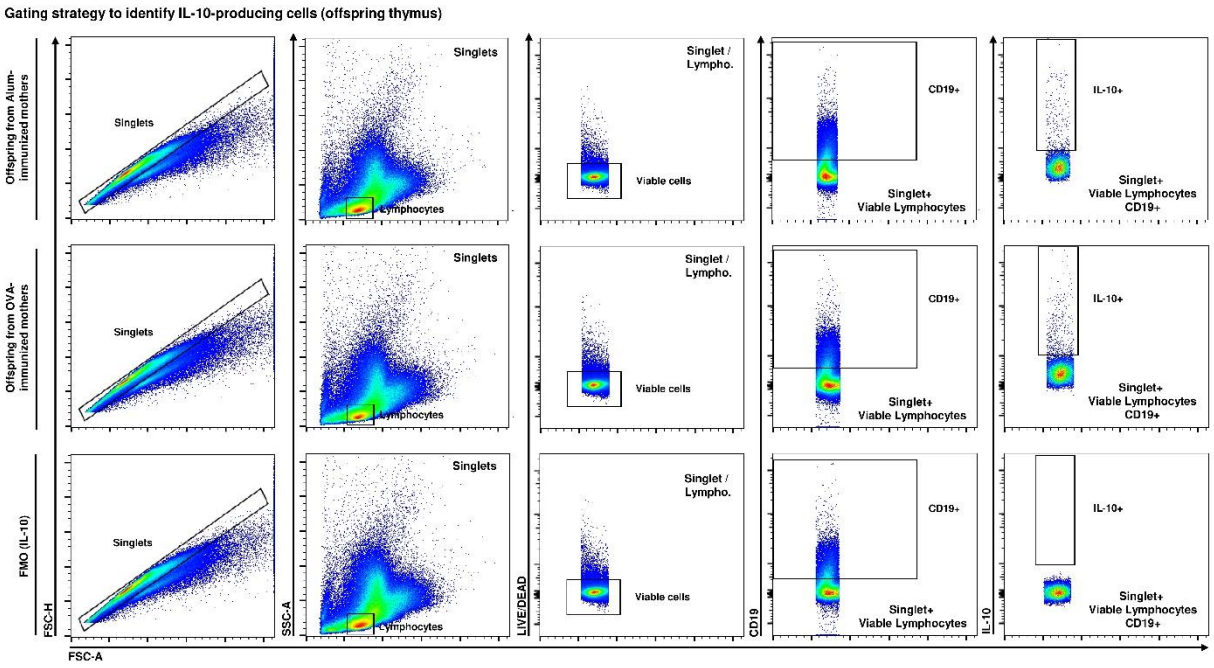

Figure S6. Gating strategy to identify offspring thymus IL-10-producing B cells. Each sample was acquired using a single-cell (Singlet) gate (determined by SSC-A/SSC-H parameters), a lymphocyte gate (determined by relative SSC-A/FSC-A). and a viable-cell gate (determined by LIVE/DEAD staining). Upper and middle panels illustrate the gating strategy to identify IL-10-producing B cells on each group (Alum-immunized or OVA-immunized offspring). CD19+ cells were gated to determine B cells, and IL-10 expression was evaluated on CD19+ cells. IL-10+ cells were gated (as determined by FMO – lower panels) to identify IL-10-producing B cells (Singlet+Lymphocytes+LIVE/DEAD-CD19+IL-10+).

Figure S7

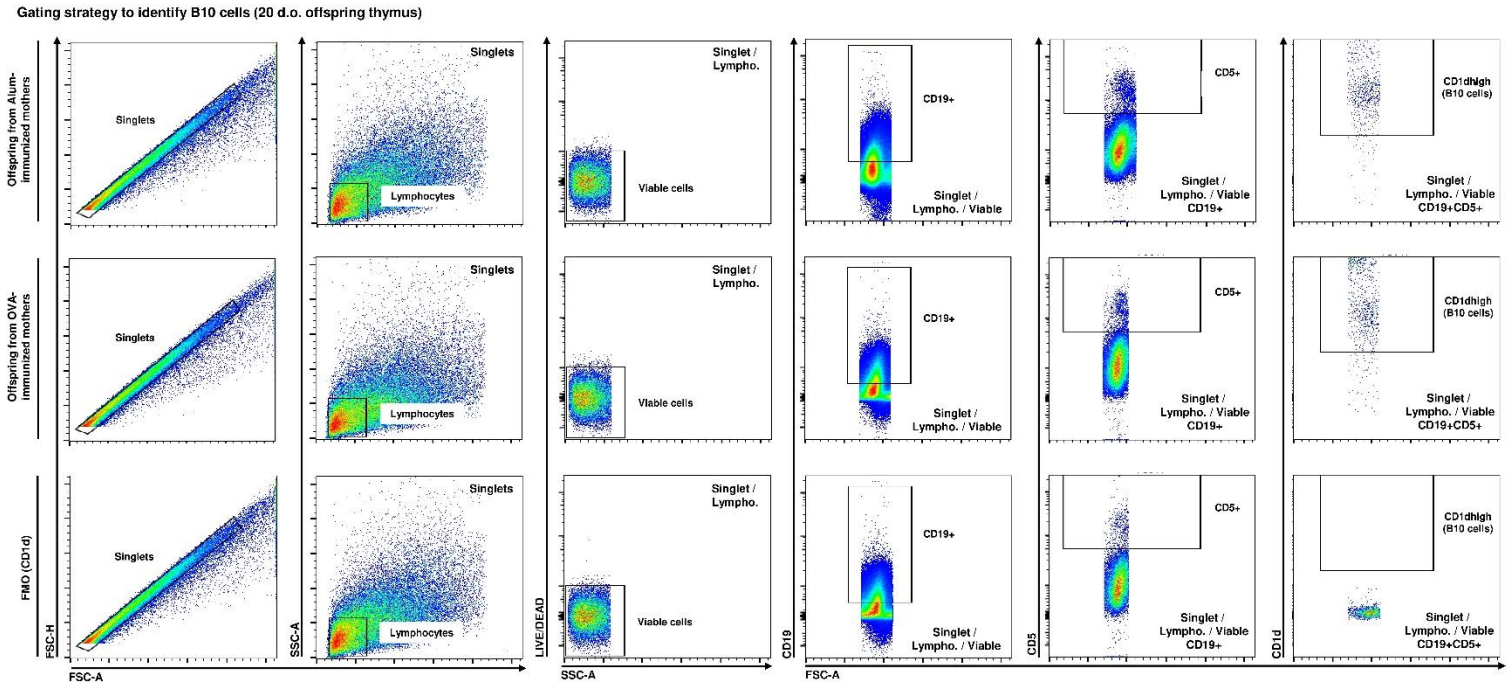

Figure S7. Gating strategy to identify 20 d.o. offspring thymus B10 cells. Each sample was acquired using a single-cell (Singlet) gate (determined by SSC-A/SSC-H parameters), a lymphocyte gate (determined by relative SSC-A/FSC-A). and a viable-cell gate (determined by LIVE/DEAD staining). Upper and middle panels illustrate the gating strategy to identify B10 cells on each group (Alum-immunized or OVA-immunized offspring). CD19+ cells were gated to determine B cells, and CD5 expression was evaluated on CD19+ cells. The expression of CD1d was evaluated on CD19+CD5+ cells, and CD1d^{high} cells were gated (as determined by FMO – lower panels) to identify B10 cells (Singlet+Lymphocytes+LIVE/DEAD-CD19+CD5+CD1d^{high}).

Figure S8

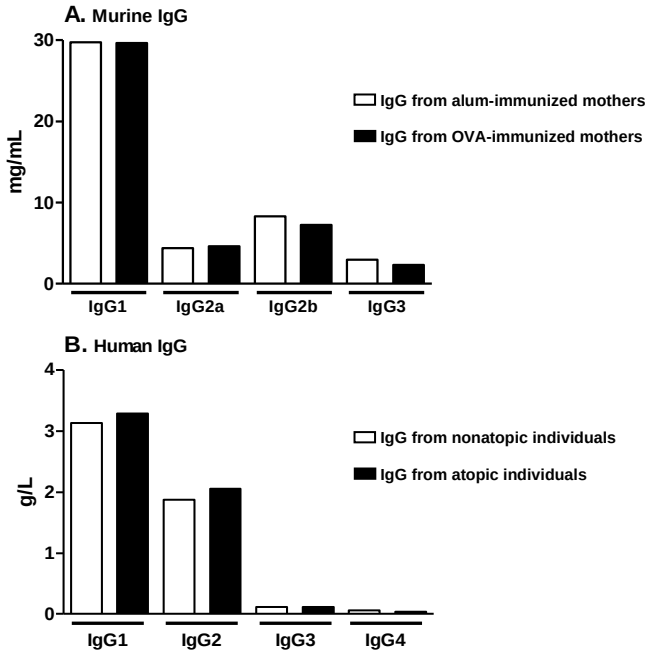

Figure S8. Murine e human IgG subclasses. Purified IgG from OVA-immunized (n=25) or Alum -immunized (n=25) females were pooled, and total IgG1, IgG2a, IgG2b, and IgG3 were evaluated by ELISA (A). IgG from atopic (n=18) or nonatopic (n=17) adults was pooled, and total IgG1, IgG2, IgG3, and IgG4 were evaluated by RID (B).