Interindividual Variation in Mitotic Recombination

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Summary

Mitotic recombination (MR) between homologous chromosomes is a mutational event that results in loss of heterozygosity in half of the segregants at mitosis. Loss of heterozygosity may have important biological consequences. The purpose of this study was to describe human variation in the spontaneous frequency of MR. Using an immunoselection technique for isolating the somatic mutations that result in loss of expression of one of the codominant alleles at the HLA-A locus, we have measured the frequency and molecular basis of somatic mutations in lymphocytes from a population of young adults. Mutations were classified as being the result of intragenic changes, major deletions, or MR. Here we show that the MR mutation frequency in females was significantly greater than that in males but that intragenic mutation frequency showed no association with sex. Individual variation in MR frequency ranged over more than two orders of magnitude and was not normally distributed. Furthermore, the observed number of individuals from whom no mutants resulting from MR were obtained was significantly greater than was expected. The endogenous level of MR may be under genetic control. Given the association of loss of heterozygosity with cancer initiation and progression, low endogenous MR may confer a reduced lifetime risk of cancer, and the converse may apply.

Introduction

Mitotic recombination (MR) is the result of reciprocal exchange of genetic material between nonsister chromatids of homologous chromosomes in mitotic cells. MR is an important genetic event since, on average, half of the daughter cells will have loss of heterozygosity (LOH) distal to the recombination site after mitosis. Cumulative LOH may be an important contributor to aging, to cancer initiation and progression, and to differential expression of the phenotype in genetic disease (Qian and Germino 1997). In previous studies, we have shown that MR is a relatively common mutational event accounting for approximately one-third of spontaneous mutations resulting in the loss of expression of one of the codominant HLA-A alleles in peripheral-blood lymphocytes (Morley et al. 1990). The spontaneous frequency of MR increases with age (Grist et al. 1992). The frequency of MR increases in human cells in response to a variety of DNA-damaging agents, which indicates that MR may be an intended or unintended by-product of DNA-repair mechanisms, as is well described in lower eukaryotes. However, the normal variation of spontaneous frequency of MR has not been described in any higher eukaryote. Since it may be hypothesized that high endogenous levels of MR will predispose certain individuals to cancer, we investigated the frequency of MR in a large number of individuals within a narrow age range.

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Subjects and Methods

Study Population

Blood samples were obtained, through the Red Cross Blood Bank (Adelaide, South Australia), from young healthy donors and were typed by immunocytotoxicity to identify individuals who were heterozygous for either HLA-A2 or HLA-A3 or both. Only the age and sex of each individual were additionally recorded. In the study population of 105 individuals, 43 were male, with a mean ± SD age of 23.6 ± 3.4 years, and 62 were female, with a mean ± SD age of 23.7 ± 3.2 years. The study was approved by the Committee on Clinical Investigation of the Flinders Medical Centre (Ethics Committee).

HLA-A Mutation Assay

The HLA-A mutation assay was performed as described elsewhere (McCarron et al. 1989). In brief, lym-

Table 1

Somatic MFs ($\times 10^{-6}$) at the HLA-A Locus in Young Adults

Mutation Category	Somatic MFs (No. of Informative Individuals) in Study Population[a]			Sex Difference[b]
	Males	Females	Total	
	MF (N)	MF (N)	MF (N)	
All[c]	22.7 (43)	27.8 (62)	25.4 (105)	NS
Intragenic[c]	22.5 (29)	18.1 (37)	19.9 (66)	NS
MR[d]	3.3 (39)	8.2 (58)	5.8 (97)	$P = .022$
Deletion	NC (25)	NC (33)	NC (58)	

[a] NC = not calculated (see text).
[b] NS = not significant.
[c] Geometric-mean mutation frequency.
[d] Median mutation frequency.

phocytes isolated from peripheral blood were subjected to immunoselection against either the HLA-A2 or HLA-A3 epitope, by use of complement-fixing antibodies BB7.2 (as anti–HLA-A2 antibody) (American Type Culture Collection) and XI23 (as anti–HLA-A3 antibody) (a gift from Dr. Anne Martin of Centre Régional de Transfusion Sanguine, Rennes, France) and rabbit complement. Control lymphocytes were exposed to complement alone and to medium alone. Cells were then plated, in 96-well microtiter plates, at cell densities of 2 cells/well for control cells and 2×10^4 cells/well for cells immunoselected with antibody and complement. The culture medium contained conditioned medium (a source of interleukin-2) and irradiated feeder cells (1×10^4 cells/well). The cells were cultured at 37°C in 10% CO_2, and plates were scored for clonal growth in microwells ≤21 d. Cells from wells showing positive growth were tested with antibody and complement to confirm that they had lost the selected phenotype and were mutant. The cloning efficiency for selected and nonselected cells was calculated, by use of Poisson statistics, from the number of negative wells in the microtiter plates, and the spontaneous-mutation frequency was calculated from the ratio of cloning efficiency in the presence of selection to that in its absence. DNA was obtained from each mutant clone, and the molecular nature of the mutation was determined by study of polymorphic loci on chromosome 6p. Mutations were classified as being due to one of the following: (1) intragenic mutation within the HLA-A gene, (2) a major deletion, or (3) MR, according to retention of heterozygosity or LOH at polymorphic loci located both within HLA-A and distally. Mutant clones showing retention of heterozygosity both at a polymorphism in intron 3 of HLA-A (Firgaira et al. 1994) and at a distal locus (either *FXIII* or *D6S89*) have been shown to have intragenic point mutations or small deletions (Male 1997). Retention of

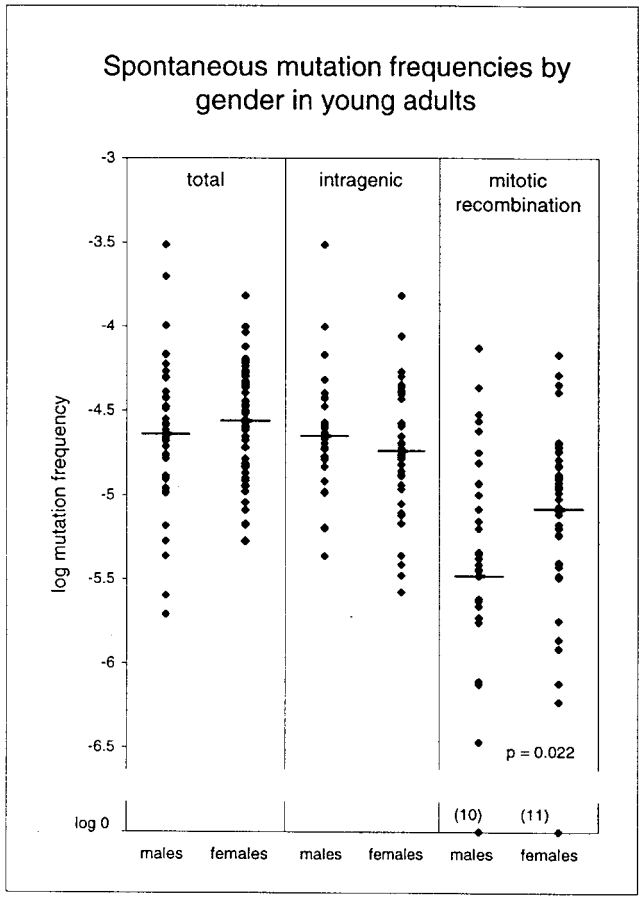

Figure 1 Spontaneous MF, measured at the HLA-A locus in human lymphocytes, in young adults. *Left panel,* Spontaneous MF in a population of 43 males and 62 females 18–30 years of age. The geometric-mean MF is shown for each sex. *Center panel,* Spontaneous MF resulting from intragenic point mutations or small deletions within the HLA-A gene in 29 males and 37 females with informative polymorphisms. The geometric-mean MF is shown for each MF. *Right panel,* Spontaneous MF resulting from MR events in 39 males and 58 females from the population. The median MF is shown for each.

heterozygosity distally but LOH at HLA-A was taken to represent a major deletion of genetic material, and distal LOH was taken to indicate a mutation arising from MR. This approach to classification of mutations has been validated elsewhere (Joseph et al. 1993).

Results

Some individuals were not informative at one or more of the loci, and the number of informative individuals for each class of mutation is indicated in the text. A total of 1,929 clones were obtained from 105 individuals. Mutation frequencies (MFs), both for the population as a whole and by sex, are given in table 1, and the individual data are presented in figure 1. Cells from seven

individuals who were HLA-A2/A3 heterozygotes were selected at both alleles. Only the geometric mean of MFs for these seven individuals was used in the calculation of the mean MFs in the population. Geometric-mean MFs are given for total MF and for intragenic MF, both of which were normally distributed (Kolmogorov-Smirnov test). There was no significant difference between the spontaneous total MF seen in males and that seen in females ($P = .1$, Wilcoxon rank-sum test).

The majority of mutant clones were due to intragenic mutations. In 66 informative individuals, 815 of 1,135 clones were due to intragenic mutation. The mean proportion of intragenic mutations per individual was 71.2%. The observed sex difference in the intragenic MF was not significant.

Individual variation in MR was not normally distributed, and the median values of MR, both for the population as a whole and by sex, are given in table 1. There was a significant sex difference in the frequency of mutations resulting from MR, with females exhibiting a higher MR frequency than was seen in males ($P = .022$, Wilcoxon rank-sum test). The female:male sex ratio of the median MF of MR was 2.5:1.

MR was observed in 528 (29.4%) of 1,795 clones from 97 informative individuals, a finding that is in excellent agreement with our previously cited results from a smaller sample (Morley et al. 1990). The mean proportion of MR events per individual was 26.4%. Of the 97 informative individuals, there were 21 from whom no MR mutant clones were recovered. Studies of some individuals would be expected to have yielded no MR clones by chance alone, given the number of clones recovered in each assay. Using Poisson statistics, we modeled the number of individuals expected to show zero MR mutant clones, both on the basis of the actual number of recovered clones and on the basis of various estimates of the expected proportion of MR mutant clones. The observed number of such individuals exceeded that which was expected, regardless of whether or not analysis was restricted to assays in which ≥ 10 clones were analyzed (table 2).

Fifty-eight individuals were informative for deletion mutation. Of 1,015 clones, 27 (2.7%) were the result of deletional events. This number precludes a meaningful calculation of the mean or median MF for this class of mutation. However, under the hypothesis that 2.7% of spontaneous mutations were due to deletion, a statistical analysis (Poisson statistic) showed that the number of individuals displaying no deletion was in agreement with the number that was expected (data not shown).

Discussion

Spontaneous somatic MF has been studied at several loci. At the *hprt* locus, we and others have found that interindividual variation exists and that the MF is $\sim 3 \times 10^{-6}$ in young adults (Morley et al. 1983) and increases with age (Trainor et al. 1984). The majority of mutations resulting in the inactivation of the *hprt* locus arise from point mutations or small deletions within the *hprt* gene (Burkhart-Schultz et al. 1996). The *hprt* gene is located on the X chromosome and consequently is hemizygous in males and functionally hemizygous in females. It is likely that MR does not occur on the X chromosome. The delay in replication during S-phase between the X chromosomes in females may preclude MR, or segregation of MR may be lethal because of dosage reasons. Accordingly, the study of mutations at X-linked loci does not demonstrate the full spectrum of potential mutational events.

There have been several studies of the frequency and spectrum of mutations at autosomal loci. Mutations involving glycophorin A and resulting in loss of the M or N blood type have been measured, by flow cytometry, in erythrocytes, and the results (Jensen et al. 1986; Akiyama et al. 1995) suggest levels of total mutations and presumed MR mutations that are similar to those reported here. However, the molecular basis of the mutations cannot be determined in erythrocytes. Flow cytometry has also been used to assess MF at the T-cell–antigen receptor (Kyoizumi et al. 1990) and at the

Table 2

Observed and Expected Numbers of Individuals with 0 MR Mutants

Total No. of Individuals	No. of Observed Individuals with 0 MR Mutants	Mean Proportion of MR	No. of Expected Individuals with 0 MR Mutants	Probability of No. Observed
In all assays: 97	21	.20	15.9	.126
		.25	12.1	.013
		.30	9.6	.001
		.35	7.8	6.6×10^{-6}
In assays with ≥ 10 clones: 67	8	.20	2.5	.004
		.25	1.3	6.4×10^{-5}
		.30	.67	5.6×10^{-7}
		.35	.36	5.1×10^{-9}

HLA-A gene (Kushiro et al. 1992). In both these reports, the total MFs were observed to be up to an order of magnitude higher than those reported here. However, in the few mutant clones subsequently analyzed at the molecular level, the proportion of MR mutants was in excellent agreement with the mean population proportion of MR reported here. MFs at the autosomal adenine phosphoribosyl transferase (*aprt*) gene have been determined, in four obligatory heterozygotes for APRT deficiency. The molecular basis of mutations in T-cell clones was determined in two of these individuals in whom MR was responsible for the majority of mutations recovered (Gupta et al. 1997).

The present study has demonstrated an interindividual variation in MR, exceeding two orders of magnitude. Given the relatively young age of the unselected individuals in our study, we believe that it is unlikely that the differences that we have observed in the frequencies of MR are due to differing environmental exposures to mutagens. The data suggest a bimodal distribution of MR frequency, with ~10% of individuals having an MR frequency that is very low or zero and with 90% of individuals having an approximately log-normal distribution near a median of 5.8×10^{-6}, with the median proportion of mutations resulting from MR in an individual being 31%. At the upper extreme, MR becomes the dominant type of somatic mutation in lymphocytes from some individuals. For example, in one individual, MR was responsible for 74% of 58 mutant clones analyzed. To what extent these clones represent either truly independent mutations in this individual or in vivo expansion of one or more mutated lymphocytes is presently unknown, although we (McCarron et al. 1989) and others (Gupta et al. 1997) have previously observed potential in vivo clonality only in a minority of cases. Indeed, recent (unpublished) studies from our laboratory demonstrate that, among unselected T cells in normal individuals, sibling T-cell clones are present at very low levels (95% confidence interval [CI] 0%–3.1%). Even so, the presence of sibling clones does not bias the best estimate of MF in a population, although it increases the variance (Morley 1996).

We have identified a group of individuals in whom the endogenous absolute frequency of MR is truly very low or zero. If MR does not occur in some individuals, this may be due to the presence of a lethal recessive gene that is distal to HLA-A and that is syntenic with the nonselected allele. Such a putative locus would become homozygous after recombination, resulting in death of the cell and failure to recover mutants. It is also possible that a form of F_1 hybrid resistance that is mediated by natural killer cells and that is known to function in the mouse might select against homozygous HLA class I–expressing cells in individuals with certain HLA haplotypes. Alternatively, there may be genetic variation that suppresses MR. We are unable to distinguish between these possibilities in this study, although we do favor genetic variation as an explanation for our observations.

Genetic control of MR is poorly described and merits further study, since individuals who display a significantly elevated MR frequency may be predisposed to premature aging and cancer. Bloom syndrome is associated with a high level of chromosomal instability and a greatly increased risk of cancer (German 1997). An elevated frequency of MR in Bloom syndrome has been inferred from flow-cytometric studies (Langlois et al. 1989) and has been observed in T-cell clones (Kusunoki et al. 1994). The gene mutated in Bloom syndrome (i.e., *Blm*) would appear to have a normal function suppressing MR. *Blm* and genes with gene products known to interact with *Blm* protein may be candidate genes for polymorphisms giving rise to biological variation in MR in the human population. More generally, failure or lack of fidelity of other mechanisms, such as DNA-repair processes, in maintaining the integrity of the genome may contribute to the frequency of MR.

In this study, we have made the novel observation of a sex difference in MR that shows a median female:male ratio of 2.5:1. In humans, mieotic recombination also occurs at a higher rate, per physical distance of DNA, in females than in males. The genomewide female:male meiotic recombination is 1.61:1. For chromosome 6, the ratio is 1.96:1, and in the region between the centromere and HLA-A, the ratio is reported to be 3.3:1 (Dib et al. 1996). These latter figures are based on 186 informative meioses and, thus, on only ~60 events in the region between the centromere and HLA-A. We have assessed, in 97 individuals, 528 MR events over the same genetic interval, in deriving our estimate of median MR frequency. The MR events that we have identified are overwhelmingly independent events distributed across ~1% of the genome. It seems likely that this interval is sufficiently large to be broadly representative of the genome as a whole.

It is interesting that both meiotic recombination and MR events are more common in females than in males. Since MR presumably occurs in diploid germ cells that undergo several hundred cell divisions by the end of male fertility, it may be that accumulated LOH after MR is a contributing factor to the reduction of fertility with advancing age and that selection might weakly favor males with a reduced frequency of MR. There is no commonly accepted explanation for the observed sex imbalance in meiotic recombination. However, if there is conservation of genetic mechanisms controlling both meiotic recombination and MR, then the benefit that suppression of MR has in the production of male gametes may explain the excess of female meiotic recombination.

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