A High Sequence Variety in the Immunoglobulin C α Hinge Region among Old World Monkeys

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(Accepted March 13, 1998)

Abstract Immunoglobulin C α gene encodes a constant region of heavy-chain of IgA antibody, and the hinge region is located between CH1 domain and CH2 domain. The hinge region functions to increase efficiency of antigen binding by varying distance between two antigen recognition sites. The constant region of IgA has been considered to be conservative, but we previously reported that immunoglobulin C α genes of great apes showed evolutionary hypervariability mainly in the hinge region (Kawamura and others, 1990). To know whether this hypervariability is also the case among Old World monkeys, we carried out PCR-SSCP analysis for macaque, baboon, and leaf monkey. The result clearly showed evolutionary hypervariability in the hinge region among Old World monkeys. Adding to this inter-species variability, intra-species variabilities were also observed in multiple species such as rhesus macaque, crab-eating macaque, Assamese macaque, and hamadryas baboon. Furthermore, polymorphism was likely shared by multiple macaque species (trans-species polymorphism). These results suggest the indispensability of hypervariability in the hinge region for these animals. Some kind of positive selection, like the overdominance at the MHC loci (Klein and others, 1993) might be responsible for this hypervariability.

Keywords: IgA, C α , hinge, hypervariability, Old World monkey

Introduction

The immunoglobulin C α gene encodes the heavy-chain constant region of the immunoglobulin IgA molecule which consists of three CH domains and a hinge region. The hinge region links Fab including antigen-recognition sites with Fc including effector sites, and it can vary the distance between two antigen-recognition sites with certain flexibility. The C α gene has three exons encoding CH1, CH2 and CH3 domains, and the hinge region is encoded at the beginning of second exon. Hominoids have two C α genes in their genomes except for orangutan, whereas Old World monkeys have one C α gene

(Kawamura and others, 1990 and references therein). Sequence comparison of hominoid C α genes revealed the hypervariability of the hinge region that might be caused by frequent remodeling associated with reiterated structure (Kawamura and others, 1990). Hypervariability is not only in the variable region of immunoglobulin, but also in the constant region, that has been considered to be conservative. If hypervariability in the hinge confers to better function of the IgA hinge, primates other than hominoids also expected to have sequence hypervariability at the hinge region of IgA. We thus carried out PCR-SSCP analysis to see the evolutionary variability of IgA in Old World monkeys, including macaques (rhesus, crab-eating, Taiwan, stump-tailed, pig-tailed, Assamese, Barbary, and Tibetan), hamadryas baboon, leaf monkeys (silvered, banded, white-thighed, and Javan), and black-and-white colobus.

Methods

The hinge and its flanking regions of immunoglobulin C α gene including CH2 exon and first intron were amplified by PCR. Total genomic DNAs for PCR templates were prepared from peripheral blood by standard procedure (Sambrook and others, 1989). Primers used for PCR were: OL381 (5'-TCT GAC CAG TTC AGG CCA TCT C-3') and OL382 (5'-GGC CGG CGC AGC GAC AGT-3'). These primers based on crab-eating macaque genomic sequence (Kawamura and others, 1992; DDBJ/EMBL/Genbank database accession number X53705) to amplify the hinge region with minimum flanking region. OL381 primer was endlabeled with ^{32}P and then used for PCR-SSCP (single strand conformation polymorphism) analysis. PCR cycle was 30 to 40 cycles of: 95°C 1min, 55°C 30sec and 72°C 2min with a preheat step of 95°C 10min. Other conditions were following manufacturer's instruction (AmpliTaq Gold, PE Applied Biosystems). Electrophoresis was performed with denaturing gel (8% acrylamide, 7M urea) for SSCP (single strand length polymorphism) analysis. The SSCP analysis was performed by using non-denaturing gel with 5% acrylamide, 5% glycerol, 0.5 $\times$ TBE buffer at 16°C constant temperature.

Tested Old World monkey species were: Barbary macaque (*Macaca sylvanus*), Tibetan macaque (*Macaca thibetana*), stump-tailed macaque (*Macaca arctoides*), rhesus macaque (*Macaca mulatta*), pig-tailed macaque (*Macaca nemestrina*), Taiwan (Formosan) macaque (*Macaca cyclopis*), Assamese macaque (*Macaca assamensis*), crab-eating macaque (*Macaca fascicularis*), hamadryas baboon (*Papio hamadryas*), black-and-white colobus (*Colobus polykomos*), silvered leaf monkey (*Presbytis cristata*), banded leaf monkey (*Presbytis melalophos*), white-thighed leaf monkey (*Presbytis femolaris*), and Javan leaf monkey (*Presbytis comata*). These species name basically follow the Napier and Napier (1985).

Results

Figure 1 shows the genomic organization of the immunoglobulin C α hinge region of crab-eating macaque and PCR strategy for SSCP analysis. PCR reaction using OL381 and OL382 gave products from all tested Old World monkeys. We analyzed PCR products by

Figure 1 Schematic diagram of C α gene of crab-eating macaque. Rectangles indicate exons corresponding to each CH domains. The hinge region is located at the beginning of the second exon. PCR reaction was done by using OL381 (located at the first intron: asterisk indicates end-labeling) and OL382 (located at the second exon) primers set which amplifies around 90 bp product, including the complete hinge region. PCR products were then analyzed by SSCP.

Figure 2 PCR results on SSLP (a) and SSCP (b) among various kinds of macaques. PCR fragment size was indicated at the left panel. All bands in SSLP gel appear as doubled bands, probably caused by Taq polymerase nature (one nucleotide addition). Lanes 1 to 3: rhesus macaque, lanes 4 to 7: crab-eating macaque, lanes 8 to 10: Taiwan macaque, lanes 11 to 14: stump-tailed macaque, lanes 15 to 17: pig-tailed macaque, lanes 18 to 20: Assamese macaque, lane 21: Barbary macaque, and lane 22: Tibetan macaque. Each individual has two alleles at maximum, that is consistent with previous report that Old World monkeys have one C α gene in its genome (Kawamura and others, 1990).

using SSLP and SSCP method. Figure 2 is the result of various macaque species, which shows inter-specific and intra-specific variations among them. Fig. 2a shows size variations of PCR products. In rhesus macaque lanes, two size variants (93 and 87 base; corresponding to 27 and 21 base in the hinge) can be observed. In crab-eating macaque lanes, two size variants are observed that are similar to those of rhesus macaque. Because lane 4 has two bands, this individual is heterozygous. As well as rhesus and crab-eating macaque, Taiwan, pig-tailed, Assamese, and Barbary macaque also have two size variants, and several individuals are heterozygous. SSCP results (Fig. 2b) show further sequence variation in these macaque species. For example, lanes 1 and 2 are same size but show different mobility in SSCP gel. This indicates these two individuals have different allele, and they are homozygous each. Other macaques also show polymorphism within species. All four lanes of crab-eating macaque show different bands. Stump-tailed macaque has no polymorphism in terms of size variation, but has sequence variations (see Fig. 2b). Adding to these observation, some alleles are shared by several macaque species. For example, lanes 3, 4, 9, 10, 15, 17, 18, 20, and 21 having 93 base bands show identical mobility in SSCP gel. Polymorphism is thus shared by these macaques. On the other hand, species-specific alleles also exist, for example, lanes 11 and 13.

We did further analysis of each macaque species to know the extent of intra-specific sequence variation. Figure 3 shows the intra-specific variation in crab-eating macaque. These samples are randomly collected from various populations. They are unusually polymorphic, more than half of individuals are heterozygous. Judging from mobility difference in SSCP gel, there are at least 8 alleles in this panel, most of them are 87 base allele. Figure 4 is the result of rhesus macaque, also showing a high sequence variation within spe-

Figure 3 PCR-SSLP (a) and SSCP (b) results of crab-eating macaque. Samples of lanes 1, 2, and 3 are identical to those of lanes 4, 5, and 6 of Fig. 2, respectively.

Figure 4 PCR-SSLP (a) and SSCP (b) results of rhesus macaque. Samples of lanes 1, 6, and 7 are identical sample to those of lanes 1, 2, and 3 of Fig. 2, respectively. Lanes 10 and 11 are from same individuals of lanes 3 and 7, respectively, but collected at different times.

Figure 5 PCR-SSLP (a) and SSCP (b) results of Assamese macaque. These samples are different individuals from those of Fig. 2. Samples of lanes 8 to 13 are from a natural population (Thai Kanchana buri). Others are from different populations.

cies, at least 7 alleles can be identified here. Adding to 93 and 87 base allele, lane 8 has another unique allele that is 9 base longer than 93 base allele. Figure 5 is the result of Asamese macaque, that also shows a high extent polymorphism. Lanes 8 to 13 are individuals from a natural population whereas others are random sample from various populations. In any case, they are polymorphic and there are at least 5 alleles. Hamadryas baboons are polymorphic as well as macaque species, at least 3 different alleles can be observed (Fig. 6). Individuals of lanes 3, 4, 5, 7, and 8 are offsprings of that of lane 1, and those of lanes 6 and 8 are offsprings of that of lane 2. Their genetic patterns follow Mendelian hereditance, and that suggests there was no somatic rearrangement event.

Figure 6 PCR-SSLP (a) and SSCP (b) results of hamadryas baboon. Most of them are heterozygous, only lane 6 is homozygous.

Within Old World monkeys, leaf monkeys are rather different from macaques and baboons in terms of genetic distance and ecological behavior. We analyzed four Asian leaf monkeys and one African colobus species. They have also evolutionary hypervariability and intra-species variations (Fig. 7). Multiple silvered leaf monkeys obtained from one population are available (lanes 1 to 4), and they have 2 variants (Fig. 7b). Banded leaf monkeys (lanes 7, 8 and 10) also show polymorphism, and allele in lane 8 is quite similar to those of silvered leaf monkeys. Black-and white colobus have at least three different size alleles, and two of them gave very weak bands, possibly because PCR primers designed for crab-eating macaques did not work effectively. Although same size bands (93bp) appeared in both leaf monkeys and crab-eating macaques (Fig. 7a), they are quite

different at the sequence level (Fig. 7b).

Figure 7 PCR-SSLP (a) and SSCP (b) results of leaf monkeys and colobus. Crab-eating macaque samples (lanes 13 to 15: identical to lanes 4 to 6 of Fig. 2) are located side by side for comparison. Lanes 1 to 4: silvered leaf monkey, lanes 5 and 6: white-thighed leaf monkey, lanes 7, 8, and 10: banded leaf monkey, lane 9: Javan leaf monkey, lanes 11 and 12: black-and white colobus. Note that sometimes shadow appeared on gel, such as one indicated by an arrow in Fig. 7a. This band does not correspond to an allele, but it might be caused by some conformational problem and is easily distinguishable from normal bands.

Discussion

We carried out PCR-SSCP analysis and found both evolutionary hypervariability and high extent of polymorphism in the immunoglobulin C α hinge region of Old World monkeys. This hypervariability can be observed in different species, although they have evolved under different environments. This hypervariability is not created by somatic mutation, because alleles are accurately transmitted from parents to offsprings (Fig. 6). It is likely that this evolutionary hypervariability of inter-and intra-species is caused by some kind of positive selection. The immunoglobulin gene shows shared polymorphism among multiple macaque species. MHC has just similar characteristics, and that is caused by over-dominance selection (Klein and others, 1993). The reason for unusual IgA polymorphism

is not apparent so far, but two explanations are possible. One is that increasing variety at the hinge region is advantageous for further antigen recognition, and another is that increasing variety at the hinge region is necessary for maintaining IgA activity. The latter explanation is more likely, because there are many class of IgA protease produced by pathogenic bacteria (Plaut and others, 1975; Kilian and others, 1979; Male, 1979). Furthermore, two conflicting reports for enzymatic activity on IgA of rhesus macaque (Proctor and Manning, 1990; Reinholdt and Kilian, 1991), are consistent with our findings. Taking these results together, we conclude that the hinge region of IgA is evolutionarily hypervariable among Old World monkeys, and that might be a consequence of some kind of positive selection.

Acknowledgments

This study was supported by Grants-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and the Cooperation Research Program of Primate Research Institute, Kyoto University. We thank Dr. T. Ishida for providing samples of crab-eating macaques.

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