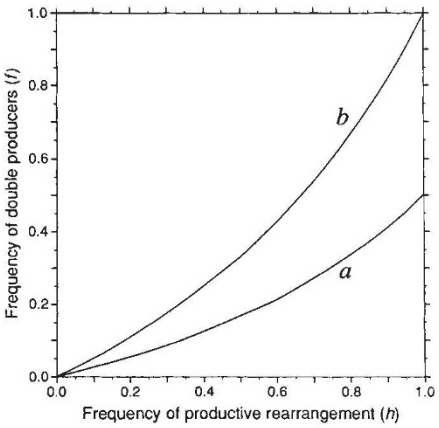
B cells derived from them. The cellular selection model predicts a frequency of double producers that is twice as great for newly arising B cells and a general, perhaps major, decrease in that frequency for spleen B cells.

Kitamura and Rejewsky present f as 20–25 per cent for newly generated B cells in the bone marrow, which decreases to about 6 per cent in the spleen. For spleen cells the observed value of f is compatible with both models. For newly generated B cells in the bone marrow, the feedback model predicts that f should be less than 10 per cent if h is less than 1/3; the cellular selection model predicts a value of about 20 per cent.



Expected frequency, f, of double producers as a function of the frequency, h, of productive *VDJ* rearrangements for two models of allelic exclusion. *a*, Feedback model; *b*, cellular selection model.

For technical reasons, a neomycin-resistance gene (neo^r) is inserted into the mutant allele; the authors discuss how that artifice might result in the values for bone marrow being too high. Without going into the validity of this point, we would like to point out that the decrease in the fraction of double producers cannot be explained in this way. The decrease in f value from bone marrow to spleen is *ipso facto* cellular selection; this is a direct consequence of the undisputed fact that spleen B cells are the descendants of bone marrow B cells.

We are left with the difference between the knockout heterozygotes and normal, allotype heterozygotes. Unfortunately, no results were reported for normal, newly generated B cells[1]. For normal spleen B cells, $f = 0.3\%$, a value that ought to be different from 6% even though no statistics are given. If the authors are willing to contemplate that the inserted neo^r gene might be responsible for the (to them) surprisingly high fraction of double producers in newly arisen bone marrow B cells, perhaps they should at least entertain the possibility that the neo^r gene might also be responsible for the (to us) surprisingly high value in spleen B cells. Be that as it may, there is clearly a component of cellular selection against double producers.

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SIR — Kitamura and Rajewsky[1] described the effects of disrupting production of the normal membrane form of the immunoglobulin μ heavy chain (μ_m) on allelic exclusion. We are concerned that readers of their paper might infer that it supports the concept of feedback inhibition, because many will equate the authors' conclusion that "μ_m signals allelic exclusion" with "μ_m stops heavy-chain rearrangement". We wish to point out that, although the authors have shown clearly that μ_m production affects the frequency of allelically excluded B cells, our understanding of the mechanism of allelic exclusion remains unsettled.

As outlined by Kitamura and Rajewsky[1], allelic exclusion in part reflects the $V(D)J$ rearrangement process itself, in that the joining of the variable-region segments permits the loss of coded or addition of uncoded nucleotides, with the result that most rearrangements are expected to create out-of-frame joints. Were this the only contribution to allelic exclusion, one would still expect that about one-third (see below) of the immunoglobulin-producing cells would have two functional alleles, whereas in fact the frequency of mature double-producing cells is usually quoted as less than 1 per cent. Several explanations have been proposed to account for this low value. One type of explanation, that of feedback inhibition, invokes control of the rearrangement process, whereby the protein product of a functional rearrangement, such as μ_m, immediately inhibits further rearrangement. Other explanations involve post-rearrangement effects, for example that cells producing a double dose of immunoglobulin are less viable, or that double producers which therefore bear surface immunoglobulin with non-uniform binding sites proliferate less after stimulation with specific antigen than do single producers.

The experimental system used by Kitamura and Rajewsky to detect feedback inhibition involved measuring the frequency of double-producing cells in mice heterozygous at the heavy-chain locus: the *a* allele was disrupted so that it could not yield μ_m; the *b* allele was normal. Taking into account the possible stop codons in the *D* segments, the authors calculated the expected frequency of *a*–*b* double producers among IgM-positive cells as 12 per cent if μ_m inhibits rearrangement. To minimize the possible complications of post-rearrangement selection against double producers, they measured the fraction of double producers among newly generated B cells, reporting values of 20 and 25 per cent. These values were described as being greatly above the theoretical expectations, by which we suppose that the authors meant greatly above the expectations for a mouse in which a mechanism of feedback inhibition was operating.

They went on to propose an explanation for the high frequency of producers, namely that the disrupted allele was more prone to rearrangement than the normal allele, perhaps because it was rendered more accessible by the SV40neo transcription unit used to disrupt the μ_m gene; other explanations, some of which invoke artefacts of the culture system, are also possible.

This risk of artefact notwithstanding, we wish to point out that if there is no feedback inhibition of rearrangement, the expected frequency of double producers among IgM-positive cells is 24 per cent. The close agreement of this prediction with the reported values emphasizes the very interesting possibility that allelic exclusion arises because of post-rearrangement selection against double producers.

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RAJEWSKY *ET AL.* REPLY — We[1] did not mean to show "another case of allelic inclusion", as Wabl and Steinberg put it, but to test whether expression of the membrane-bound antibody heavy (H) chain of class μ inhibits further H-chain V-region (VH) gene rearrangement during B-cell development — a specific version of the 'feedback' model of allelic exclusion[2,3]. For this purpose, we used mice heterozygous for a null mutation in the membrane exon of the μ-chain. After polyclonal activation, B cells pro-