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Molecular cloning of cDNAs encoding dog high-affinity IgE receptor α , β , and γ chains

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The high-affinity receptor for IgE (Fc ϵ RI), which is mainly expressed on mast cells and basophils, plays a crucial role in triggering an IgE-mediated allergic reaction. Fc ϵ RI is a tetrameric complex consisting of a single IgE-binding α subunit, a single β subunit, and two disulfide-linked γ subunits (Blank et al. 1989). Some human Fc ϵ RI, however, have been found to be composed of only α and γ subunits without an association with a β subunit (Maurer et al. 1994). Fc ϵ RI α belongs to the immunoglobulin (Ig) superfamily and serves as an IgE-binding subunit lacking signal-transducing ability. Fc ϵ RI β is a unique four-time transmembrane-spanning protein with both amino- and carboxy-terminal tails in its cytoplasm. Fc ϵ RI γ is a type I transmembrane protein with a short extracellular domain and is also a component of other receptor complexes, such as the Fc γ receptor and the T-cell antigen receptor (Paolini et al. 1995). The intracellular domains of both β and γ

subunits possess immunoreceptor tyrosine-based activation motifs (ITAM) that bind cytoplasmic protein tyrosine kinases and are involved in signal transduction (Jouvin et al. 1994). Not only because of their use in premedical trials of anti-allergic drugs but also because they naturally experience many allergic diseases, dogs have been considered a valuable animal model. In the present study we therefore cloned three subunits of dog Fc ϵ RI cDNAs in order to establish a molecular basis for the research on an IgE-mediated allergy in dogs.

We isolated full-length cDNA clones encoding dog Fc ϵ RI α and β subunits from a dog mastocytoma cell line (LuMC) cDNA library by using a polymerase chain reaction (PCR)-amplified partial dog Fc ϵ RI α cDNA fragment and a human Fc ϵ RI β probe, respectively. A coding region of dog Fc ϵ RI γ cDNA clone was also isolated by PCR using cDNA derived from a dog mesenteric lymph node mRNA as a template. The primer sequences were: 5'-T(A/G)AGAG(C/T)(A/G)AACCTGTGTAC(C/T)T-3' and 5'-CC(A/C/T)GT(C/G)TCCACAGCAAACAGAAT-3' for dog Fc ϵ RI α , and 5'-CCCA(A/G)GATGAT(C/T)(C/T)CAGC(A/C/G)GTG-3' and 5'-AAGCTA(C/T)TG(G/T)GGTGGTTT(C/T)TC-3' for Fc ϵ RI γ . PCR conditions were 30 cycles of 94°C for 1 min, 37°C for 2 min, and 72°C for 3 min, followed by a final extension at 72°C for 10 min.

The overall identity of the predicted amino acid sequence of a dog α subunit with mouse, rat, and human α subunits was 42.0%, 46.2%, and 54.4%, respectively (Fig. 1A). Although Fc ϵ RI α was the least conserved subunit of Fc ϵ RI between species, the extracellular region of dog Fc ϵ RI α also contained two characteristic Ig C2-domains, including four highly conserved cysteine residues that participate in the formation of two intra-chain disulfide bonds. The length of the cytoplasmic region diverged highly between species.

Like the β subunit in other species, the dog β subunit had no amino-terminal hydrophobic signal sequences but contained four hydrophobic segments sug-

The nucleotide sequence data reported in this paper have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers D16413, AB017552, and AB017553

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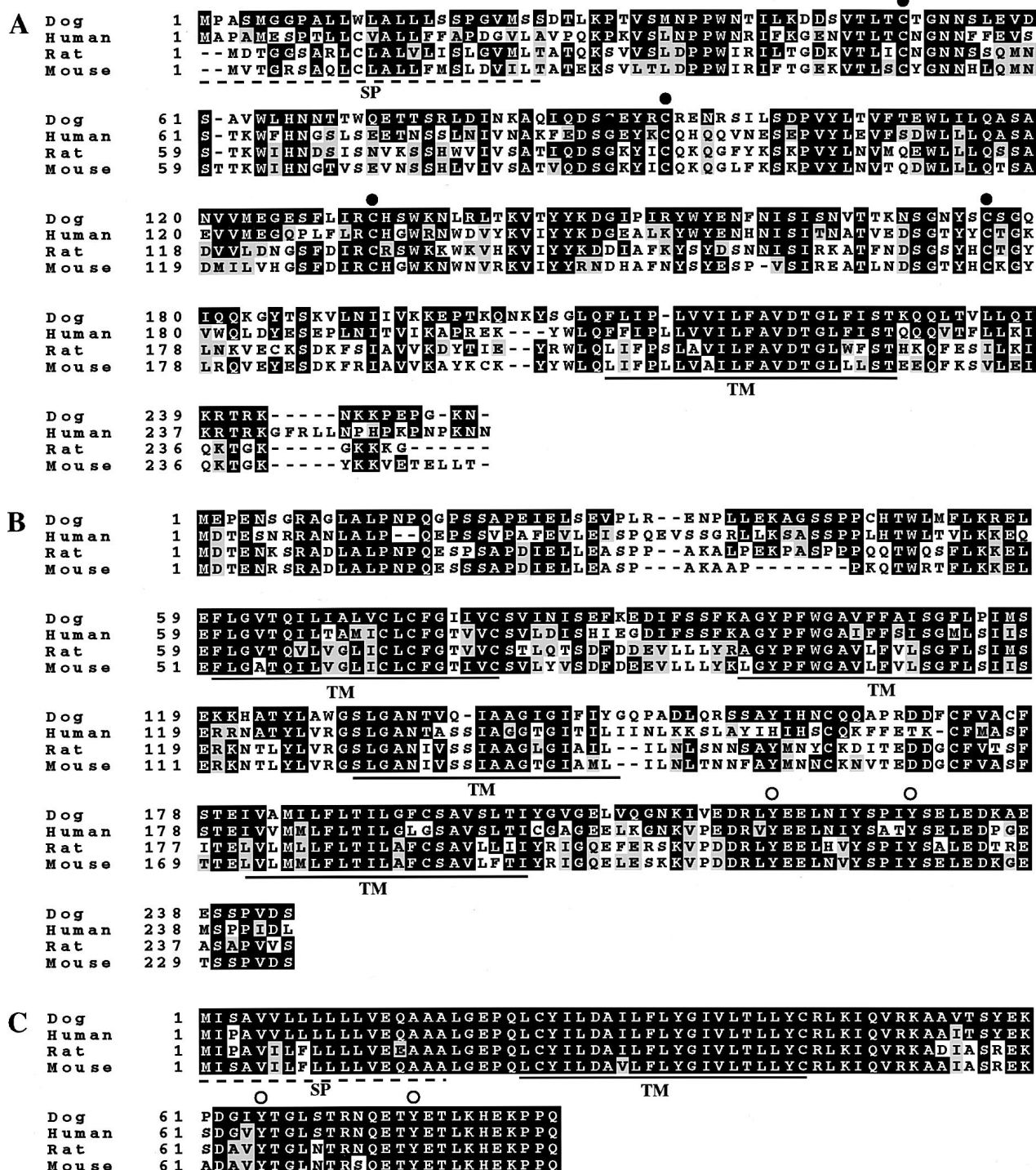


Fig. 1A-C Alignment of deduced amino acid sequences of dog **A** FcεRI α, **B** β, and **C** γ subunits with their mouse, rat, and human counterparts. Identical and similar amino acids are indicated by black and grey shading, respectively. Dashes denote gaps introduced to optimize similarity. The numbers in the left-hand column indicate the position of amino acid residues. The N-terminal signal peptides (SP) are indicated by dashed lines, and the transmembrane domains (TM) are underlined. The conserved cysteine residues for intrachain disulfide-bonds are shown by closed circles, and the conserved tyrosine residues within ITAM are shown by open circles

gestive of transmembrane domains (Fig. 1B). The overall identity of the predicted amino acid sequence of dog β subunit with its mouse, rat, and human counterparts was 58.8%, 56.3%, and 60.2%, respectively. The carboxy-terminus of the dog β subunit also contained two tyrosine residues within ITAM.

The FcεRIγ showed the highest amino acid sequence conservation between species, as compared with other two subunits of FcεRI (Fig. 1C). The overall identity of the predicted amino acid sequence of the

dog γ subunit with its mouse, rat, and human counterparts was 87.2%, 86.0%, and 95.3%, respectively. Two ITAM found in the intracellular domain of the γ subunit were precisely conserved among species.

The molecular information on dog Fc ϵ RI subunits presented here offers an opportunity to compare the requirement for the surface expression of Fc ϵ RI and also the possible association of the polymorphism of the Fc ϵ RI chain with a susceptibility to atopy (Shirakawa et al. 1994) between humans and dogs.

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