



PERGAMON

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Developmental and Comparative Immunology 28 (2004) 61–75

www.elsevier.com/locate/devcompimm

**Developmental
& Comparative
Immunology**

Cloning of IgE from the echidna (*Tachyglossus aculeatus*) and a comparative analysis of ϵ chains from all three extant mammalian lineages[☆]

Molly Verneris^{a,b}, Maria Aveskog^{a,b}, Lars Hellman^{a,b,*}

^aDepartment of Cell and Molecular Biology, Immunology Programme—Biomedical Center, University of Uppsala,
P.O. Box 596, Uppsala S-751 24, Sweden

^bResistentia Pharmaceuticals AB, P.O. Box 853, Uppsala S-753 23, Sweden

Received 14 May 2002; revised 21 March 2003; accepted 29 March 2003

Abstract

In continuation of our evolutionary studies of immunoglobulin (Ig) expression, we present here the cloning of IgE from a monotreme, the short-beaked echidna (*Tachyglossus aculeatus*). Including echidna IgE, 15 ϵ chain sequences have been isolated and each of the three mammalian lineages (placentals, marsupials and monotremes) is now represented by at least two sequences. Phylogenetic analyses based on all available ϵ chains and a selection of other mammalian Ig isotypes (IgM, IgA and IgG) were generated using three different algorithms. The resulting trees strongly support the Theria hypothesis, which states that the monotreme lineage was the first of the three extant mammalian lineages to appear in evolution. Furthermore, to increase our understanding of IgE we have done a detailed comparative analysis, with focus on primary structure, potential *N*-glycosylation, charge distribution and conservation of residues in the putative receptor-binding site. The overall structure of IgE, i.e. four constant domains and the positions of putative disulfide-bridge formations, are conserved, as is an *N*-glycosylation site in the third constant domain. An increased homology was observed in the putative receptor-binding site, which suggests an important function for the IgE/Fc ϵ RI interaction. IgE has been found exclusively in mammals, but it is present in all extant mammalian lineages. This, together with the overall conservation of structure, indicates that IgE appeared as a separate isotype early in mammalian evolution and that structural maintenance may have a selective advantage.

© 2003 Elsevier Ltd. All rights reserved.

Keywords: Immunoglobulins; IgE; ϵ chain; Mammals; Monotreme; Evolution; Fc ϵ RI interaction; *N*-glycosylation

Abbreviations: Ig, immunoglobulin; bp, base pair; pI, isoelectric point; NJ, neighbor joining; ML, maximum likelihood; MP, maximum parsimony.

[☆] The sequence presented is submitted to GenBank, accession number AY099258.

* Corresponding author. Address: Department of Cell and Molecular Biology, Immunology Programme—Biomedical Center, University of Uppsala, P.O. Box 596, Uppsala S-751 24, Sweden. Tel.: +46-18-471-4532; fax: +46-18-471-44347.

E-mail address: lars.hellman@icm.uu.se (L. Hellman).

1. Introduction

Mammals express up to five major immunoglobulin (Ig) classes, IgM, IgD, IgG, IgE, and IgA, and some of them (IgG and IgA) are frequently expressed from more than one gene. The individual genes encoding the heavy chain constant region, that is, μ , δ , γ_1 , γ_2 , γ_3 , γ_4 , ϵ , α_1 or α_2 in humans, define the isotype

of the Ig and unique structures in the heavy chain constant region of each isotype account for the isotype-specific effector functions in humoral immunity, e.g. interaction with isotype-specific receptors on various immune cells, activation of complement and transfer across the placenta or through epithelial layers.

IgE was the last human isotype to be isolated and characterized due to its low abundance in sera [1–3]. The concentration of IgE in normal human sera is between 10 and 400 ng/ml, whereas IgG is present at concentrations that are 30,000 higher (9.5–12.5 mg/ml) [4]. However, the IgE response is powerfully amplified through its interactions with two structurally unrelated receptors: the high affinity receptor, FcεRI, which engages IgE with a 1000-fold higher affinity ($K_a \approx 10^{10} \text{ M}^{-1}$) than does the low affinity receptor, FcεRII or CD23 ($K_a \approx 10^7 \text{ M}^{-1}$). FcεRI is homologous to a family of Ig receptors that belong to the Ig superfamily, whereas CD23, being a member of the C-type lectin superfamily, is unique among Ig receptors [5]. CD23 is found on a variety of cells and depending on the expressing cell type, its function as a receptor for IgE varies from mediating cellular cytotoxicity to regulating antigen presentation and IgE production [5–7]. FcεRI is expressed primarily by mast cells in the tissues and basophils in the blood, but has been reported on Langerhans cells and other cell types in humans, see Ref. [8] for a review. IgE and its receptors are believed to have evolved as a mechanism for protection against parasites [5], and several human seroepidemiological studies have shown that the presence of antigen-specific IgE correlates with acquired immunity toward certain parasitic helminth infections [9–11]. However, the importance of the IgE/FcεRI interaction in parasitic infections remains controversial [12,13]. In contrast, the central role of the IgE/FcεRI interaction in allergic reactions is well established and a lot of research has been devoted to increasing our understanding of the interaction. The solution of the crystal structure of the human IgE-Fc fragment bound to FcεRIα has contributed important insights [14] and opened a new field of therapeutic approaches for intervention in atopic diseases.

So far, IgE as well as IgG have been found exclusively in mammals. However, IgY, the major isotype found in amphibians and birds (and probably

reptiles), shares characteristics with both mammalian IgG and IgE and appears to have a common phylogenetic history [15,16]. An ancestral IgY probably duplicated sometime early in mammalian evolution and formed two separate Ig classes: the major serum antibody IgG and IgE [15,16].

Apart from the placental mammals (eutherians), which are the best studied with regard to Ig expression, there are two other extant mammalian lineages, the marsupials (metatheria) and the monotremes (prototheria). The short-beaked echidna or spiny anteater (*Tachyglossus aculeatus*) belongs to the monotremes, a group of egg-laying mammals that are represented by only three, highly specialized, extant species; the duck-billed platypus and two species of echidna. Because they lay eggs, the monotremes have been regarded as primitive mammals, representing a stage in evolution between reptilian and placental mammals. Nevertheless, the monotremes do possess all of the major mammalian features, including a well-developed fur coat, mammary glands, a four-chambered heart, a single dentary bone and three bones in the middle ear.

The major lymphoid organs of the echidna (spleen, thymus and the appendix) are located in the same anatomical situations as in other mammals and they are comparable in histological structure to those of therian mammals (eutherians and metatherians) [17]. However, in the echidna, as in the platypus, small and very numerous nodules, described as intermediate in histological organization between the jugular-bodies of the amphibian and the eutherian lymph nodes, are found at sites where lymph nodes would be expected in other mammals [17,18], suggesting that monotremes have a somewhat more primitive immune system than therian mammals. Early studies of Igs in echidna established that the major Ig classes were very similar to human IgG and IgM [19]. This was recently confirmed by the cloning of echidna IgG1 [20] and IgM [21].

Recently, we cloned IgE, as well as two IgG isotypes [22] and two IgA isotypes (Verneris et al., manuscript in preparation) of the platypus, another monotreme, which revealed that all modern post-switch isotypes found in mammals appeared before the evolutionary separation of monotremes from the other mammalian lineages. However, the evolutionary relationships among the three mammalian

lineages are not yet fully resolved. The Marsupionta hypothesis, supported mainly by mitochondrial data, suggests that marsupials and monotremes are sister lineages [23–26]. In contrast, the Theria hypothesis proposes that marsupials and placentals are sister lineages and that the monotremes constitute the most divergent lineage, i.e. was the first of the extant mammalian lineages to appear in evolution. The Theria hypothesis emerged initially from morphological data and has more recently gained additional support from paleontological data [27,28] and a number of nuclear gene sequences [21,22,29–36].

To continue our evolutionary studies of Igs, we present here the cloning of the ϵ chain of echidna IgE. Including echidna IgE, 15 ϵ chains have been characterized and at least two species from each of the three mammalian lineages are now represented. Hence, in an attempt to increase our understanding of IgE, a key player in allergy with a somewhat elusive physiological function, we have conducted a detailed comparative analysis of all of the ϵ chain sequences determined so far, with focus on primary structure, potential disulfide-bridge formation, charge distribution and glycosylation. Furthermore, due to the central role of the IgE/Fc ϵ RI interaction in atopic allergy, the conservation of residues in the putative Fc ϵ RI-binding regions among the various ϵ chains was examined, and possible consequences of mutations in interspecies IgE/Fc ϵ RI interactions are discussed. In addition, to further resolve the evolutionary relationships among the extant mammalian lineages we conducted phylogenetic analyses based on all available ϵ chain sequences and a selection of other mammalian Ig isotypes (IgM, IgA and IgG).

2. Materials and methods

2.1. Isolation of full-length cDNA for echidna IgE

Total cellular RNA from echidna splenocytes was isolated by the guanidium thiocyanate method [37] and poly(A)⁺ RNA was purified using oligo dT-coupled magnetic beads according to the manufacturer's protocol (PolyATtract, mRNA Isolation System II, Promega, Madison, WI, USA). Double-stranded cDNA was synthesized using the Time-saver cDNA synthesis kit (Pharmacia-Biotech, Uppsala, Sweden)

and ligated into the single *Eco*RI site of the λ -gt 10 vector. The recombinant vectors were packaged into phage particles using an in vitro packaging system (Stratagene, La Jolla, CA, USA).

Approximately 50 000 plaques from this unamplified library were transferred to Hybond N+ filters (Amersham Int., Buckinghamshire, England) and screened with a ³²P-labeled fragment of approximately 470 base pairs (bp), encoding a region stretching from the CH3 to CH4 central regions of the platypus ϵ chain [22]. The filters were washed at medium stringency (2 × SSC, 0.5% SDS) and autoradiography was performed for 24–48 h on Kodak Exomat AR film (Eastman–Kodak Company, Rochester NY, USA). Positive plaques were purified and the inserts were excised by *Not*I cleavage and subcloned into Bluescript SK(+/-) (Stratagene, La Jolla, CA, USA). The cDNA clones obtained were sequenced from both ends using T7 and T3 primers. Based on these sequences, new sequence primers were designed and used to complete the entire insert sequences.

2.2. Phylogenetic analysis

The amino acid sequences of the constant regions from all presently available IgE sequences, along with a selection of IgM, IgG, IgA and IgY sequences, were aligned using the CLUSTAL X program [38], which performs progressive pairwise comparisons in order of divergence, initiating with the most closely related sequence. To visualize the relationship of the echidna ϵ chain with the constant region of other Ig molecules, we constructed a bootstrap tree based on 1000 independent calculations using CLUSTAL X and the neighbor-joining (NJ) algorithm. To resolve the Marsupionta /Theria debate, two additional unrooted trees were constructed using the maximum likelihood (ML) and maximum parsimony (MP) algorithms. The sequences were aligned in the CLUSTAL X program as mentioned earlier. A bootstrap tree based on 1000 independent calculations was then generated using the ML algorithm in the PHYLIP package [39]. The ML tree was constructed with PROTML [40] under the Dayhoff [41] and JTT [42] models. The PAUP* program version 4.0b10 [43] was used to generate the MP tree and heuristic searches using 1000 bootstraps were performed. The ML and MP trees were drawn with the TREEVIEW program [44].



The GenBank accession numbers for the included amino acid sequences are listed below

IgM: axolotl (X68700), xenopus (M20484), turtle (U53567), duck (U27213), chicken (X01613), echidna (AF416952), opossum (AF108226), possum (AF157618), mouse (X03690), cow (U63637) and human (X149940).

IgA: duck (U27222), chicken (S40610), echidna (AF416951), platypus α_1 (AY055778), platypus $\text{Ig}\alpha_2$ (AY055779), opossum (AF108225), possum (AF027382), mouse (J00475), rabbit α_{12} (X82108–X82118), pig (U12594), dog (L36871), macaque (X53705), gorilla α_1 (X53703), human α_1 (J00220) and human α_2 (J00221).

IgY: axolotl (X69492), duck (X65219) and chicken (X07174).

IgG: echidna γ_1 (AF416949), platypus γ_1 (AY055781), platypus γ_2 (AY055782), opossum (AF035195), possum (AF191648), mouse γ_1 (J00453), mouse γ_{2a} (J00470), mouse γ_{2b} (J00461), mouse γ_3 (J00451), rat γ_{2a} (M28669), rat γ_{2b} (M28671), rat γ_{2c} (X07189), hamster (U17166), rabbit (L29172), sheep γ_2 (X70983), pig γ_1 (U03778), pig γ_{2a} (U03779), pig γ_{2b} (U03780), pig γ_3 (U03781), pig γ_4 (U03782), human γ_1 (J00228), human γ_2 (J00230), human γ_3 (X03604) and human γ_4 (K01316).

IgE: echidna (AY099258), platypus (AF055780), opossum (AF035194), possum (AF157617), mouse (X01857), rat (K02901), sheep (M84356), pig (U96100), horse I (U15150), horse II (AJ305046), cow (U63640), dog (L36872), cat (AF162134), chimpanzee (M15398), orangutan (M15399) and human (L00022).

3. Results and discussion

A partial platypus IgE cDNA clone of approximately 470 bp, stretching from the CH3 to CH4 central regions of the platypus ϵ chain, had been isolated earlier using a PCR-based cloning strategy and degenerate primers [22]. This platypus ϵ chain fragment was used as probe to screen an echidna spleen cDNA library, and screening of about 50 000 plaques resulted in 16 positive signals. Seven positive clones were selected for further analysis, and the complete nucleotide sequence of one full-length clone was determined (Fig. 1).

Like the previously characterized ϵ chains, the echidna ϵ chain consists of four constant domains and all of the cysteine residues involved in the various inter- and intra-chain disulfide-bridge formations that are conserved among most mammalian ϵ chains occur also in echidna IgE (Figs. 1 and 2). The variable region (V-region) of the echidna IgE clone (Fig. 1) was compared with a selection of mammalian V-region sequences and was found to group with the previously characterized platypus clones, all of which belong to clan III of mammalian V-region sequences [45] (data not shown).

The amino acid sequence of the echidna ϵ chain was compared with the available IgE sequences and was found to share the highest degree of sequence homology, 83% identity, with the ϵ chain of another monotreme, the platypus (*Ornithorhynchus anatinus*). Interestingly, the sequence identity between the two monotreme–IgE sequences varies quite significantly over the different domains. The highest homology is observed in the CH2 domain (90%) and the CH1 domain (87%), whereas the homology is significantly

Fig. 1. Structure of the echidna IgE heavy-chain cDNA clone (GenBank AY099258). A schematic map is depicted at the top of the figure, the constant domains are shown by striped boxes, the J-segment (J) and the signal sequence (S) are represented in black, the variable region (V) in light gray and the D-segment (D) in dark gray. The approximate positions of intra-chain disulfide bridges are indicated by brackets, and cysteine residues involved in inter-chain disulfide bridges connecting the two heavy chains and the heavy chain with the light chain are denoted by H and L, respectively. The entire insert sequence from the original lambda clone, excluding the linker sequence, is shown below the schematic map, the tentative positions of the exon boundaries, based on the exon–intron structures of mouse, rat, human, and dog ϵ chain genes, are indicated by vertical lines. A black line below the sequence indicates the position of the J-segment. Cysteine residues involved in putative intra-chain disulfide bridges are marked by asterisks, and the various inter-chain disulfide bridges are indicated by thick brackets to the right of the sequence, the cysteine residues involved in putative inter-chain disulfide bridges are indicated by H or L as above. Potential N-glycosylation sites are denoted by open circles and a small arrow marks the position of the signal sequence cleavage site.

		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10		20		30		40		50		60		70		80		90		100		110		120		130		140		150	
		10																													

lower in the CH3 domain (79%) and the CH4 domain (75%). This is somewhat curious, since, in general, the CH3 domain is the most conserved domain among all available ϵ chains, whereas the CH1 domain appears to be the least conserved ϵ chain domain. The sequence divergence from the echidna ϵ chain is greatest in rodent and primate IgE, exemplified by mouse and human IgE, which share only 40 and 44% identity with echidna IgE, respectively.

Despite a significant sequence variation among the different ϵ chains, the overall IgE structure is generally conserved. The most striking deviations are found in the rodent ϵ chains, which lack one of the intra-chain disulfide bridges in the CH1 domain (positions 15 and 108 in Fig. 2) and have an extended C-terminus (Fig. 2). These characteristic features of rodent IgE probably appeared in a common rodent ancestor after the major radiation of the placental mammals. Interestingly, the finding that a marsupial ϵ chain, that of the Australian brushtail possum, also lacks the second intra-chain disulfide bridge in the CH1 domain (positions 15 and 108 in Fig. 2) suggests that this disulfide bridge has been lost at least twice during mammalian evolution [46]. The mouse ϵ chain is further distinguished by the presence of an extra cysteine residue in the CH2 domain (positions 181 in Fig. 2), indicating a third inter-chain disulfide bridge in this domain of mouse IgE, whereas only two are present in all other characterized ϵ chains (positions 125 and 217 in Fig. 2).

The constant region of the echidna ϵ chain contains four potential *N*-glycosylation sites; two in the CH2 domain, one in the CH3 domain and one in the CH4 domain (Fig. 1). One of these sites, the site in position 287 of the CH3 domain, is conserved among all of the various ϵ -chain sequences determined so far (Fig. 2), suggesting that glycosylation at this position is of importance for protein structure and/or solubility.

The carbohydrate content of human IgE is relatively high, approximately 12%, and based on studies of human IgE myelomas (IgND and IgPS), four to six of the seven potential *N*-glycosylation sites appear to be glycosylated [47,48]. The CH3 domain contains three potential glycosylation sites: N371, N383 and N394 according to Ref. [47]. N371 appears to be incompletely glycosylated and N383 is probably unglycosylated due to steric hindrance [47,49]. The third site, N394, corresponds to the generally conserved site (position 287 in Fig. 2). It contains a high-mannose oligosaccharide [47,50] that is buried between the two CH3 domains [51] and may thus be inaccessible to the processing enzymes in the Golgi. The three to five remaining sites in human IgE are exposed on the surface of the protein and are considered to contain complex oligosaccharides [47,48].

The conserved asparagine residue (N394/position 287 in Fig. 2) is located in the Fc ϵ RI binding region of human IgE-Fc, but the involvement of the attached carbohydrate in the interaction appears to be limited [14]. Furthermore, aglycosylated and deglycosylated recombinant IgE fragments have been reported to retain Fc ϵ RI binding [52,53]. Nevertheless, removal of the conserved *N*-glycosylation site by point mutagenesis (N394T or N394Q) abolished Fc ϵ RI binding [54,55]. The loss of binding could be attributed to the mere substitution of the asparagine residue rather than to the loss of carbohydrate. However, sequential deglycosylation studies under non-denaturing conditions have shown that deglycosylation of N394 correlates with reduced Fc ϵ RI reactivity [55]. Hence, although glycosylation may not be required for the receptor interaction it may be important to ensure a proper conformation of the receptor-interacting region.

An *N*-glycosylation site that corresponds to the conserved site in IgE is also present in other Ig

Fig. 2. Amino acid sequence alignment of the 15 presently available ϵ chains. The top sequence is generated by the amino acid residues that are represented in the majority of the sequences. Residues that match with the consensus sequence are indicated by a dash, the positions of the exon/intron boundaries (based on sequences in which gene structure has been determined) are indicated by gray arrow heads above the sequence. All of the cysteine residues involved in putative inter- and intra-chain disulfide bridge formations are marked by asterisks and the intra-chain disulfide bridges are indicated by brackets. Cysteine residues involved in the formation of inter-chain disulfide bridges connecting the heavy chains and the heavy chain with the light chain are denoted by H and L, respectively. For GenBank accession numbers see Section 2.

isotypes, including amphibian and mammalian IgM (not echidna IgM), IgG and avian IgY. However, no *N*-glycosylation site is present in the corresponding region of amphibian IgY or in IgA. Glycosylation at this site in human IgG (N297) has been shown to be critical for maintaining Fc γ -receptor interactions [56].

The approximate positions of the potential *N*-glycosylation sites in all ϵ chains isolated so far are shown in Fig. 3. The monotreme and the marsupial ϵ chains contain rather few potential glycosylation sites compared to the ϵ chains of eutherian mammals in general (Fig. 3). Furthermore, the ϵ chains of monotremes and the marsupials have no *N*-glycosylation site in the CH1 domain, whereas two or three sites are present there in eutherian IgE. Conversely, some eutherian ϵ chains (those of primates, pig and horse) lack potential *N*-glycosylation sites in the CH4 domain. Hence, although additional glycosylation of the various IgE molecules may have some importance, the positioning of these additional sites appears to be less restricted.

In general, most of the sequence conservation observed among the various ϵ chains is located near the cysteine residues and other structurally important amino acid residues such as tryptophan and proline. The most common substitutions are serine/threonine and other substitutions among analogous amino acid

residues. Furthermore, a considerable number of substitutions occur among charged amino acid residues, resulting in a significant variation in charge. The charge distribution (based on amino acid sequence) over the different domains at pH 7.0 and the isoelectric point (pI) values are shown in Table 1. The echidna, together with the platypus and the marsupial ϵ chains, all have a substantial negative net charge, resulting in pI values at or below 6.0, whereas the eutherian ϵ chains have pI values between 6.5 and 8.2. Interestingly, there appears to be some correlation between charge distribution and potential *N*-glycosylation sites (Fig. 3 and Table 1). Domains that lack *N*-glycosylation sites or have fewer sites compared to the corresponding domain of other species often have a more pronounced negative charge (low pI) based on the amino acid sequence. Conversely, although not as clear, a more positive charge in comparison with the same domains in other species frequently correlates with an increased number of potential *N*-glycosylation sites. Hence, the actual pI values for the various IgEs may be more similar than what can be discerned from the amino acid sequence alone due to the presence of sialic acid-containing complex oligosaccharides.

The crystal structure of the human IgE-Fc fragment bound to Fc ϵ RI revealed that one dimeric IgE-Fc molecule binds asymmetrically to two separate sites

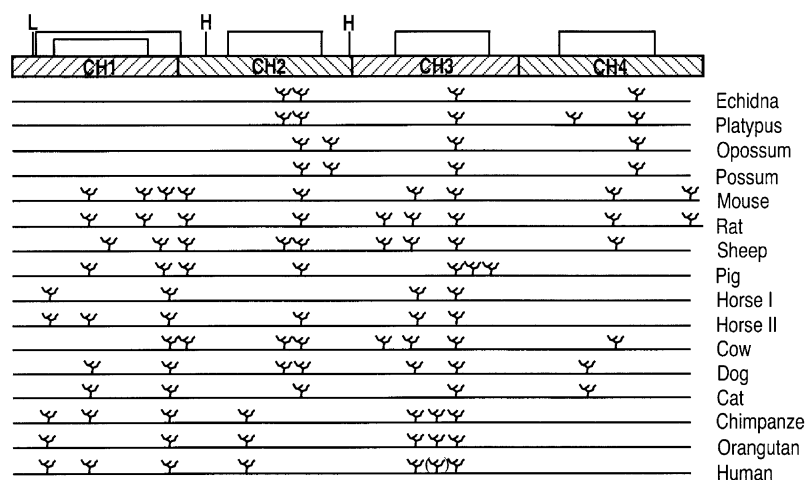


Fig. 3. Potential glycosylation sites in the constant region of the various IgE heavy-chains. The constant regions of the presently available IgE heavy-chains are indicated as lines below a schematic map of the overall structure of the constant region of IgE (in scale). The approximate positions of the potential glycosylation sites in the respective IgE chains are indicated by forks. Horse I and horse II represents different allotypes of horse IgE. For GenBank accession numbers see Section 2.

Table 1

Charge at pH 7.0 and isoelectric points (pI) of the different ϵ chain domains and the entire ϵ chains from the various species, based on the primary amino acid sequences without post-translational modifications

	CH1		CH2		CH3		CH4		ϵ chain	
	Charge at pH 7.0	pI	Charge at pH 7.0	pI	Charge at pH 7.0	pI	Charge at pH 7.0	pI	Charge at pH 7.0	pI
Echidna	– 3.0	4.6	– 2.6	5.9	– 4.8	4.7	– 0.8	6.4	– 10.9	5.3
Platypus	– 3.2	4.4	– 0.7	6.6	– 6.0	4.4	3.7	8.9	– 6.0	5.9
Opossum	– 6.0	4.2	– 1.4	6.5	– 4.0	4.6	0.9	7.4	– 10.3	5.7
Possum	– 7.8	3.9	3.3	8.5	– 5.8	4.5	1.4	7.6	– 8.7	6.0
Mouse	4.2	9.0	– 4.6	5.2	– 0.5	6.7	2.2	8.5	1.6	7.3
Rat	1.2	7.9	0.8	7.4	– 1.5	6.3	6.2	9.6	6.9	8.2
Sheep	– 0.9	6.3	– 1.7	6.1	– 1.6	6.1	4.2	9.1	0.2	7.0
Pig	1.0	7.8	– 0.7	6.6	– 0.8	6.3	3.4	8.9	3.0	7.7
Horse	5.0	9.0	– 5.7	4.6	– 3.8	4.9	3.0	8.9	– 1.3	6.7
Cow	– 1.0	5.8	– 1.7	6.1	– 0.8	6.5	0.6	7.4	– 2.8	6.5
Dog	1.3	7.8	– 1.7	6.1	– 0.5	6.7	3.2	8.9	2.5	7.5
Cat	2.1	8.2	– 1.7	6.1	– 3.6	5.1	3.4	8.9	0.4	7.1
Chimpanzee	2.1	8.2	– 5.9	4.4	9.4	10.4	0.5	7.4	6.4	8.2
Orangutan	1.4	7.8	– 6.1	4.3	8.4	10.2	0.5	7.4	4.5	7.9
Human	1.1	7.8	– 5.7	4.6	8.4	10.2	1.4	8.0	5.4	8.0

on one Fc ϵ RI α [14]. The two binding sites on Fc ϵ RI α and engage one C ϵ 3 domain each, explaining the 1:1 stoichiometry of the interaction. The residues in the respective C ϵ 3 domains that are involved in the interaction overlap, but are not identical. Twelve amino acids from one of the C ϵ 3 domains interact with receptor site 1, whereas 10 amino acids from the other C ϵ 3 domain interact with site 2 (Fig. 4). Furthermore, binding to Fc ϵ RI appears to induce a conformational change in the Fc region of IgE, indicating conformational flexibility in this region and suggesting that the IgE/Fc ϵ RI interaction might be achieved by an induced fit [51,57]. Apparently, this conformational change also involves the C ϵ 2 domain, and its presence markedly reduces the dissociation rate of the IgE/Fc ϵ RI complex [57–59]. Furthermore, although the C ϵ 4 domain is not directly involved in the Fc ϵ RI α interaction, this domain appears to be important for dimerization and proper folding of the C ϵ 3 domain [52,53,60].

The amino acid residues in the various ϵ chains that correspond to the receptor-binding residues in human IgE are shown in Fig. 4. In general, the conservation over the receptor-binding region is significantly higher than that over the entire ϵ chain. For example, the receptor-binding region of the echidna shares 50 and 56% homology with those of mice and humans,

respectively, whereas the homology over the entire ϵ chain is 40 and 44%, respectively. Of the 18 residues that are involved in the Fc ϵ RI interaction, six are identical in all 15 species (Fig. 4). The putative receptor-binding regions of the rodent ϵ chains are the most divergent, sharing only eight to ten residues with the corresponding regions of primate and monotreme IgE. Most of the substitutions are among analogous amino acid residues, but there are some noteworthy exceptions. For example, in the rodent ϵ chains positions 227 and 254 are occupied by a hydrophobic residue (Ile) and a relatively polar residue (Gln), respectively, whereas in all other ϵ chains a hydrophilic residue occurs at position 227 and a small hydrophobic residue at position 254 (Fig. 4). Three allotypes of the horse ϵ chain have been described [61, 62], all of which have a non-analogous substitution to a lysine residue at position 288 (Fig. 4). In addition, in one of the horse allotypes, Horse I (GenBank U15150, [62]), position 224 is occupied by a proline residue, whereas an arginine residue occurs at this position in all other ϵ chains (Fig. 4). However, most of the non-analogous substitutions are observed in the primate ϵ chains, which differ from all other ϵ chains at three positions: N222, P255 and R286 (Fig. 4).

Despite the differences in the receptor-binding regions of primate and rodent IgE, which in this

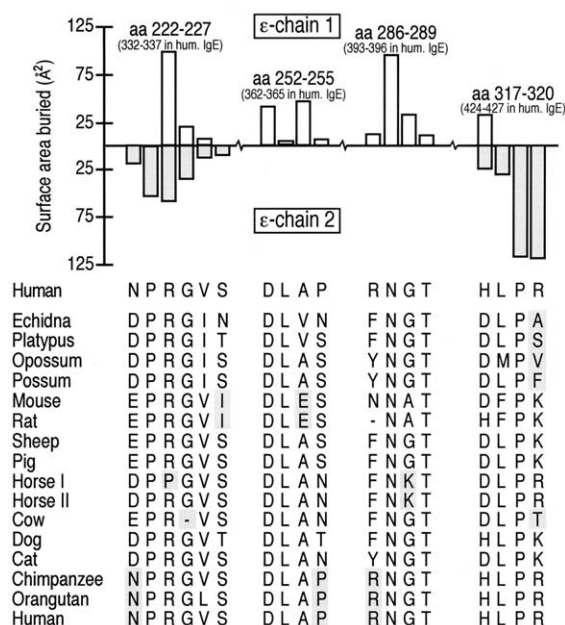


Fig. 4. Conservation of the amino acid residues involved in the interaction between IgE and FcεRI. Based on the crystal structure of the human IgE-Fc fragment bound to FcεRIα [14]. The plot of the buried surface area of residues in the CH3 domain of IgE is adopted from Garman et al. [14], the residues buried in site 1 (top, white bars) and site 2 (bottom, gray bars), are shown. The amino acid residues are numbered according to the consensus sequence in Fig. 2. Attached carbohydrate contributes 50 Å² of the buried surface area of N287. The amino acid residues in the various IgE sequences that correspond to the FcεRI-interacting residues of human IgE (based on the sequence alignment in Fig. 2) are shown; Non-analogous substitutions are shaded in gray. Horse I and horse II represent different allotypes of horse IgE. For GenBank accession numbers see Section 2.

respect constitute the most divergent species, rodent IgE cross-reacts with the human FcεRI receptor [63, 64]. However, mouse IgE cannot engage human CD23 [52,65]. The binding site on IgE for CD23 has also been mapped to the Cε3 domain and appears to overlap with, but is distinct from, the FcεRI site [65]. Furthermore, human IgE cannot bind to either of the rodent receptors: FcεRI [66] or CD23 [65]. Cross-reactivity between IgE from other species and the human FcεRI has also been reported. Using the Prausnitz–Kustner reaction, IgE-mediated reactivity was observed in human and rhesus monkey skin upon passive transfer of dog IgE, whereas neither human nor rhesus monkey IgE sensitized canine skin [67].

The structural basis for these inter-species interactions is unknown. Although speculative, it is tempting to link the apparent species specificity of human (primate) IgE to the non-analogous substitutions found in the receptor-binding region. On the other hand, the explanation may lie in the FcεRI receptor: the human receptor may have more promiscuous binding properties than the rodent and canine FcεRI. In recent studies, point mutations within the IgE-interacting regions of human FcεRIα were shown to differentially affect the binding of human and mouse IgE, and these differences appeared to be linked to the aforementioned non-analogous substitutions in the receptor-binding region of rodent and primate IgE (Fig. 4) [68].

Phylogenetic trees based on the amino acid sequences of the available ε chains along with a selection of IgG, IgY, IgA and IgM sequences were generated using three different algorithms (see Section 2). Trees created with the NJ and ML algorithms are presented in Fig. 5(a) and (b), respectively. The monotreme Ig isotypes group together and form a separate branch within each isotype branch in all trees (Fig. 5(a) and (b)). In the tree generated by the NJ algorithm the monotreme-IgE branch is shared with marsupial IgE sequences, suggesting that monotremes and marsupials are sister lineages and thus supporting the Marsupionta hypothesis [23–25]. However, all other isotypes (IgM, IgA and IgG) support the opposing Theria hypothesis, which proposes that marsupials and eutherians are sister lineages and that the monotremes constitute the most divergent mammalian lineage (Fig. 5(a)). Furthermore, in trees created with the ML (Fig. 5(b)) and MP algorithm (data not shown) the branches for all monotreme isotypes, including IgE, are clearly separated from those of their therian counterparts.

The evolutionary separation of the marsupials from the placental mammals is generally dated back to approximately 130–175 million years ago [21,29,69], whereas studies based on IgM and protamine sequences estimate the time of divergence of the monotremes from the other mammalian lineages to around 150–170 million years ago [21,29]. This latter estimate fits quite well with dating based on fossil records and anatomical features [27,28,70]. Considering the distance in evolutionary time to the events that led to the monotreme and marsupial lineages,

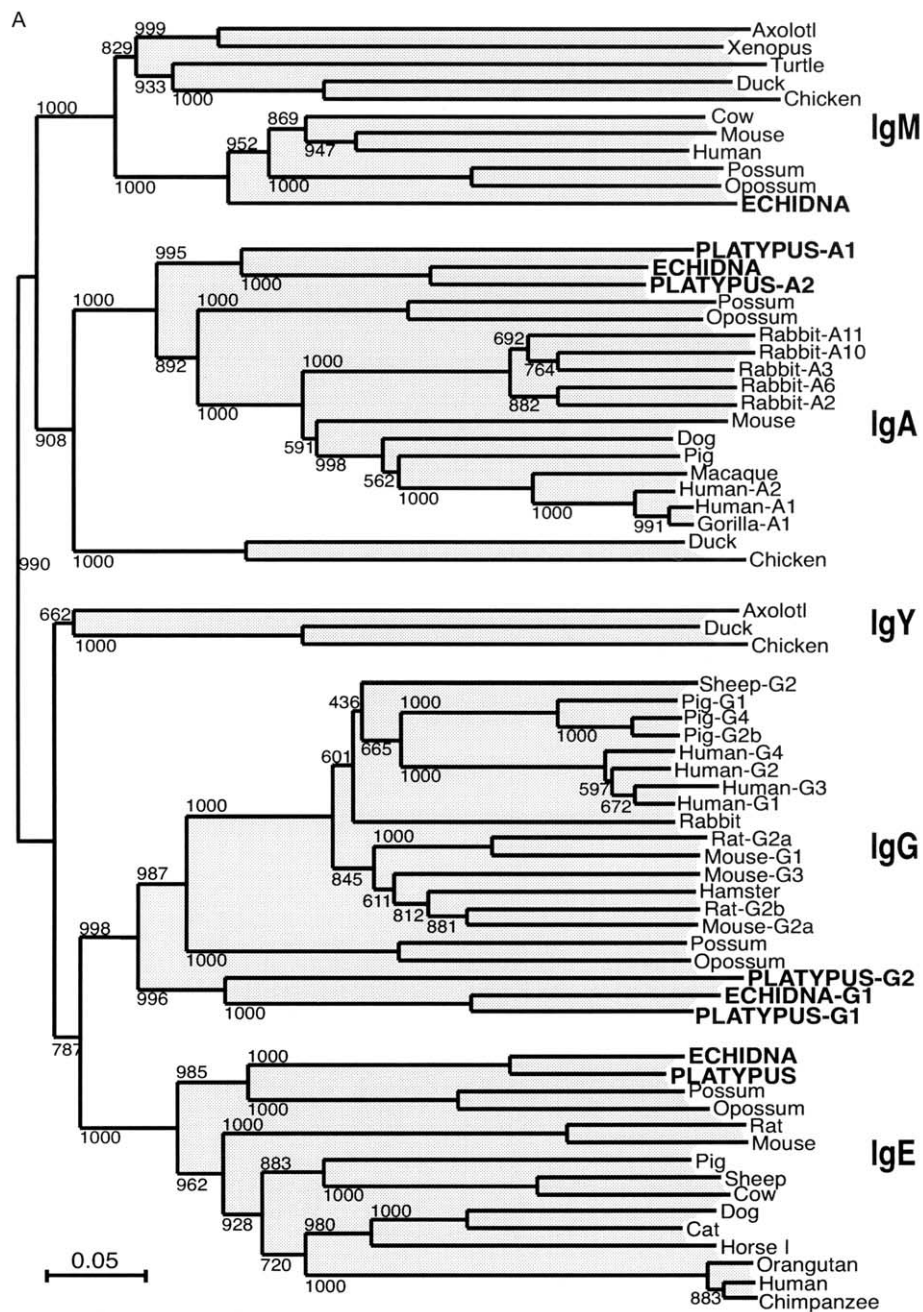


Fig. 5. Phylogenetic trees generated from amino acid sequence alignments of the presently available ϵ chains along with a selection of IgG, IgY, IgA and IgM sequences. (a) An unrooted tree using the NJ algorithm. The bootstrap values indicated at each branch point are based on 1000 replicates, the sequence distance is represented by the length of the branches according to the scale at the bottom of the figure, which corresponds to a sequence divergence of 5%. (b) An unrooted tree using the ML algorithm. For GenBank accession numbers and program versions see Section 2.

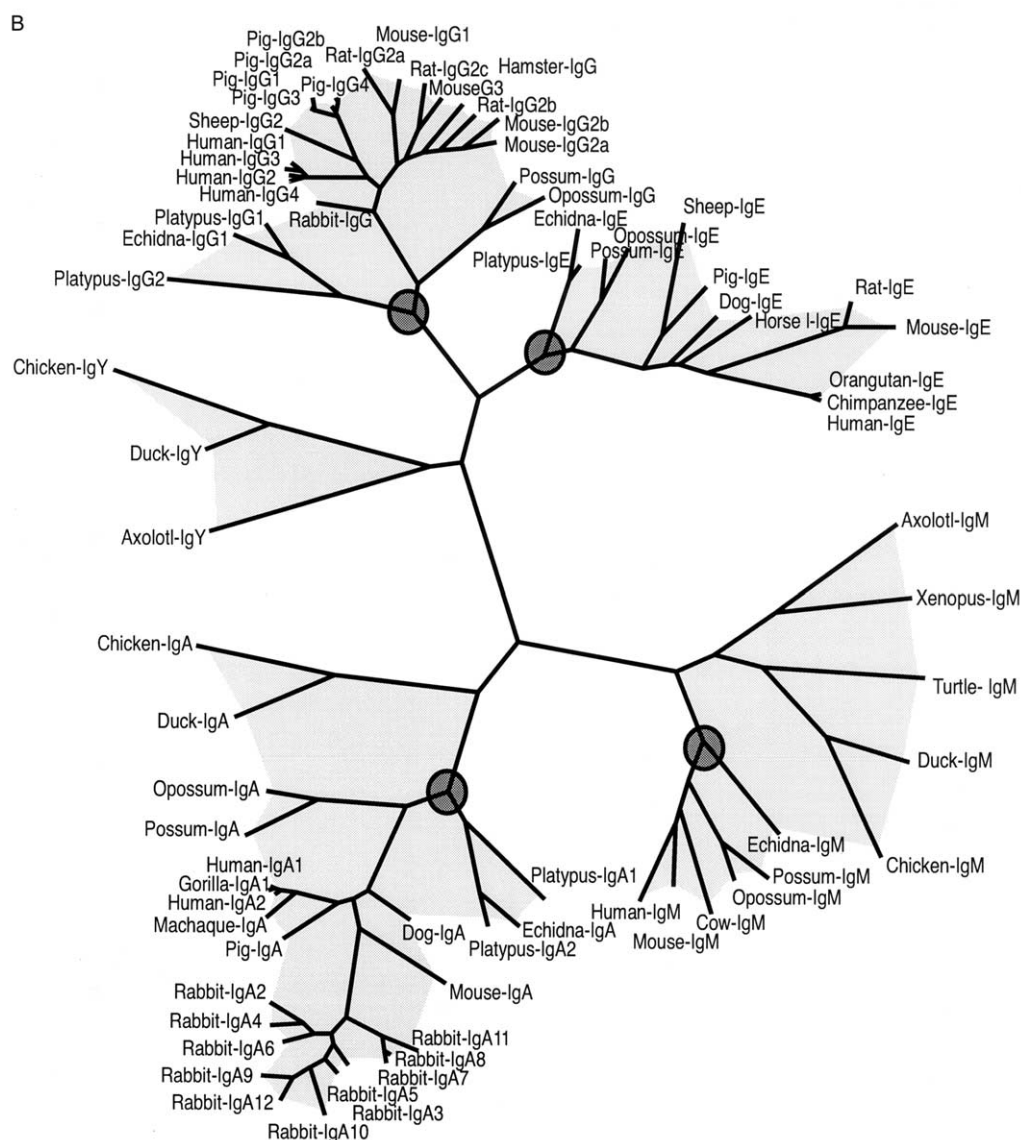


Fig. 5 (continued)

respectively, these events apparently occurred within a relatively short time period of about 20–40 million years, thus explaining conflicting results and the difficulties in resolving the Marsupionta/Theria debate with only a few sequences. Nevertheless, the Theria hypothesis is supported by an increasing number of nuclear gene sequences [29–33], including genes encoding Ig isotypes (IgM, IgA and IgG)

[20–22] and now also IgE, at least in trees generated by the ML or MP algorithms.

In conclusion, IgE has been found exclusively in mammals, but the presence of IgE in all three mammalian lineages reveals that IgE appeared as a separate isotype very early in mammalian evolution. All mammalian post switch isotypes, including IgE, were probably present in a common mammalian

ancestor in essentially their present form at least 150–170 million years ago, but may have appeared as early as 310–330 million years ago, the estimated time of divergence from the early reptile lineages [69]. Despite a significant sequence variation among the different ϵ chains, there appears to be a relatively strong pressure to maintain the overall structure of IgE. A duplication of an ancestral IgY in the lineage leading to the evolution of mammals probably resulted in an advantageous separation of the major effector functions characteristic of present day IgG and IgE. Therefore, limiting the role of the major plasma Ig isotype (IgG) in the activation of mast cells and basophils may have had a selective advantage, thereby explaining the maintenance of both IgG and IgE in all extant mammalian lineages.

Acknowledgements

We would like to thank Dr David Eaker for linguistic revision. This investigation was supported by a grant from the Swedish Natural Sciences Research Council (B-BU 9400-301).

References

- [1] Ishizaka K, Ishizaka T. Identification of gamma-E-antibodies as a carrier of reagenic activity. *J Immunol* 1967;99:1187–98.
- [2] Johansson SG, Bennich H, Wide L. A new class of immunoglobulin in human serum. *Immunology* 1968;14:265–72.
- [3] Bennich HH, Ishizaka K, Johansson SG, Rowe DS, Stanworth DR, Terry WD. Immunoglobulin E. A new class of human immunoglobulin. *Immunochemistry* 1968;5:327–8.
- [4] Capra JD, Frazer JK. Immunoglobulins: structure and funktion. In: Paul WE, editor. *Fundamental Immunology*, 4th ed. Philadelphia: Lippincott–Raven Publishers; 1999. p. 60.
- [5] Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;366:421–8.
- [6] Bonnefoy JY, Aubry JP, Gauchat JF, Graber P, Life P, Flores RL, Mazzei G. Receptors for IgE. *Curr Opin Immunol* 1993;5:944–9.
- [7] Mudde GC, Bheekha R, Bruijnzeel KC. Consequences of IgE/CD23-mediated antigen presentation in allergy. *Immunol Today* 1995;16:380–3.
- [8] Kinet JP. The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931–72.
- [9] Hagan P, Blumenthal UJ, Dunn D, Simpson AJG, Wilkins HA. Human IgE, IgG4 and resistance to infection with *Schistosoma haematobium*. *Nature* 1991;349:243–5.
- [10] Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 1991;21:2679–86.
- [11] Dunne DW, Butterworth AE, Fulford AJC, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to infection. *Eur J Immunol* 1992;22:1483–94.
- [12] King CL, Xianli J, Malhotra I, Liu S, Mahmoud AA, Oettgen HC. Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *J Immunol* 1997;158:294–300.
- [13] Jankovic D, Kullberg MC, Dombrowicz D, Barbieri S, Caspar P, Wynn TA, Paul WE, Cheever AW, Kinet JP, Sher A. Fc epsilonRI-deficient mice infected with *Schistosoma mansoni* mount normal Th2-type responses while displaying enhanced liver pathology. *J Immunol* 1997;159:1868–75.
- [14] Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS. Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. *Nature* 2000;406:259–66.
- [15] Warr GW, Magor KE, Higgins DA. IgY: clues to the origins of modern antibodies. *Immunol Today* 1995;16:392–8.
- [16] Musmann R, Wilson M, Marcuz A, Courtet M, Du Pasquier L. Membrane exon sequences of the three *Xenopus* Ig classes explain the evolutionary origin of mammalian isotypes. *Eur J Immunol* 1996;26:409–14.
- [17] Diener E, Ealey EH. Immune system in a monotreme: studies on the Australian echidna (*Tachyglossus aculeatus*). *Nature* 1965;208:950–3.
- [18] Connolly JH, Canfield PJ, McClure SJ, Whittington RJ. Histological and immunohistological investigation of lymphoid tissue in the platypus (*Ornithorhynchus anatinus*). *J Anat* 1999;195:161–71.
- [19] Atwell JL, Marchalonis JJ, Ealey EH. Major immunoglobulin classes of the Echidna (*Tachyglossus aculeatus*). *Immunology* 1973;25:835–46.
- [20] Belov K, Hellman L, Cooper DW. Characterization of immunoglobulin gamma 1 from a monotreme, *Tachyglossus aculeatus*. *Immunogenetics* 2002;53:1065–71.
- [21] Belov K, Hellman L, Cooper DW. Characterisation of echidna IgM provides insights into the time of divergence of extant mammals. *Dev Comp Immunol* 2002;26:831–9.
- [22] Verneris M, Aveskogh M, Munday B, Hellman L. Evidence for an early appearance of modern post-switch immunoglobulin isotypes in mammalian evolution (II), cloning of IgE, IgG1 and IgG2 from a monotreme, the duck-billed platypus, *Ornithorhynchus anatinus*. *Eur J Immunol* 2002;32:2145–55.
- [23] Janke A, Gemmell NJ, Feldmaier-Fuchs G, von Haeseler A, Paabo S. The mitochondrial genome of a monotreme—the platypus (*Ornithorhynchus anatinus*). *J Mol Evol* 1996;42:153–9.

- [24] Janke A, Xu X, Arnason U. The complete mitochondrial genome of the wallaroo (*Macropus robustus*) and the phylogenetic relationship among Monotremata, Marsupialia, and Eutheria. *Proc Natl Acad Sci USA* 1997;94:1276–81.
- [25] Penny D, Hasegawa M. Molecular systematics. The platypus put in its place. *Nature* 1997;387:549–50.
- [26] Kirsch JA, Mayer GC. The platypus is not a rodent: DNA hybridization, amniote phylogeny and the palimpsest theory. *Philos Trans R Soc Lond B Biol Sci* 1998;353:1221–37.
- [27] Hu Y, Wang Y, Luo Z, Li C. A new symmetrodont mammal from China and its implications for mammalian evolution. *Nature* 1997;390:137–42.
- [28] Luo ZX, Cifelli RL, Kielan-Jaworowska Z. Dual origin of tribosphenic mammals. *Nature* 2001;409:53–7.
- [29] Retief JD, Winkfein RJ, Dixon GH. Evolution of the monotremes. The sequences of the protamine P1 genes of platypus and echidna. *Eur J Biochem* 1993;218:457–61.
- [30] Kullander K, Carlson B, Hallbook F. Molecular phylogeny and evolution of the neurotrophins from monotremes and marsupials. *J Mol Evol* 1997;45:311–21.
- [31] Messer M, Weiss AS, Shaw DC, Westerman M. Evolution of the monotreme: phylogenetic relationship to marsupials and eutherians, and estimation of divergence dates based on α -lactalbumin amino acid sequences. *J Mamm Evol* 1998;5: 95–105.
- [32] Lee MH, Shroff R, Cooper SJ, Hope R. Evolution and molecular characterization of a beta-globin gene from the Australian Echidna *Tachyglossus aculeatus* (Monotremata). *Mol Phylogenet Evol* 1999;12:205–14.
- [33] Killian JK, Buckley TR, Stewart N, Munday BL, Jirtle RL. Marsupials and Eutherians reunited: genetic evidence for the Theria hypothesis of mammalian evolution. *Mamm Genome* 2001;12:513–7.
- [34] Belov K, Zenger KR, Hellman L, Cooper DW. Echidna IgA supports mammalian unity and traditional Therian relationship. *Mamm Genome* 2002;13:656–63.
- [35] Miska KB, Harrison GA, Hellman L, Miller RD. The major histocompatibility complex in monotremes: an analysis of the evolution of Mhc class I genes across all three mammalian subclasses. *Immunogenetics* 2002;54:381–93.
- [36] Miska KB, Hellman L, Miller RD. Characterization of β 2-microglobulin coding sequence from three non-placental mammals: the duckbill platypus, the short-beaked echidna, and the grey short-tailed opossum. *Dev Comp Immunol* 2003; 3:247–256.
- [37] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156–9.
- [38] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–82.
- [39] Felsenstein J. PHYLIP 3.6; 2002. <http://evolution.gs.washington.edu/phylip.html>
- [40] Adachi J, Hasegawa M. Molphy Version 2.3. Programs for molecular phylogenetics based on maximum likelihood. *Comput Sci Monogr* 28, Tokyo: Institute of Statistical Mathematics; 1996.
- [41] Dayhoff MO, Schwartz RM, Orcutt BC. A model of evolutionary change in proteins. In: Dayhoff MO, editor. *Atlas of Protein Sequence Structure*. Washington DC: National Biomedical Research Foundation; 1978. p. 345–52.
- [42] Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* 1992;8:275–82.
- [43] Swofford DL. PAUP: phylogenetic analysis using parsimony. Version 3.1.1. Distributed by the Illinois Natural History Survey, Champaign, IL; 1993.
- [44] Page RD. TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 1996;12: 357–8.
- [45] Johansson J, Aveskogh M, Munday B, Hellman L. Heavy chain V region diversity in the duck-billed platypus (*Ornithorhynchus anatinus*): long and highly variable complementarity-determining region 3 compensates for limited germline diversity. *J Immunol* 2002;168:5155–62.
- [46] Belov K, Harrison GA, Miller RD, Cooper DW. Molecular cloning of the brushtail possum (*Trichosurus vulpecula*) immunoglobulin E heavy chain constant region. *Mol Immunol* 1999;36:1255–61.
- [47] Dorrington KJ, Bennich HH. Structure-function relationships in human immunoglobulin E. *Immunol Rev* 1978;41:3–25.
- [48] Baenziger J, Kornfeld S, Kochwa S. Structure of the carbohydrate units of IgE immunoglobulin I. Over-all composition, glycopeptide isolation, and structure of the high mannose oligosaccharide unit. *J Biol Chem* 1974;249: 1889–96.
- [49] Young RJ, Owens RJ, Mackay GA, Chan CM, Shi J, Hide M, Francis DM, Henry AJ, Sutton BJ, Gould HJ. Secretion of recombinant human IgE-Fc by mammalian cells and biological activity of glycosylation site mutants. *Protein Eng* 1995;8: 193–9.
- [50] Fridriksson EK, Beavil A, Holowka D, Gould HJ, Baird B, McLafferty FW. Heterogeneous glycosylation of immunoglobulin E constructs characterized by top-down high-resolution 2D mass spectrometry. *Biochemistry* 2000;39: 3369–76.
- [51] Wurzburg BA, Garman SC, Jardetzky TS. Structure of the human IgE-Fc C epsilon 3-C epsilon 4 reveals conformational flexibility in the antibody effector domains. *Immunity* 2000; 13:375–85.
- [52] Helm BA, Sayers I, Higginbottom A, Machado DC, Ling Y, Ahmad K, Padlan EA, Wilson AP. Identification of the high affinity receptor binding region in human immunoglobulin E. *J Biol Chem* 1996;271:7494–500.
- [53] Basu M, Hakimi J, Dharm E, Kondas JA, Tsien WH, Pilson RS, Lin P, Gilfillan A, Haring P, Braswell EH, et al. Purification and characterization of human recombinant IgE-Fc fragments that bind to the human high affinity IgE receptor. *J Biol Chem* 1993;268:13118–27.
- [54] Sayers I, Cain SA, Swan JR, Pickett MA, Watt PJ, Holgate ST, Padlan EA, Schuck P, Helm BA. Amino acid residues that

- influence Fc epsilon RI-mediated effector functions of human immunoglobulin E. *Biochemistry* 1998;37:16152–64.
- [55] Da Luz J. Aspects of *N*-glycosylation in human IgE. Department of Medicin, Karolinska Institutet. ISBN 91-7349-130-6. Stockholm: Repro Print AB; 2002.
- [56] Jefferis R, Lund J, Pound JD. IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. *Immunol Rev* 1998;163: 59–76.
- [57] Wan T, Beavil RL, Fabiane SM, Beavil AJ, Sohi MK, Keown M, Young RJ, Henry AJ, Owens RJ, Gould HJ, Sutton BJ. The crystal structure of IgE-Fc reveals an asymmetrically bent conformation. *Nat Immunol* 2002;3: 681–6.
- [58] McDonnell JM, Calvert R, Beavil RL, Beavil AJ, Henry AJ, Sutton BJ, Gould HJ, Cowburn D. The structure of the IgE Cepsilon2 domain and its role in stabilizing the complex with its high-affinity receptor FcepsilonRIalpha. *Nat Struct Biol* 2001;8:437–41.
- [59] Novak N, Bieber T. To bend or not to bend. *Nat Immunol* 2002;3:607–8.
- [60] Bjorklund JE, Schmidt M, Magnusson CG. Characterisation of recombinant human IgE-Fc fragments expressed in baculovirus-infected insect cells. *Mol Immunol* 2000;37: 169–77.
- [61] Wagner B, Siebenkotten G, Radbruch A, Leibold W. Nucleotide sequence and restriction fragment length polymorphisms of the equine Cepsilon gene. *Vet Immunol Immunopathol* 2001;82:193–202.
- [62] Navarro P, Barbis DP, Antczak D, Butler JE. The complete cDNA and deduced amino acid sequence of equine IgE. *Mol Immunol* 1995;32:1–8.
- [63] Hempstead BL, Parker CW, Kulczycki Jr A. Characterization of the IgE receptor isolated from human basophils. *J Immunol* 1979;123:2283–91.
- [64] Conrad DH, Wingard JR, Ishizaka T. The interaction of human and rodent IgE with the human basophil IgE receptor. *J Immunol* 1983;130:327–33.
- [65] Nissim A, Schwarzbaum S, Siraganian R, Eshhar Z. Fine specificity of the IgE interaction with the low and high affinity Fc receptor. *J Immunol* 1993;150:1365–74.
- [66] Kulczycki A, Isersky C, Metzger H. The interaction of IgE with rat basophilic leukemia cells. I. Evidence for specific binding of IgE. *J Exp Med* 1974;139:600–16.
- [67] Lowenthal M, Patterson R, Harris KE. Passive transfer of IgE-mediated cutaneous reactivity in heterologous species. *Ann Allergy* 1993;71:481–4.
- [68] Mackay GA, Hulett MD, Cook JP, Trist HM, Henry AJ, McDonnell JM, Beavil AJ, Beavil RL, Sutton BJ, Hogarth PM, Gould HJ. Mutagenesis within human FcepsilonRIalpha differentially affects human and murine IgE binding. *J Immunol* 2002;168:1787–95.
- [69] Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. *Nature* 1998;392:917–20.
- [70] Archer M, Flannery TF, Ritchie A, Molnar RE. First Mesozoic mammal from Australia—an early Cretaceous monotreme. *Nature* 1985;318:363–6.