On the differentiation of mouse IgE$^+$ cells

To the Editor:

We read with interest the paper by Talay *et al.* in the April issue of *Nature Immunology*[1]. In this article, the authors use a green fluorescent protein (GFP) reporter mouse strain to establish the rules that govern immunoglobulin E (IgE) responses *in vivo*. After carefully reading the manuscript, we believe that there are several issues that challenge the validity of their experimental model and, thus, their conclusions.

The reporter construct used by Talay *et al.* has two features that require careful confirmation[1]. First, the construct adds an exon encoding human membrane IgE (M1′) to the existing exons encoding mouse membrane IgE. The resulting mouse-human chimeric IgE molecule is an altered IgE molecule that is longer by 52 amino acids. This humanized mouse molecule should not be the first choice of model with which to study normal mouse IgE responses. The longer mouse-human chimeric IgE molecule cannot be considered to reflect normal IgE structure, aggregation and signaling in the absence of comparative studies, which are missing in the present manuscript[1], or the previously published article by these authors describing the humanized mouse strain[2]. Second, the authors replace the IgE 3′-polyadenylation site. The 3′-polyadenylation site in IgE is unusual in its sequence and organization, and it has been shown that a point mutation that creates a consensus site changes the ratio of membrane IgE to secreted IgE by tenfold[3]. This by itself could explain some of the unexpected data presented in the manuscript by Talay *et al.*[1]. Furthermore, there is no comparison of the ratio of membrane IgE to secreted IgE generated by the targeted allele versus that generated by the normal allele.

Another potential issue with the study by Talay *et al.*[1] is that not all GFP$^+$ cells are IgE$^+$. The safest confirmation of a membrane IgE reporter mouse strain would be to correlate GFP expression with surface staining with an antibody to mouse IgE. However, such IgE staining is absent throughout this manuscript[1]. The only staining with antibody to IgE (anti-IgE) in the manuscript is one panel of intracellular staining. Instead of anti-IgE, the authors use an antibody to the human domain M1′. Although the M1′ staining data in **Figure 1c** of the article is described as indicating that the cultured cells "expressed membrane IgE"[1]. The shift in fluorescence intensity of the sample stained with anti-M1′ relative to that of the control sample is minimal. The overlay of the flow cytometry of cells from *Nippostrongylus brasiliensis*–infected mice with anti-M1′ shows almost perfect overlap with the that of isotype-matched control antibody on the left of the anti-M1′ curve and, in addition, 'piling up' of many GFP$^+$ cells completely negative for M1′ along the vertical axis (Supplementary Fig. 4b of the article)[1]. Thus, a substantial proportion of GFP$^+$ cells seem to be negative for M1′ in *N. brasiliensis*–infected mice. These GFP$^+$ cells probably express immunoglobulins of other isotypes.

A published paper by Yang *et al.* uses a different IgE reporter system[4]. In contrast to the model proposed by Talay *et al.*, Yang *et al.* show that IgE$^+$ germinal-center cells diminish rapidly during an ongoing response, whereas IgG1$^+$ germinal-center cells persist[4]. Furthermore, IgE$^+$ plasma cells have considerably fewer mutations than do IgE$^+$ germinal-center cells, an indication that highly mutated IgE$^+$ germinal-center cells are not part of the main IgE response. It remains most likely that, as proposed before, class switching to IgE is linked to a plasma-cell differentiation program and that IgG1$^+$ germinal-center cells contribute to the IgE$^+$ plasma-cell compartment[5,6]. In fact, we have shown that mice deficient in IgG1 have a considerable impairment in their ability to generate affinity-matured IgE antibodies[6]. Although the reporter allele used by Yang *et al.* is less manipulated than that used by Talay *et al.*, the ratio of membrane IgE to secreted IgE produced by the targeted allele in the mice studied by Yang *et al.*[1] is two- to threefold higher than that produced by the normal allele encoding IgE, and this allele is, possibly, altered even further in the mice used by Talay *et al.* The differences between the data obtained by Talay *et al.* and the data of other groups could reflect substantial differences in the activity of the longer humanized molecules used by Talay *et al.* relative to that of normal mouse IgE molecules.

In conclusion, we find that the report by Talay *et al.* is lacking in several important ways and therefore would like to bring to the attention of *Nature Immunology* readers the existence of alternative explanations for most of their observations.

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The authors declare no competing financial interests.

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Talay *et al.* reply:

We welcome the opportunity to address and clarify the concerns raised by Lafaille and colleagues about our study[1] and its differences with their work (by Erazo *et al.*[2]).

We were careful to assess whether the introduction of the bicistronic reporter gene encoding enhanced GFP and the exon segment encoding human IgE membrane (M1′; inserted to assess the effects of targeting membrane IgE$^+$ cells via the M1′ segment) into the locus encoding