CORRESPONDENCE

Decreased Stability of the O Allele mRNA Transcript of the ABO Gene

To the Editor:

We previously reported a method for genotyping the major alleles of the ABO blood group locus.[1] We subsequently developed a reverse transcription-polymerase chain reaction (RT-PCR) method to assess the relative expression of the alleles of the ABO gene. Unexpectedly, we observed markedly reduced expression of the O allele in normal AO or BO genotype individuals.

Mononuclear cells from seven adult bone marrows and 15 fetal umbilical cord bloods were examined for ABO expression. RNA was isolated, reverse-transcribed with random hexamers and amplified by PCR. The primers used amplify nucleotides 12 through 329 of the ABO cDNA sequence[2,3] spanning the site at position

261 where the nonfunctional O allele has a single base deletion.[4] The deletion creates a *Kpn*I restriction endonuclease site in the O allele, where there is a *Bst*EII site present in the A and B alleles. Ten microliter aliquots of the RT-PCR product were digested with 10 units of *Kpn*I or *Bst*EII and resolved on 8% polyacrylamide gels (Fig 1).

Digestion of the 316bp O allele RT-PCR product with *Kpn*I results in a band of 249bp, whereas no digestion of the product occurs with *Bst*EII. Likewise, digestion of the A or B alleles with *Bst*EII results in a 249bp product, and there is no digestion with *Kpn*I. The five OO genotype individuals all expressed the O allele and the two AA genotype individuals both expressed the A allele. However, when the ten AO and five BO genotype individuals were examined, the amount of the O allele was considerably diminished relative to that of the A or B allele (see lanes 3 through 8, Fig 1). Thus, although the O allele is expressed in OO genotype individuals, the majority of the steady-state mRNA of the ABO gene is comprised of the A or B allele in individuals heterozygous for the O allele. Similar patterns of expression were also seen for the hematopoietic and colon carcinoma cell lines; KCl22, LIM2412 (genotype AO), LIM1215 (BO), and K562, HEL, and LIM 2405 (OO).

RT-PCR products from AO and BO heterozygotes digested with *Bst*EII gave a residual undigested fraction whereas AA RT-PCR products digested completely. This result was repeatable, and consistent with the presence of a small amount of O allele. This result and the apparent absence of a digested band corresponding to the O allele in some *Kpn*I digests could be explained by the formation of heteroduplexes. The small amount of PCR product corresponding to the O transcript would almost entirely be found in heteroduplexes. These heteroduplexes would be resistant to digestion with *Kpn*I and would remain in the undigested band corresponding to A or B.

The markedly reduced steady-state O allele mRNA level in heterozygotes may be explained by lower stability of the O transcript. The O allele has a single base deletion that leads to a premature stop codon at nucleotide 352, and subsequent early termination of translation.[4] Apparent instability of mRNA transcripts with premature stop codons has been documented for a number of genes in a wide range of species. The mechanisms behind this phenomenon are not completely understood.[5,6,7] The decreased levels are not due to differential transcription as mutant and normal alleles are transcribed at the same rate, and the loss of the transcript with the premature stop codon has been shown to take place after splicing.[6,7] Interestingly, reduced levels of the O allele were also seen by us in a OO_2 heterozygote (data not shown). The transcript of the O_2 allele has no premature stop codon[8] and thus would be expected to have similar stability to the A and B alleles.

The results seen here for the ABO gene strikingly resemble those reported for the retinoblastoma tumour suppressor gene (RB1).[5] Whereas the normal RB1 allele was expressed in the lymphocytes of patients carrying germline mutations resulting in premature stop codons, the expression of mutant RB1 alleles was not detected. However, in tumors where the normal allele was physically lost, the mutant allele was easily detectable. The authors proposed that transcription of the mutant allele was increased to compensate for loss of the functional product.

Similarly, it seems that the stability of the O mRNA is reduced, and therefore it could be proposed that the amount of transcript, or functional gene product, either directly or indirectly regulates ABO transcription. In OO genotype individuals, transcription of the gene would be increased to compensate for the decreased stability of the O mRNA. This is consistent with Northern analysis which shows approximately equivalent amounts of ABO mRNA in cell lines derived from A, B, and O blood group individuals.[2] Further analysis of the transcriptional regulation of the ABO gene is required to understand the mechanisms underlying these observations.

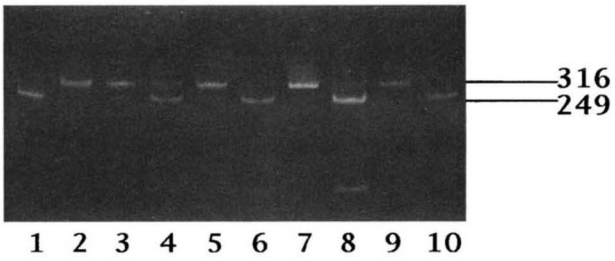

Fig 1. ABO RT-PCR product after diagnostic restriction enzyme digestion. cDNA from five individuals whose genotypes were OO (lanes 1, 2), AO (lanes 3, 4), AO (lanes 5, 6), BO (lanes 7, 8) and AA (lanes 9, 10) was amplified. RT-PCR products were digested with *Kpn*I (lanes 1, 3, 5, 7, 9), or *Bst*EII (lanes 2, 4, 6, 8, 10).

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