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SUPPLEMENTAL DISCUSSION

Why is there a subpopulation of "high" expressing transgenic B cells in B-TG mice?

The expression of transgenic FcγRIIb in B-TG mice is detectable on all B cells and is strictly B cell specific. The majority (80–90%) express a twofold increase in FcγRIIb, whereas the remaining 10–20% B cells have an ~10-fold increase in expression, as determined by FACS using two independent anti-FcγRIIb antibodies (2.4G2 and Ly17.1; Fig. 1 C). Although this is so, the distinct profile of transgene expression within this population is difficult to explain. This was confirmed by sorting the high and "low" populations based on 2.4G2 staining, and then detecting the level of the V5-tagged transgene by both Western blotting and intracellular staining using an anti-V5 antibody. As expected, more V5 expression could be detected in the high population (unpublished data).

This expression pattern is highly unlikely to be caused by the integration site of the transgene, as three separate founders were generated, with all of them showing the same expression pattern (Fig. S1 and not depicted). The high B-TG B cells were detectable in all of the tissues assessed (spleen, lymph node, bone marrow, and blood), and we show by immunohistolology that 2.4G2-high B cells are scattered throughout the spleen in TG mice (Fig. S3). Therefore, the high population is not confined to a particular tissue location. The level of expression does not correlate with a particular subset of B cells. We have shown equal distribution of high and low transgenic B cells throughout various B cell subsets (B220, CD19, CD22, CD23, CD21, IgM, IgD, CD5, CD138, and I-A^b), and found no correlation with the activation status of B cells as assessed by activation markers (CD40, CD80, and CD86; Fig. 1 D and not depicted).

Upon activation with intact anti-IgM antibodies, the levels of FcγRIIb expression are slightly reduced on all transgenic B cells, and the high population merges back into the lower population (as determined by 2.4G2 staining). It has similarly been reported that in an unrelated transgenic mouse, where the transgene (choramphenicol acetyltransferease) is also regulated by the V_H promoter and IgH intron enhancer, that stimulation of B cells with anti-IgM antibodies reduces transgene activity (1).

Expression of the high population is detectable at the pro–B cell stage of B cell development ($B220^+IgM^-CD43^{high}$) and at all stages of differentiation up to mature B cells ($B220^+IgM^+IgD^+$; unpublished data). This timing is consistent with the onset of Ig heavy chain rearrangement and when the V_H promoter and μ and 3′κ enhancers are required (2). Despite this unusual pattern of transgene expression, all B cells from B-TG mice overexpress functional FcγRIIb, and this is exclusive to this cell type.

REFERENCES

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