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LETTER

Mouse IgE clone SPE-7 can contain functional mouse IgG

To the editor,

The hybridoma-produced mouse IgE anti-DNP antibody clone SPE-7 is one of the most widely used molecules to study IgE biology and function. At the same time, SPE-7 has exhibited some unique characteristics such as the ability to trigger mast cell activation and survival independent of a cross-linking antigen,[1,2] the potential to bind DNP-unrelated antigens through conformational diversity,[3] and an increased capability to bind mouse Fcγ receptors.[4] Here, we compared IgE clone SPE-7 to other mouse IgE clones using enzyme-linked immunosorbent assays (ELISA), biolayer interferometry (BLI), liquid chromatography—mass spectrometry (LC–MS), and cell-based in vitro assays with mouse bone-marrow derived mast cells (BMMCs) and spleen-derived mouse B cells.

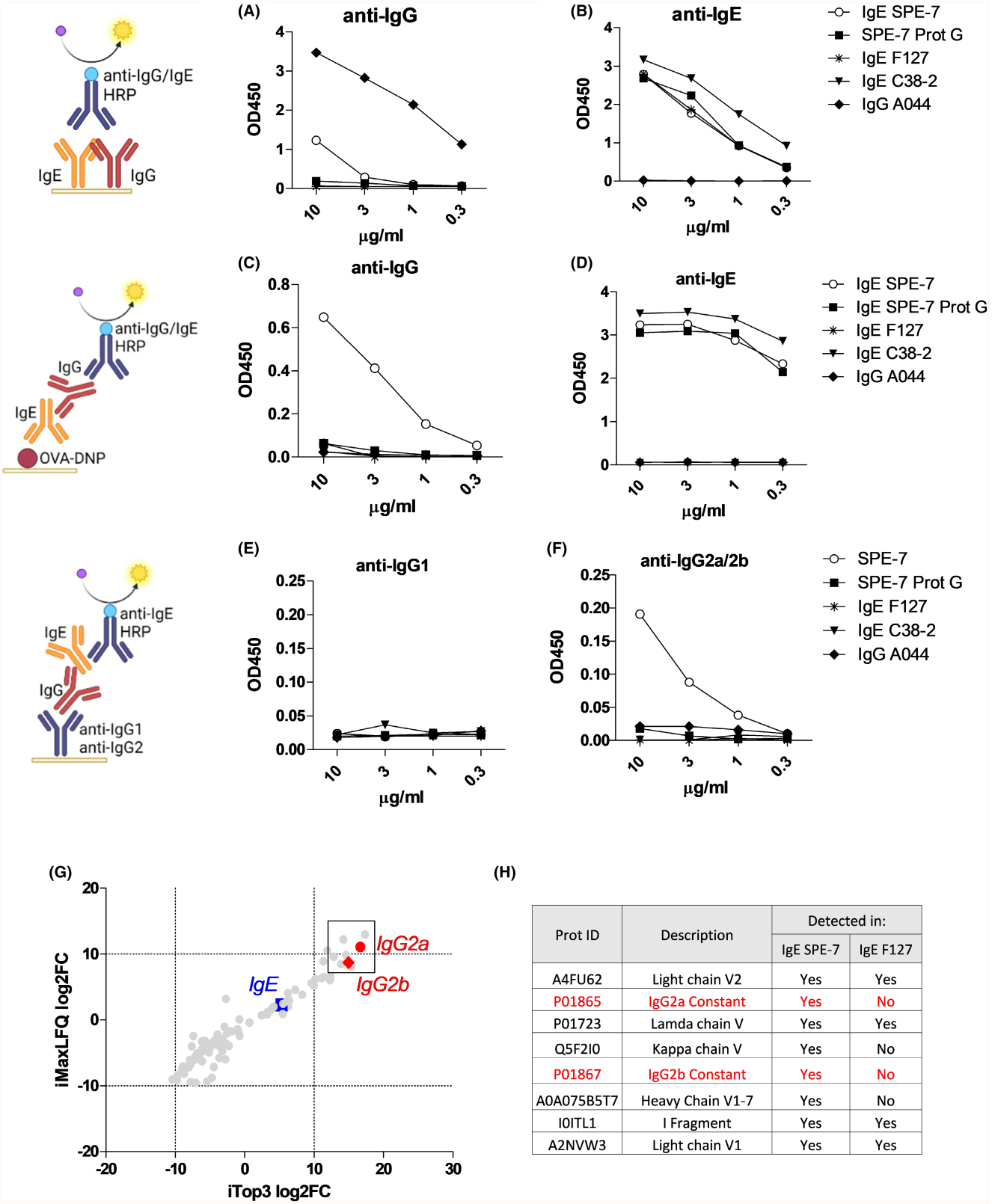
Surprisingly, we found that in contrast to other mouse IgE clones, SPE-7 was recognized by an anti-IgG detection antibody (Figure 1A–D, Figure S1). These IgG signals were lost by purification of SPE-7 using protein G columns. IgG present in SPE-7 could be captured with an anti-IgG2a/b antibody (but not with anti-IgG1) and detected with an anti-IgE antibody, suggesting the presence of IgG2a/b-IgE complexes in SPE-7 (Figure 1E,F). Similarly, SPE-7 ultracentrifugation reduced the contaminating IgG in SPE-7 (Figure S1).

The presence of IgG in SPE-7 was confirmed by LC–MS. We compared the cumulative distribution between samples using MaxLFQ and Top3 normalization protocols (suppl. Methods, Figure S1). Figure 1G shows an increased prevalence of IgG2a/b in IgE SPE-7 compared to IgE F127, and both IgG2a and IgG2b showed up in the top 8 up-regulated hits summarized in Figure 1H.

To test if IgE SPE-7 can functionally bind to IgG receptors, we displayed FcγRI (CD64) on BLI chips (Figure 2A) or on ELISA plates (Figure 2B). In both cases SPE-7, but no other IgE clone, showed binding activity to FcγRI. Using flow cytometry analysis, we next tested whether IgG influences the binding of IgE SPE-7 to the IgE receptor CD23 expressed on spleen-derived B cells. Indeed, Figure 2C shows that binding of IgE SPE-7 to CD23 was increased compared to other IgEs, or compared to IgG-depleted SPE-7.

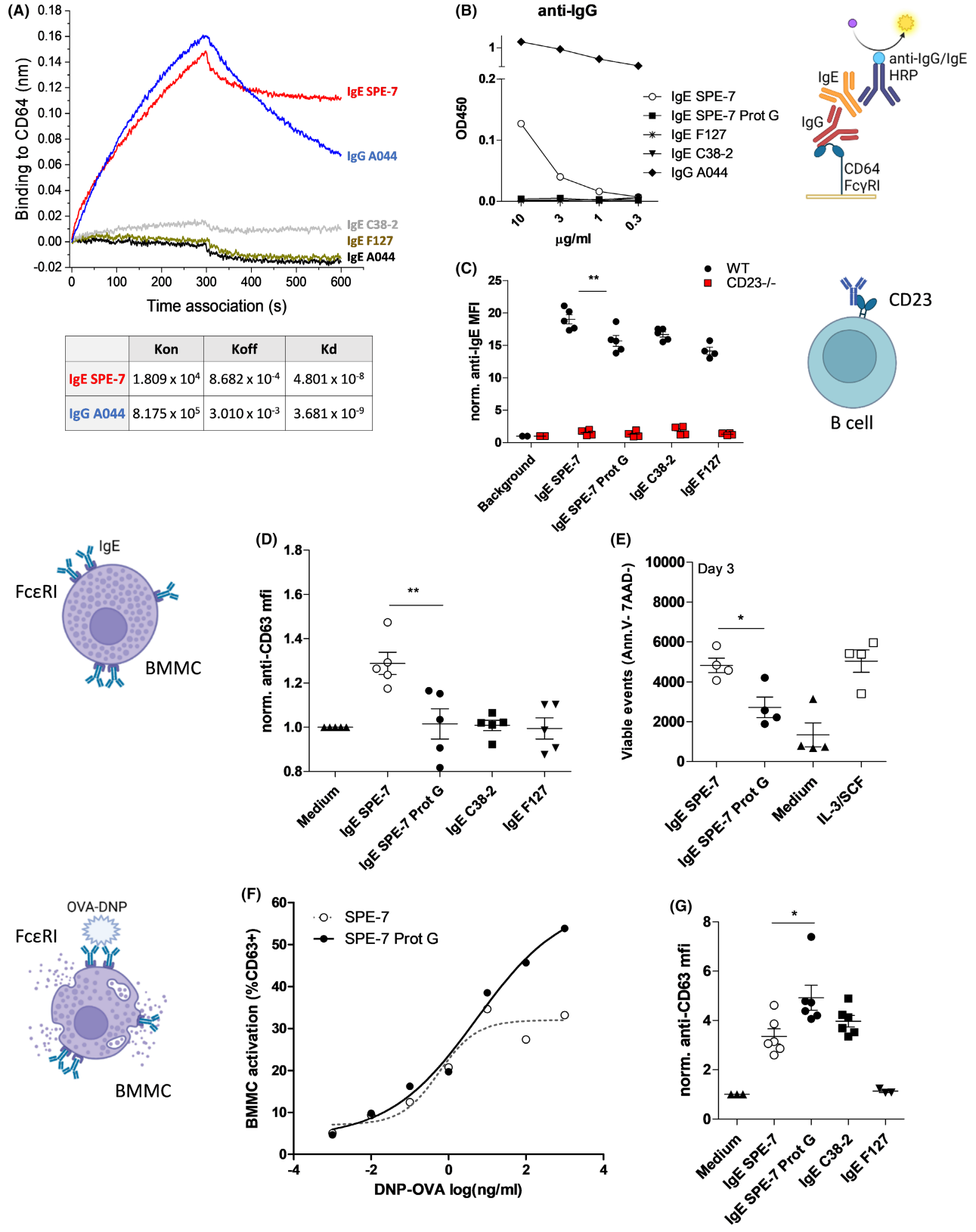
The binding of SPE-7 to FcεRI-expressing BMMCs at 4°C was not significantly altered by IgG removal (Figure S2). When BMMCs were incubated with the different IgEs at 37°C, activation marker CD63 was slightly but consistently up-regulated by SPE-7 but not by IgG-depleted SPE-7 or control IgEs (Figure 2D). In survival tests over 3 days in absence of IL-3/SCF, IgE SPE-7 but not IgG-depleted IgE SPE-7 increased BMMC survival compared to medium controls (Figure 2E). Finally, in classical antigen-dependent degranulation assays, IgG depletion led to a striking increase in the ability of IgE SPE-7 to de-granulate mast cells (Figure 2F,G).

In conclusion, we unveil that purified monoclonal IgE SPE-7 can contain mouse IgG antibodies that form complexes with IgE and alter its functional profile. Our results are in line with previous studies that attributed the unique effects of SPE-7 to the presence of trimeric aggregates.[1] Our findings challenge the concept that IgE exerts antigen-independent effects on mast cell degranulation and survival. Moreover, the extent to which mouse IgE can bind to FcγRs needs to be carefully re-evaluated. The physiological relevance for IgG-IgE complexes is still given, as natural IgG anti-IgE autoantibodies and IgG-IgE complexes occur in mice and humans.[5] Our findings are important to consider in the production of therapeutic IgE antibodies, which are gaining interest in cancer therapy.[6] Contaminating IgGs may alter the functional and pharmacological profile of these therapeutic IgEs. Future studies will investigate how IgG makes its way into hybridoma-produced IgE clones.



Prot ID	Description	Detected in:	
		IgE SPE-7	IgE F127
A4FU62	Light chain V2	Yes	Yes
P01865	IgG2a Constant	Yes	No
P01723	Lamda chain V	Yes	Yes
Q5F2I0	Kappa chain V	Yes	No
P01867	IgG2b Constant	Yes	No
A0A075B5T7	Heavy Chain V1-7	Yes	No
I0ITL1	I Fragment	Yes	Yes
A2NVW3	Light chain V1	Yes	Yes

FIGURE 1 Hybridoma IgE clone SPE-7 contains mouse IgG2. (A, B) Dilutions of IgE/IgG were coated on ELISA plates and detected with (A) anti-IgG or (B) anti-IgE antibodies. Shown is the anti-Ig OD450. (C, D) OVA-DNP was coated on ELISA plates. Dilutions of IgE/IgG clones were incubated and detected with (C) anti-IgG or (D) anti-IgE. Shown is the anti-Ig OD450. (E, F) ELISA plates were coated with (E) anti-IgG1 or (F) anti-IgG2a/2b antibodies, and dilutions of IgE/IgG were incubated and detected with anti-IgE. Shown is the anti-IgE OD450. (G, H) IgE/IgG clones were investigated by LC–MS. Shown are (G) the combined Top3 and MaxLFQ fold changes for IgE clone SPE-7 versus IgE clone F127 and (H) a list of the top 8 up-regulated hits in SPE-7. Schemes were created with Biorender.



	Kon	Koff	Kd
IgE SPE-7	1.809×10^{4}	8.682×10^{-4}	4.801×10^{-8}
IgG A044	8.175×10^{5}	3.010×10^{-3}	3.681×10^{-9}

FIGURE 2 IgG alters the functional profile of IgE clone SPE-7. (A) IgG/IgE binding to BLI-displayed FcγRI and estimated Kon/Koff and Kd values (B) IgG/IgE binding to ELISA-displayed FcγRI. Shown is the OD450. (C) IgE binding assay in wild type or CD23−/− B cells. Shown is the anti-IgE MFI ± SEM measured by FACS. (D) BMMCs were incubated with IgE. Shown is the normalized anti-CD63 MFI ± SEM after 2 h measured by FACS. (E) Shown are AnnexinV and 7-AAD negative events after 3 days measured by FACS. (F) BMMC degranulation assay. Shown are CD63+ cells with OVA-DNP dilution after 20 min measured by FACS. (G) Shown is the normalized mean ± SEM anti-CD63 MFI after 20 min measured by FACS. Schemes created with BioRender. Statistical significance was tested using one-way ANOVA with Dunnett's test. $^{*}p \leq 0.05$,$^{**}p \leq 0.01$.

AUTHOR CONTRIBUTIONS

P.E. conceptualized and supervised the study. M.S. and M.Z. purified IgE and performed ELISA. M.S. and P.E. performed cell-based assays. M.V. performed biolayer interferometry. A.C.U. and S.B.L. performed mass spectrometry. P.E. wrote the manuscript, A.C.U., M.S. and M.V. edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in relation to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.