V β 8⁺ T cells by SEB or SEB-MHC class II complexes.

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Spontaneous mutation rate

SIR — We have described a mouse *in vivo* mutagenicity assay capable of detecting mutations at the *Dlb-1* locus which encodes the *Dolichos biflorus* lectin receptor expressed in the epithelium of the small intestine¹. In this intestinal mutagenicity test (IMT), clonal descendants of embryonal intestinal stem cell precursor cells mutated *in utero* can be identified histochemically as unstained groups of enterocytes on villi in otherwise positively stained epithelium of adult animals (8–12 weeks of age) using peroxidase-labelled lectin. The test permitted us to quantify spontaneous mutation rates in untreated mice as well as rates of induced mutations. Our results, derived from scorings of intact preparations (Fig. 1) of complete small intestines, indicated that spontaneous mutation rates are considerably

FIG. 1. Regions of duodenum from ENU-treated *dlb-1^a/dlb-1^b* mouse *in utero*. Most of the villi appear brown following histochemical staining using peroxidase-labelled *Dolichos biflorus* agglutinin. Fully separated and mucus-free gut preparation showing a mutated crypt (arrow) bearing the *dlb-1^a/dlb-1^b* genotype feeding two distinct adjacent villi. Mutated villi occurring in distinct patches of villi which fail to bind the lectin, thus appearing unstained. Scale bars: a, 0.5 mm; b, 1 mm.

lower than reported by Winton *et al.*², who used mice aged 3–26 weeks in a similar biological test system involving different methodological approaches. We therefore reassessed levels of spontaneous mutations at the *Dlb-1* locus by scoring whole-mount preparations of the mouse gut as described¹, using mice aged up to 56 weeks (Fig. 2). The slope of the curve was estimated to be 0.01, whereas a slope of 0.27 was estimated from Fig. 2 of Winton *et al.*². We estimate a spontaneous mutation rate of 0.5 mutations per 10⁴ villi per year, whereas the estimate given by Winton *et al.* is 13.5 mutations per 10⁴ villi per year.

Although the reasons for this discrepancy are unclear, it seems that the different methods used to carry out the assay may have contributed to the considerable differences in the results obtained. The large standard deviations reported by Winton *et al.*² (their Fig. 2) may reflect technical difficulties in scoring subsamples of gut preparations (about 5 per cent of the total gut) to determine spontaneous mutation rates. By contrast, we have shown that because of the sporadic distribution of mutant villi and mutant villus patches in the gut it is essential to score the entire intestine and to generate undamaged gut preparations in which the histochemical staining extends from the crypt orifices to the tips of the villi¹ (Fig. 1). We have therefore developed methods that eliminate sample variability associated with subsampling, incomplete tissue staining and sporadic tissue damage. Our method is reliable, as shown by the minuscule standard deviations of our data (Fig. 2).

Mutant villi occur either isolated or in small and spatially isolated patches¹. The former arise as a result of mutations in stem cells in individual crypts² or in embryonal intestinal precursor cells. Their descendants are thought to be deposited in neighbouring or closely adjacent crypts, thus causing the appearance of patches of mutant villi. Thus, in assessing the number of mutational events in gut preparations we have scored mutant villus patches as single mutational events rather than counting each mutated villus within a patch separately¹. Winton *et al.* scored unstained ribbons on immediately adjacent villi arising from an apparent common centre (a crypt) resulting from single events, whereas villi in patches that were not immediately adjacent were scored as resulting from various events. Adult guts display both mutated villus patches as well as single mutated crypts stemming from single mutational events occurring during embryogenesis or in adult mice, respectively. Therefore, the scoring technique used by Winton *et al.* presumably contributed to an inflated estimate of spontaneous mutation rates.

This suggestion is strengthened by a recent publication by Winton *et al.*³, who scored numbers of mutated crypts from the abluminal side of the gut, rather than mutated ribbons on villi, which led to estimates of the spontaneous mutation rate of about 10-fold

FIG. 2 Whole-mount preparations of small intestine (pylorus to ileum) from four mice were scored each at 8 and 12 weeks of age and three intestines at 56 weeks. Mutations were observed as spatially isolated patches of one to two striped villi which were scored as separate mutation events. The total number of villi was estimated by taking measurements from four intestinal preparations. Using this estimate, the mutation scores per intestine were adjusted to mutations per 10⁴ villi and plotted against time. Data are presented with error bars smaller than the symbols used (●). The resulting slope fitted to our data is 0.01, whereas that of Winton *et al.*² (their Fig. 2) is 0.27 (○).

lower than in their earlier studies² (assuming an average of 10 crypts per villus⁴), and thus are similar to our observations¹. Additional inconsistencies exist between results of Winton *et al.*^{2,3} regarding mutation rates induced in response to ethylnitrosourea (ENU) (50 mg kg⁻¹) treatment, differing about 50-fold depending on their scoring procedure. Such discrepancies emphasize the need for a standardized scoring procedure to determine quantitatively the potential of mutagenic agents in this assay, particularly if used for human risk assessments.

Using a different approach, Griffiths *et al.*⁵ observed no spontaneous mutations in the colon using glucose-6-phosphate dehydrogenase as a marker gene in sections containing about 400,000 crypts. Even though these results may not be comparable directly with our data, they are not surprising: assuming an average of 10 crypts per villus⁴, our estimate of 0.5 mutations per 10⁴ villi per year may be converted to 0.5 mutations per 10⁵ crypts per year. Hence, there is little chance of observing significant numbers of mutated crypts in a sample of 400,000 and even less so considering that the age of the mice used by Griffiths *et al.* was less than one year⁵. Our findings corroborate those of Griffiths *et al.*, indicating that the rate of spontaneous mutation in the intestine is extremely low.

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