Immunogenetics (2002) 53:907–913
DOI 10.1007/s00251-001-0412-x

ORIGINAL PAPER

**Massimiliano Del Pero · Michele Boniotto
Dario Zuccon · Piero Cervella · Andrea Spanò
Antonio Amoroso · Sergio Crovella**

β-Defensin 1 gene variability among non-human primates

Received: 11 October 2001 / Revised: 26 November 2001 / Published online: 24 January 2002

Abstract Defensins are a recently described family of peptides that play an important role in innate immunity. Recent studies have shown that defensins exhibit a broad spectrum of antimicrobial activities against bacteria and fungi. Three families have been identified so far in mammals, α-defensins, β-defensins and θ-defensins, presumably derived from a common ancestral defensin. A long-term study on the evolution of these multigene families among primates has been undertaken to investigate: (1) the degree of interspecific differentiation; (2) the genetic mechanisms responsible for the variability of these molecules; and (3) the possible role of different environmental factors in their evolution. Nucleotide sequences have been obtained from great and lesser apes, several African and Asian catarrhine monkeys and one New World monkey. A comparison of rates of synonymous and nonsynonymous (amino-acid changing) nucleotide substitution indicates that the primate β-defensin 1 gene evolved under a pattern of random nucleotide substitution as predicted by the neutral theory of molecular evolution. These results are not consistent with the hypothesis that the primate β-defensin 1 gene has diversified in response to changes in the microbial species to which a given host is exposed. Analyses of interpecific variability have yielded some insights about the pattern of molecular evolution of the gene among primates. Humans and great apes present high levels of sequence similarity, differing in only one amino acid residue in the mature peptide. Compared with these taxa, hylobatids and cercopithecids exhibit 3–4 amino acid substitutions, some of which increase the net charge of the active molecule.

Keywords Defensins · Molecular evolution · Molecular adaptation · Innate immune system · Antimicrobial peptide

Nucleotide sequence data reported are available in the GenBank database. Sequences are identified by the following accession numbers: *Pan troglodytes* (AY033735–AY033749), *Gorilla gorilla* (AY033734–AY033750), *Pongo pygmaeus* (AY033736–AY033751), *Hylobates concolor* (AY033737–AY033752), *H. lar* (AY033738–AY033753), *H. moloch* (AY033739–AY033754)), *Presbytis cristata* (AY033740–AY033755), *P. obscurus* (AY033741–AY033756), *P. melalophos* (AY033742–AY033757), *Cercopithecus aethiops* (AY033744–AY033758), *C. preussi* (AY033743–AY033759), *C. erythrogaster* (AY033745–AY033760), *Papio anubis* (AY033747–AY033761), *Macaca fascicularis* (AY033746–AY033762), *M. mulatta* (AF014016), and *Saguinus oedipus* (AY033748–AY033763)

M. Del Pero · D. Zuccon · P. Cervella
Dipartimento di Biologia Animale e dell'Uomo,
Università di Torino, Via Accademia Albertina 13/17,
10123 Torino, Italy

M. Boniotto · A. Spanò · A. Amoroso · S. Crovella (✉)
Dipartimento di Scienze della Riproduzione e dello Sviluppo,
Ospedale infantile "Burlo Garofolo", Università di Trieste,
Via dell'Istria 65/1, 34137 Trieste, Italy
e-mail: crovella@burlo.trieste.it
Tel.: +39-040-3785538, Fax: +39-040-3785540

Introduction

The appearance of bacterial strains resistant to antibiotics has renewed scientific interest in the study of the antimicrobial peptides of the innate immune system. The notion of innate immunity refers to the host response occurring directly following microbial invasion. The process relies on the innate ability of the host organism to discriminate self from infectious nonself by means of a broad spectrum of recognition molecules directed against microbial molecules (Medzhitov and Janeway 1997). A further feature of the innate immune system in mammals appears to be its role in stimulating the subsequent, clonal response of adaptive immunity. During the last two decades, inducible antimicrobial peptides have been described in all multicellular organisms investigated (Hoffmann et al. 1999), yielding more than 400 peptides in total. Defensins form a major group of small (M_r 3,500–4,500), protease-resistant, cationic antimicrobial peptides identified so far in mammals, insects and plants (Broekaert et al. 1995; Ganz and Lehrer 1994; Stolzenberg et al. 1997). According to the spatial position of six conserved cysteine residues, the disulfide-bonding pat-

tern, the structure of the precursor, and the mode and region of expression, two main categories of defensins have been distinguished, referred to as α- and β-defensins. α-Defensins have been isolated in neutrophils and intestinal Paneth cells, while β-defensins are expressed in many epithelial tissues (Harder et al. 1997a; Stolzenberg et al. 1997; Zhao et al. 1996). β-Defensins show a broad spectrum of antimicrobial activities directed against several bacteria, fungi, and enveloped viruses. These molecules, like most other antimicrobial peptides, form channel-like structures in lipid bilayers, thus altering the cell membrane permeability of the microorganism (Gazit et al. 1996; Kagan et al. 1990; Matsuzaki 1998).

Impaired function in β-defensins has been shown to contribute to the pathophysiology of recurrent airway infections in patients affected by cystic fibrosis (Goldman et al. 1997). Recent studies also reported a key role for β-defensins in attracting both immature dendritic cells and memory T cells, and thus stimulating the clonal response of the adaptive (antibody- and T-cell-mediated) immune system (Yang et al. 1999).

In humans, four types of β-defensins have been described so far: β-defensin 1 (HBD-1), which is constitutively expressed in kidney tubules and at lower levels in the pancreas, lungs and genitourinary tract (Bensch et al. 1995; Diamond et al. 1996; Goldman et al. 1997; Schonwetter et al. 1995; Zhao et al. 1996); β-defensin 2 (HBD-2), the expression of which is primarily induced in skin and other epithelia during inflammation (Harder et al. 1997b; Hiratsuka et al. 1998; Liu et al. 1998; Mathews et al. 1999); β-defensin 3 (HBD-3), which is secreted by keratinocytes and epithelial cells of the respiratory tract after inflammatory stimuli or after contact with gram-negative or gram-positive bacteria (Harder et al. 2001); and β-defensin 4 (HBD-4) which is expressed in testis, stomach, uterus, neutrophils, thyroid, lung and kidney (Garcia et al. 2001).

β-Defensin 1 is probably the best described molecule of its family among vertebrates. The mature HBD-1 is a peptide of 36 amino acids that is cleaved from a primary translation product containing a signal peptide (20 amino acids), a propiece (12 amino acids), and the mature peptide (Bensch et al. 1995). Human β-defensin 1 gene consists of two relatively small exons separated by a large intron of 6,962 bp. The first exon, 128-bp long, encodes the signal sequence. The second 234-bp exon encodes the propiece and the mature peptide (Liu et al. 1997). Otherwise, human β-defensin genes have three exons and a longer propiece, the charge of which is typically balanced with the mature domain.

The β-defensin gene family has been mapped in close chromosomal proximity to the gene cluster for the α-defensins in both human and mouse (Liu et al. 1997; Morrison et al. 1998). Thus, despite a lack of DNA sequence similarity between α and β defensin genes, the physical linkage of these two gene families is strong evidence for a common evolutionary origin. Moreover, the degree of sequence similarity between mammalian and avian (chicken and turkey) β-defensin genes (Brockus et al. 1998; Harwig et al. 1994) suggests that the ancestral gene duplication, from which the α- and β-defensin families originated, occurred before the separation of the mammalian and avian lineages (Liu et al. 1997).

The evolutionary mechanisms underlying the diversification of mammalian defensins at the amino acid level are still unknown. Some authors have suggested that defensin genes have diversified in a species-specific manner as a consequence of changes in the microbial community to which a host was exposed while adapting to a new niche (Hughes and Yeager 1997). In order to test this hypothesis we have studied the evolution of these multigene families among primates to investigate (1) the degree of interspecific differentiation; (2) the genetic mechanisms responsible for the variability of these molecules; and eventually, (3) the possible role of different environmental factors in their evolution. Here we report the sequences of PCR amplified products obtained from the β-defensin 1 coding regions of 16 primate species, and the deduced amino acid sequences of the respective precursor peptides.

Materials and methods

Both exons of the β-defensin 1 gene and short portions of their flanking untranslated and intronic regions have been investigated in 16 primate species for interspecific polymorphisms. The primate sample analyzed here was composed of three species of great apes (*Pan troglodytes*, *Gorilla gorilla*, and *Pongo pygmaeus*), three species from the family Hylobatidae (*Hylobates concolor*, *H. lar*, and *H. moloch*), eight species of Cercopithecidae (*Macaca fascicularis*, *Papio anubis*, *Cercopithecus aethiops*, *C. erythrogaster*, *C. preussi*, *Presbytis cristata*, *P. obscurus*, and *P. melalophos*) and one platyrrhine species (*Saguinus oedipus*). Hairs from *Pan troglodytes*, *Gorilla gorilla* and *Pongo pygmaeus* were collected at the Zoo Negara Malaysia (Kuala Lumpur); muscle and bone samples were collected from deceased specimens of *H. concolor*, *H. lar*, *H. moloch*, *P. cristata*, *P. obscurus*, *P. melalophos*, *M. fascicularis*, *M. mulatta* and *Papio anubis*, while liver samples of *C. aethiops*, *C. erythrogaster* and *C. preussi* were collected at the Mulhouse Zoo. Genomic DNAs were extracted from liver, bone and muscle tissues using the phenol/chloroform protocol (Sambrook et al. 1989). DNA was extracted from hairs using Chelex 100 extraction (Walsh et al. 1991). Genomic DNA amplification of HBD-1 homologs was performed using the primers designed on the basis of the published human sequence. For the first exon, the primer sequences were, respectively, forward 5′-TGGAAGCCTCTGTCAGCTCA-3′ and reverse 5′-TGCTTGTTCCTCGTCCCTTG-3′; for the second exon, the forward primer was 5′-AAGCCATGAGTCTGAAGT-3′ and the reverse primer was 5′-TTTGTGGTTCGACCTGTC-3′. For the first exon 45 cycles of PCR were carried out in a Thermal cycler 2400 (Applera Genomics) using 1 U of Taq Gold (Applera Genomics) and an annealing temperature of 55°C. For the second exon, the annealing temperature was 50°C. After 45 cycles of the PCR for *Presbytis cristata*, *P. obscurus*, *P. melalophos*, *Macaca fascicularis*, *M. mulatta*, *Papio anubis*, *Cercopithecus aethiops*, *C. erythrogaster*, *C. preussi* no PCR products were observed in a 3% agarose gel, stained with ethidium bromide. In order to obtain the amplicons for these species, a nested-PCR was performed. Briefly, 1 µl of the first PCR product was re-amplified using the primers and the conditions previously described by Dörk and Stuhrmann (1998). These primers were internal to the primers previously mentioned. DNA sequencing of PCR products was executed according to the modified Sanger dideoxy method. The samples

were purified using exonuclease I and shrimp alkaline phosphatase (pre-sequencing kit Amersham Life Science) to remove residual single strand primers and excess dNTPs from the PCR mixture which could interfere with the sequencing reaction. A thermal cycle reaction was carried out for both strands of all exons with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applera Genomics), using the primers described by Dork and Stuhrmann (1998). DNA sequences were detected and analyzed on an automated ABI Prism 310 Genetic Analyzer (Applera Genomics). Multiple alignments of the nucleotide and amino acid sequences were performed using the program CLUSTAL X (Aiyar 2000). Pairwise comparisons between nucleotide sequences were performed to investigate the extent of evolutionary constraint at the amino acid level in different regions of the molecule. The number of synonymous nucleotide substitutions per synonymous site (dS) and the number of nonsynonymous nucleotide substitutions per nonsynonymous site (dN) between orthologous sequences were computed following the method of Nei and Gojobori (1986). The neutral theory of molecular evolution (Kimura 1977) predicts that in most functional genes dS>dN as a consequence of the fact that synonymous positions are subject to far fewer functional constraints than nonsynonymous sites. On the other hand, if dN>dS then it is assumed to be evidence of positive Darwinian selection acting to promote diversity at the amino acid level (Hughes and Nei 1988). Finally, we investigated the variation in residual charge of the propiece and mature portions of the precursor among the different primate β-defensin 1 sequences. Amino acid residues were classified as positive (H, K, R), negative (D, E), or neutral (all others).

Results

Sequences obtained from the primate samples are reported in Fig. 1. Anthropoid β-defensin 1 sequences demonstrate considerable nucleotide similarity to the human coding sequence. Overall comparisons of exons 1 and 2 show a very low degree of nucleotide divergence both at the generic and family level, ranging from complete identity (among *Presbytis* species) to 2% divergence (among Cercopithecidae and great apes, respectively). When sequences from different families are compared, these values rise to about 5–6%, in the comparison of great and lesser apes, and 6–7.4% between great apes and cercopithecid species. Notably, hylobatid sequences were more similar to cercopithecids than to great apes and humans. As would be expected on the basis of phylogenetic relationships, the β-defensin 1 sequence obtained from the New World monkey was the most divergent (8–10.3%).

In similarity to HBD-1, the deduced 68 amino acid sequence from the primate β-defensin 1 open reading frames consists of a putative 20 amino acid signal sequence, a 12 residue propiece, and a mature peptide of 38 amino acids with the characteristic spacing pattern of six cysteines and at least five cationic residues (Fig. 2). Compared with HBD-1, the β-defensin 1 of chimpanzee, gorilla, hylobatids and the platyrrhine monkey all lack a positive residue in the mature peptide at position 61, while all cercopithecids have histidine instead of arginine at that position. Humans and great apes show a serine residue at position 39, while gibbons and cercopithecids present an arginine and *Saguinus* a lysine residue, both of which increase the positive charge of the mature peptide. The latter species also share the substitution of a neutral amino acid with a negative residue (Gly22/Asp22) in the propiece. Cercopithecids present the highest net positive charge (+6) in the mature peptide. In contrast, the chimpanzee and gorilla possess only four positively charged amino acids.

Table 1 shows mean values of synonymous and nonsynonymous nucleotide substitutions for comparisons among and between primate families. Sequences of the β-defensin 1 orthologous genes have been subdivided into the three regions in order to evaluate the selective pressures acting on different domains of the molecule. All comparisons show a high dS/dN ratio, suggesting that primate β-defensin 1 largely differentiated under purifying selection. Nevertheless, substitution rates in the different regions of the gene, observed between distantly related species, reveal a decrease in the dS to dN ratio, with an increase of nonsynonymous substitutions within the region encoding the mature portion of the peptide. These results may indicate that slightly different degrees of selection acted on different portions of the molecule during primate evolution.

Finally, it should be mentioned that, using the primers based on human and higher primate β-defensin 1 gene sequences, no amplified product from several distantly related prosimian species (*Microcebus murinus*, *Eulemur fulvus*, *Galago crassicaudatus*, *Nycticebus coucang*) was detected. We assume that prosimians also possess this

Table 1 Mean values of synonymous (*Ks*) and nonsynonymous (*Ka*) nucleotide substitutions obtained from comparisons of ß-defensin 1 sequences among and between primate families

	Signal sequence		Propiece		Mature peptide	
	Ks	Ka	Ks	Ka	Ks	Ka
Among primate families						
Human and pongids	0.000±0.000	0.012±0.000	0.055±0.000	0.000±0.000	0.043±0.000	0.008±0.000
Hylobatids	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.057±0.000	0.000±0.000
Cercopithecids	0.038±0.034	0.011±0.011	0.000±0.000	0.000±0.000	0.032±0.027	0.009±0.007
Between primate families						
Human and pongids vs hylobatids	0.000±0.000	0.006±0.011	0.085±0.051	0.039±0.000	0.235±0.046	0.046±0.003
Human and pongids vs cercopithecids	0.107±0.038	0.013±0.015	0.214±0.059	0.039±0.000	0.176±0.037	0.062±0.007
Hylobatidae vs cercopithecids	0.108±0.039	0.008±0.011	0.118±0.000	0.000±0.000	0.085±0.034	0.018±0.006
Saguinus vs all others	0.140±0.075	0.127±0.011	0.212±0.059	0.049±0.019	0.167±0.038	0.070±0.007

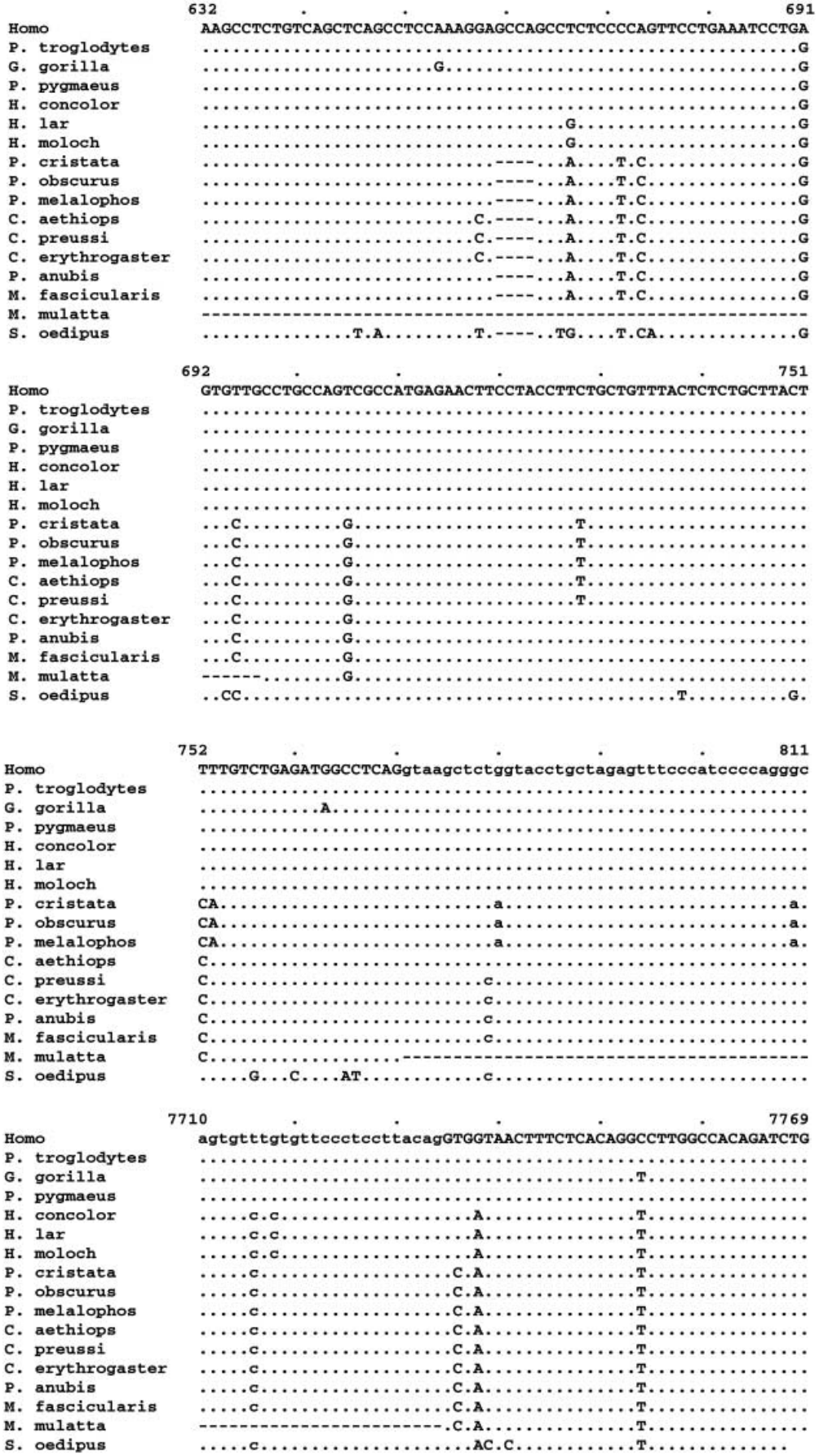


Fig. 1. Alignment of nucleotide sequences from exon 1 (positions 632–772 referred to the human sequence) and exon 2 (positions 7735–7889) of the β-defensin 1 gene. Partial intron sequences are shown in *small letters*. *Dots* indicate nucleotide identity to the human sequences by Liu et al. (1997) (GenBank accession numbers: U50930–U50931)

Fig. 1 (continued)

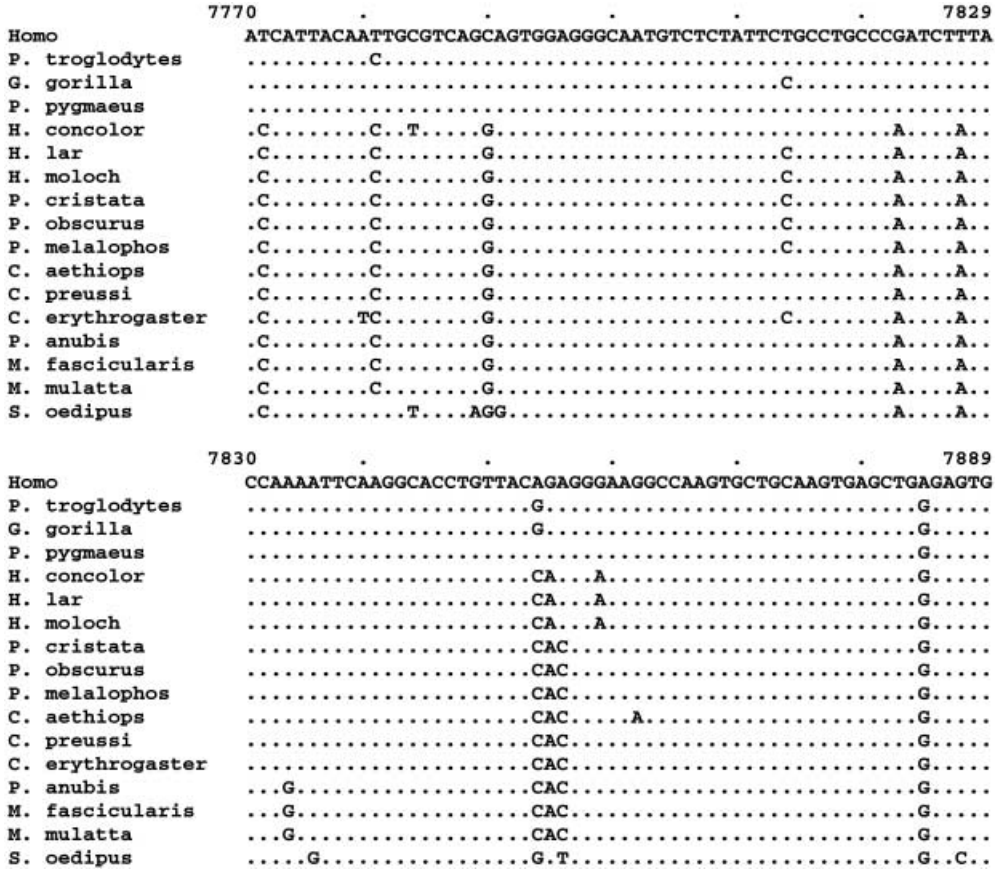
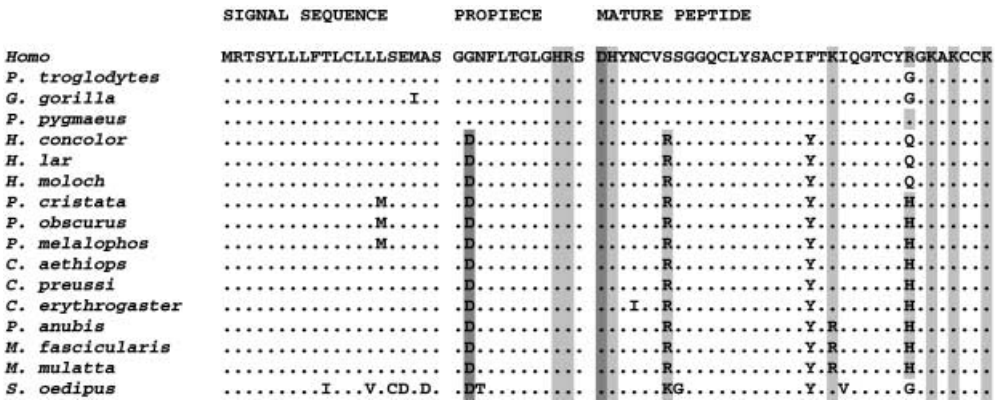


Fig. 2 Alignment of primate β-defensin 1 deduced amino acid sequences. *Dots* indicate amino acid identity to the human sequence. Positively (*light shading*) and negatively charged (*dark shading*) residues are shown



gene, but that the primer sequences we used are not conserved in prosimians.

Discussion

Comparative studies are emerging as a crucial aspect of the description of the evolutionary pattern and biology of the innate immune system. Nucleotide and amino acid sequence variation between diverse species, together with differences in tissue expression, antimicrobial activity, and relationships with other elements of the immune system, are providing insights into the biology of the innate response to microbial infection.

In this study, the variability of the β-defensin 1 gene in different primate species was investigated. In a manner similar to previously described mammalian β-defensin 1 genes (Huttner et al. 1997; Liu et al. 1997; Morrison et al. 1998), strongly conserved sequences were found among species of anthropoid primates. These results are not consistent with findings from α-defensins and other antimicrobial gene families, where interspecific variation suggested markedly different evolutionary patterns (Boman 1995; Ganz and Lehrer 1994; Hughes and Yeager 1997). Hughes and Yeager (1997) also proposed that classic defensins evolved in a species-specific manner as a response to changes in the microbial communities encountered by mammalian species during the process of adaptation to new niches.

Overall variability derived from sequence comparisons among species belonging to the same primate family show zero to three amino acid substitutions. When comparing species classified in different primate families, the hylobatids, despite sharing the same phylogen-etic lineage as humans and pongids, surprisingly show a β-defensin 1 peptide more similar to that of Old World monkeys.

All comparisons of the region encoding the mature peptide indicate that the rate of synonymous nucleotide substitution has substantially exceeded the nonsynonymous rate. These results conform to the predictions generated by the neutral theory of molecular evolution (Kimura 1977) and indicate that orthologous β-defensin 1 genes in primates have evolved without being subjected to strong pressures of directional selection, promoting a rapid diversification at the amino acid level after lineage separation.

However, most of the comparisons between families showed a consistent pattern in which dN is highest in the mature defensin, intermediate in the propiece, and lowest in the signal peptide. This trend indicates that the mature peptide is the least conserved region at the amino acid level, while the signal peptide is the most conserved. We suggest that the high rate of nonsynonymous substitution in the active region of the molecule can be accounted for if, in distantly related species, changes that could possibly affect the activity of the protein have been fixed more rapidly than in other portions of the molecule. Recently Hughes (1999) demonstrated a positive selection in β-defensin genes by comparing numerous β-defensins from Bovidae (bovine and sheep). Since we had information for only one locus (HBD-1), we were not able to hypothesize an adaptive diversification between paralogous β-defensins in primates.

Some of the substitutions observed also modify the net charge of the mature peptide. For the neutrophil storage granule defensins (α-defensins), it has been proposed that the net positive charge of the mature peptide is neutralized by a negatively charged propiece (Hughes and Yeager 1997; Michaelson et al. 1992). Inactivation of the mature peptide by the propiece has been confirmed in vitro (Valore et al. 1996) and the charge distribution in the molecule has been suggested to be an adaptation to prevent cytotoxic effects during post-translational processing. The propiece of classical defensins is also involved in peptide folding and in targeting of the mature peptide into the subcellular organelles (Liu and Ganz 1995). By contrast, β defensins are storage granule-free peptides synthesized de novo upon stimulation and possess a very short propiece without neutralizing properties (Brockus et al. 1998; Russell et al. 1996; Schonwetter et al. 1995). Accordingly, β-defensin 1 in primates presents a positively charged mature peptide not balanced by a negative propiece. Humans and great apes show no negatively charged amino acids in the propiece, whereas hylobatids, cercopithecids and the New World monkey have one negative residue (Asp22). It is remarkable that the latter species show an increase of positive charge in the active portion of the peptide (Ser39/Arg39-Lys39). This charge distribution may thus represent a case of coordinated amino acid changes (Hughes and Yeager 1997), because the presence of one negative residue in the propiece is found in the species with the highest positively charged mature peptide.

The degree of cationic charge of defensins has been suggested as a feature correlated with antimicrobial potency (Selsted et al. 1984, 1985). However, more recent studies suggest that specific molecular features, conferred by single amino acid substitutions, may have enabled different peptides to interact selectively with one or more microbial targets (Aley et al. 1994; Ouellette et al. 1994; Sawyer et al. 1988; Tang et al. 1999).

In vitro comparisons between human and mouse β-defensin 1 (mBD-1) antimicrobial activities have shown that mBD-1 is required at a much higher concentration than HBD-1 (Morrison et al. 1998). The authors postulated that these molecules may have slightly different characteristics in vivo, in terms of the extent of their effects, the pathogens against which they act, and their dependence on other elements of the defense mechanism (Morrison et al. 1998). While our data indicate a close genetic relationship between orthologous primate β-defensin 1 peptides, further investigation focusing on gene regulatory mechanisms as well as structural properties of different peptides and their biological activity will be necessary to clarify the actual significance of the variation recorded between primate species. The comparative approach will contribute to a better knowledge of defense mechanisms in species closely related to humans and may have important implications for the development of new strategies in the treatment of infectious diseases.

Acknowledgements This work was partly supported by a COFIN 2001 (PRIN) project of MURST. M. Boniotto is recipient of fellowship from University of Trieste. The authors are also greatly indebted to Nicole Bolzicco for the collaboration in the preparation of this manuscript.

References

Aiyar A (2000) The use of CLUSTAL W and CLUSTAL X for multiple sequence alignment. Methods Mol Biol 132:221–241

Aley SB, Zimmerman M, Hetsko M, Selsted ME, Gillin FD (1994) Killing of Giardia lamblia by cryptdins and cationic neutrophil peptides. Infect Immun 62:5397–5403

Bensch KW, Raida M, Magert HJ, Schulz-Knappe P, Forssmann WG (1995) hBD-1: a novel beta-defensin from human plasma. FEBS Lett 368:331–335

Boman HG (1995) Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 13:61–92

Brockus CW, Jackwood MW, Harmon BG (1998) Characterization of beta-defensin prepropeptide mRNA from chicken and turkey bone marrow. Anim Genet 29:283–289

Broekaert WF, Terras FR, Cammue BP, Osborn RW (1995) Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 108:1353–1358

Diamond G, Russell JP, Bevins CL (1996) Inducible expression of an antibiotic peptide gene in lipopolysaccharide-challenged tracheal epithelial cells. Proc Natl Acad Sci USA 93:5156–5160

Dork T, Stuhrmann M (1998) Polymorphisms of the human beta-defensin-1 gene. Mol Cell Probes 12:171–173

Ganz T, Lehrer RI (1994) Defensins. Curr Opin Immunol 6: 584–589

Garcia JR, Krause A, Schulz S, Rodriguez-Jimenez FJ, Kluver E, Adermann K, Forssmann U, Frimpong-Boateng A, Bals R, Forssmann WG (2001) Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. FASEB J 15:1819–1821

Gazit E, Miller IR, Biggin PC, Sansom MS, Shai Y (1996) Structure and orientation of the mammalian antibacterial peptide cecropin P1 within phospholipid membranes. J Mol Biol 258:860–870

Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM (1997) Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 88:553–560

Harder J, Bartels J, Christophers E, Schroder JM (1997a) A peptide antibiotic from human skin. Nature 387:861

Harder J, Siebert R, Zhang Y, Matthiesen P, Christophers E, Schlegelberger B, Schroder JM (1997b) Mapping of the gene encoding human beta-defensin-2 (DEFB2) to chromosome region 8p22-p23.1. Genomics 46:472–475

Harder J, Bartels J, Christophers E, Schroder JM (2001) Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J Biol Chem 276:5707–5713

Harwig SS, Swiderek KM, Kokryakov VN, Tan L, Lee TD, Panyutich EA, Aleshina GM, Shamova OV, Lehrer RI (1994) Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. FEBS Lett 342:281–285

Hiratsuka T, Nakazato M, Date Y, Ashitani J, Minematsu T, Chino N, Matsukura S (1998) Identification of human beta-defensin-2 in respiratory tract and plasma and its increase in bacterial pneumonia. Biochem Biophys Res Commun 249:943–947

Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA (1999) Phylogenetic perspectives in innate immunity. Science 284: 1313–1318

Hughes AL (1999) Evolutionary diversification of the mammalian defensins. Cell Mol Life Sci 56:94–103

Hughes AL, Nei M (1988) Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 335:167–170

Hughes AL, Yeager M (1997) Coordinated amino acid changes in the evolution of mammalian defensins. J Mol Evol 44:675–682

Huttner KM, Kozak CA, Bevins CL (1997) The mouse genome encodes a single homolog of the antimicrobial peptide human beta-defensin 1. FEBS Lett 413:45–49

Kagan BL, Selsted ME, Ganz T, Lehrer RI (1990) Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc Natl Acad Sci U S A 87:210–214

Kimura M (1977) Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution. Nature 267:275–276

Liu L, Ganz T (1995) The pro region of human neutrophil defensin contains a motif that is essential for normal subcellular sorting. Blood 85:1095–1103

Liu L, Zhao C, Heng HH, Ganz T (1997) The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. Genomics 43:316–320

Liu L, Wang L, Jia HP, Zhao C, Heng HH, Schutte BC, McCray PBJr, Ganz T (1998) Structure and mapping of the human beta-defensin HBD-2 gene and its expression at sites of inflammation. Gene 222:237–244

Mathews M, Jia HP, Guthmiller JM, Losh G, Graham S, Johnson GK, Tack BF, McCray PB Jr (1999) Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect Immun 67:2740–2745

Matsuzaki K (1998) Magainins as paradigm for the mode of action of pore forming polypeptides. Biochim Biophys Acta 1376: 391–400

Medzhitov R, Janeway CJ (1997) Innate immunity: the virtues of a nonclonal system of recognition. Cell 91:295–298

Michaelson D, Rayner J, Couto M, Ganz T (1992) Cationic defensins arise from charge-neutralized propeptides: a mechanism for avoiding leukocyte autocytotoxicity? J Leukoc Biol 51: 634–639

Morrison GM, Davidson DJ, Kilanowski FM, Borthwick DW, Crook K, Maxwell AI, Govan JR, Dorin JR (1998) Mouse beta defensin-1 is a functional homolog of human beta defensin-1. Mamm Genome 9:453–457

Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 3:418–426

Ouellette AJ, Hsieh MM, Nosek MT, Cano-Gauci DF, Huttner KM, Buick RN, Selsted ME (1994) Mouse Paneth cell defensins: primary structures and antibacterial activities of numerous cryptdin isoforms. Infect Immun 62:5040–5047

Russell JP, Diamond G, Tarver AP, Scanlin TF, Bevins CL (1996) Coordinate induction of two antibiotic genes in tracheal epithelial cells exposed to the inflammatory mediators lipopolysaccharide and tumor necrosis factor alpha. Infect Immun 64:1565–1568

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Sawyer JG, Martin NL, Hancock RE (1988) Interaction of macrophage cationic proteins with the outer membrane of Pseudomonas aeruginosa. Infect Immun 56:693–698

Schonwetter BS, Stolzenberg ED, Zasloff MA (1995) Epithelial antibiotics induced at sites of inflammation. Science 267: 1645–1648

Selsted ME, Szklarek D, Lehrer RI (1984) Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. Infect Immun 45:150–154

Selsted ME, Szklarek D, Ganz T, Lehrer RI (1985) Activity of rabbit leukocyte peptides against Candida albicans. Infect Immun 49:202–206

Stolzenberg ED, Anderson GM, Ackermann MR, Whitlock RH, Zasloff M (1997) Epithelial antibiotic induced in states of disease. Proc Natl Acad Sci U S A 94:8686–8690

Tang YQ, Yuan J, Miller CJ, Selsted ME (1999) Isolation, characterization, cDNA cloning, and antimicrobial properties of two distinct subfamilies of alpha-defensins from rhesus macaque leukocytes. Infect Immun 67:6139–6144

Valore EV, Martin E, Harwig SS, Ganz T (1996) Intramolecular inhibition of human defensin HNP-1 by its propiece. J Clin Invest 97:1624–1629

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, Oppenheim JJ (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286:525–528

Zhao C, Wang I, Lehrer RI (1996) Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett 369:319–322