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Cross-Reactivity and Molecular Mass of the ϵ Chains of the IgE Antibodies in Dogs, Humans, Rats, and Mice

Key Words

Cross-reactivity
IgE

Abstract

We report the cross-reactivities and comparative molecular masses of the IgE ϵ chains in humans, rats, mice, and dogs. Monoclonal human, rat, and mouse IgE, and our purified polyclonal dog IgE were used in the study. IgE of the 4 species, separated by SDS-PAGE, were analyzed by immunoblotting with polyclonal and monoclonal antihuman IgE, polyclonal and monoclonal antimouse IgE, monoclonal antirat IgE, and polyclonal antidog IgE antibodies. The polyclonal antihuman and polyclonal antimouse IgE cross-reacted with the IgE of the other 3 species, while their monoclonal forms cross-reacted with dog IgE only. Polyclonal antidog IgE cross-reacted with human and mouse IgE, while the monoclonal antirat IgE did not cross-react with any other species. 'Reverse' passive cutaneous anaphylaxis in ragweed-sensitized dogs revealed that polyclonal antihuman and polyclonal antimouse IgE were able to elicit positive skin responses, and monoclonal antihuman, antirat, and antimouse IgE antibodies were not. The molecular masses of the ϵ chains were 77 kDa for mice, 75 kDa for rats and dogs, and 70 kDa for humans.

Introduction

In 1966 IgE was discovered to be a unique class of antibody which mediates type I hypersensitivity [1]. In 1967 a human myeloma IgE was reported [2]. In 1974 and 1978 immunocytoma rat IgE [3] and hybridoma mouse IgE [4] were identified, respectively. The extremely low serum concentrations of IgE have limited the purification of polyclonal IgE. To date, IgE ϵ chains have been identified only from the polyclonal antibodies of dogs [5] and sheep [6]. The cross-reactivity of IgE among different species is an important aspect of studies of comparative immunology, IgE molecular structure, and identification of IgE in species in which IgE has yet to be identified. Previous studies have

shown that IgE antibodies from humans, monkeys, rats, mice, cattle, dogs, sheep, and other mammals have some common functional and physicochemical characteristics, and share certain antigenic determinants [7–17].

We have purified and identified the ϵ chain of dog IgE from ragweed-sensitized dog sera and have produced antidog IgE antibodies [5]. This has facilitated our study of the cross-reactivities and the physical differences of IgE in dogs and other species. Here, we report the cross-reactivities of IgE between dogs, humans, rats, and mice using immunoblotting and reverse passive cutaneous anaphylaxis (reverse PCA), as well as a comparison of the molecular masses of their ϵ chains.

Materials and Methods

Materials

Human myeloma IgE (purified PS myeloma protein [2]) was a gift from Dr. T. Ishizaka (La Jolla Institute of Allergy and Immunology). Rat immunocytoma IgE (purified IR-162 immunocytoma protein) and rat monoclonal antibodies (mAb) antimouse IgE ϵ chain (LO-ME-3) were contributed by Dr. H. Bazin (University of Louvain). The mouse anti-DNP IgE hybridoma cell line (26.82) was provided by Dr. D. H. Katz (Medical Biology Institute, La Jolla, Calif., USA). The hybridoma mouse IgE was purified from ascites and provided by Dr. G. Long (University of Manitoba). Mouse mAb antihuman IgE (clone CIA-E-7.12, ascites) which bound to the CH2 domain in the region between amino acid 218–301 was provided by Dr. A. Saxon (University of California, Los Angeles, Calif., USA). Horse antirat IgE antiserum was a gift from Dr. A. Froese (University of Manitoba). Purified polyclonal rabbit antihuman IgE was purchased from Dakopatts (Glostrup, Denmark). Goat antimouse IgE (Fc) antiserum was obtained from Immunological Labs (Tilburg, The Netherlands). Mouse mAb anti-rat IgE ϵ chain (MARA-1) was purchased from Serotec Canada (Toronto, Ont., Canada). Alkaline phosphatase-conjugated goat antirabbit IgG (heavy and light chain, H+L) and alkaline phosphatase-conjugated rabbit antigoat IgG Fc fragment were purchased from Jackson ImmunoResearch Laboratories (West Grove, Pa., USA). Alkaline phosphatase-conjugated goat antirat IgG (H+L) and antimouse IgG (H+L) were purchased from Zymed Laboratories (San Francisco, Calif., USA) and Tago (Burlingame, Calif., USA), respectively. The specificities of the anti-IgE and enzyme-conjugated antibodies used are also listed in table 1.

Prestained SDS-PAGE Standards, 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt (BCIP) and *p*-nitro blue tetrazolium chloride (NBT) were purchased from Bio-Rad (Richmond, Calif., USA).

Dog IgE-Enriched Fraction

Dog IgE-enriched fractions were purified from ragweed-sensitized dog sera as previously described [5]. Briefly, dog IgE-enriched fractions were obtained by a combination of salt precipitation, gel filtration, and affinity with concanavalin A- and Protein G-Sepharose. The fraction which contained other immunoglobulins and serum proteins had a PCA titer of 1,024 and its protein concentration was 2 mg/ml.

Preparation of Rabbit Antidog IgE Antibodies

Polyclonal antidog IgE antibody was developed, purified and identified as previously reported [5]. Rabbits were immunized by three injections at 2-week intervals with a dog IgE-enriched fraction in complete Freund's adjuvant for the first injection and in incomplete Freund's adjuvant for the other injections. The rabbit antisera were obtained 2 weeks after the third injection. The antisera were applied to a column of Sepharose-4B coupled with control dog serum proteins followed by a column of Sepharose-4B coupled with dog IgG. Rabbit IgG was obtained by chromatography on Protein G-Sepharose.

The IgE activity of the purified rabbit antibody was confirmed by reverse PCA and PCA inhibition tests. No cross-reactivity of the antibody to either dog serum proteins or dog IgG was found by ELISA in which microplates coated with dog serum proteins or IgG were incubated with the antibody followed by incubation with goat antirabbit IgG conjugate.

SDS-PAGE and Immunoblot

SDS-PAGE was performed in a discontinuous system according to the method of Laemmli [18] in a Bio-Rad mini slab gel apparatus un-

Table 1. Specificities of the anti-IgE and enzyme-conjugated antibodies used in the immunoblot

Figures	Anti-IgE antibody	Enzyme-conjugated antibody
Figure 1	Rabbit α^* dog IgE (whole molecule)	Goat α rabbit IgG (H+L)
Figure 2		
a	Rabbit α human IgE (whole molecule)	Goat α rabbit IgG (H+L)
b	Mouse mAb α human IgE ϵ CH2	Goat α mouse IgG (H+L)
Figure 3	Mouse mAb α rat IgE ϵ	Goat α mouse IgG (H+L)
Figure 4		
a	Goat α mouse IgE Fc	Rabbit α goat IgG Fc
b	Rat mAb α mouse IgE ϵ	Goat α rat IgG (H+L)
Figure 5		
Lane 1	Rabbit α dog IgE (whole molecule)	Goat α rabbit IgG (H+L)
Lane 2	Mouse mAb α human IgE ϵ	Goat α mouse IgG (H+L)
Lane 3	Mouse mAb rat IgE ϵ	Goat α mouse IgG (H+L)
Lane 4	Rat mAb α mouse IgE ϵ	Goat α rat IgG (H+L)
* α = anti.		

der reducing conditions. Resolving gels were 10% polyacrylamide, with 4% stacking gels. Samples were boiled at 100°C for 3 min in sample buffer. Two micrograms of proteins in each of the dog, human, rat and mouse IgE preparation were loaded per lane. Proteins separated by SDS-PAGE were electrophoretically transferred onto nitrocellulose membranes using transblot buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3). Free binding sites on the membranes were blocked by a 2-hour incubation with 3% BSA dissolved in 0.02 M phosphate-buffered saline, pH 7.4, containing 0.05% (v/v) Tween 20 (PBS-T). After washing 3 times with 1% BSA PBS-T the membranes were incubated for 2 h with one of the following antibodies: rabbit antidog IgE (1:200), rabbit antihuman IgE (1:500), goat antimouse IgE (1:1,500), mouse mAb antihuman IgE (1:1,500), mouse mAb antirat IgE (1:750) and rat mAb antimouse IgE (1:500). After incubation, the membranes were washed 3 times with PBS-T and then incubated with one of the following alkaline-phosphatase-conjugated antibodies: goat antirabbit IgG (1:1,500), rabbit antigoat IgG Fc (1:2,500), goat antimouse IgG (1:3,000), goat antirat IgG (1:1,500), rabbit antidog IgG (1:1,000). The membranes were washed and developed for color reaction with BCIP and NBT dissolved in alkaline phosphatase buffer, pH 9.5.

Horse antirat IgE was not used in the immunoblot, because enzyme-conjugated antihorse IgG is not commercially available.

To compare the molecular masses of the ϵ chains, dog IgE, human IgE, rat IgE, and mouse IgE were placed in different wells of the same gel. After electrophoresis, the proteins were transferred to nitrocellulose membranes and the membranes were cut into strips. The strip containing dog IgE was incubated with rabbit antidog IgE, followed by incubation with goat antirabbit IgG conjugate. The strip containing human IgE and the one containing rat IgE were incubated with mAb

Table 2. Estimation of molecular mass for the ϵ chains of different species

Species	Source	Method	ϵ chain, kDa	Authors	Year	Reference No.
Human	Myeloma (ND)	Yphantis ¹	72,500	Bennich and Johansson	1971	21
	Myeloma (PS)	Yphantis	69,800	Kochwa et al.	1972	22
	Myeloma	—	73,300	Ishizaka	1988	23
	Myeloma (PS)	SDS-PAGE + immunoblot	70,000	Peng et al.	1995	Present study
Rat	Immunocytoma	SDS-PAGE	68,000	Bazin et al.	1974	3
	Immunocytoma	SDS-PAGE + immunoblot	75,000	Peng et al.	1995	Present study
Mouse	Hybridoma	SDS-PAGE	75,000	Bose et al.	1984	24
	Hybridoma	SDS-PAGE + immunoblot	77,000	Peng et al.	1995	Present study
Sheep	Parasite-infected sheep serum	SDS-PAGE + immunoblot	72,000	Yilmaz et al.	1993	6
Dog	Allergic dog serum	SDS-PAGE + immunoblot	73,000	Peng et al.	1993	5
	Allergic dog serum	SDS-PAGE + immunoblot	75,000	Peng et al.	1995	Present study

¹ Sedimentation equilibrium techniques.

antihuman and mAb antirat IgE, respectively, followed by incubation with goat antimouse IgG conjugate. The strip containing mouse IgE was incubated with rat mAb antimouse IgE followed by incubation with goat antirat IgG conjugate.

The molecular masses of the immunoreactive proteins were determined from the standard curve of prestained SDS-PAGE standards separated in the same gel. Table 1 listed the anti-IgE and the conjugated antibodies used in the immunoblot.

Reverse PCA in Dogs

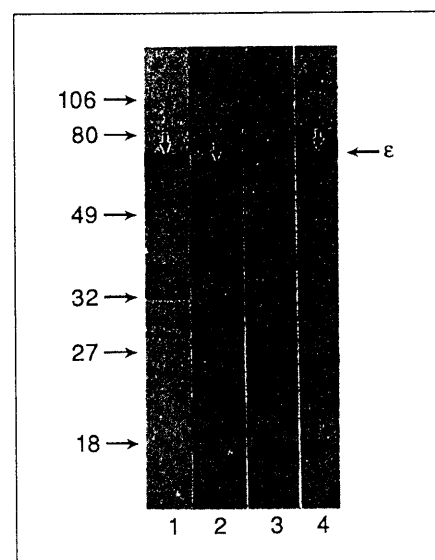
Rabbit antidog IgE, rabbit and mouse mAb antihuman IgE, horse and mouse mAb antirat IgE, goat and rat mAb antimouse IgE antibodies were 10-fold diluted in normal saline (1:10, 1:100, 1:1,000). Each dilution was injected (0.1 ml) intradermally into a ragweed-sensitized dog [5]. Normal rabbit IgG, goat IgG, mouse IgG, rat IgG and horse serum (1:10) were injected as negative controls for the relevant anti-IgE antibodies. After 20 min. the skin wheal size was measured using the largest and the orthogonal diameters. A wheal size at least 2 mm in average diameter larger than the appropriate negative control was considered to be a positive response.

Results

SDS-PAGE and Immunoblot Analysis

The cross-reactivities of the IgE in the 4 species to rabbit antidog IgE (fig. 1), to polyclonal and mAb antihuman IgE (fig. 2), to mAb antirat IgE (fig. 3), and to polyclonal and mAb antimouse IgE (fig. 4) are shown. Polyclonal dog IgE

Fig. 1. Cross-reactivities of the ϵ chains among the IgE antibodies of dogs (lane 1), humans (lane 2), rats (lane 3), and mice (lane 4) to rabbit antidog IgE in immunoblot.



cross-reacted with both polyclonal and monoclonal antihuman and antimouse IgE (fig. 2, 4, lane 1), but not with mAb antirat IgE (fig. 3, lane 1). Human myeloma IgE cross-reacted slightly with polyclonal antidog IgE (fig. 1, lane 2) and

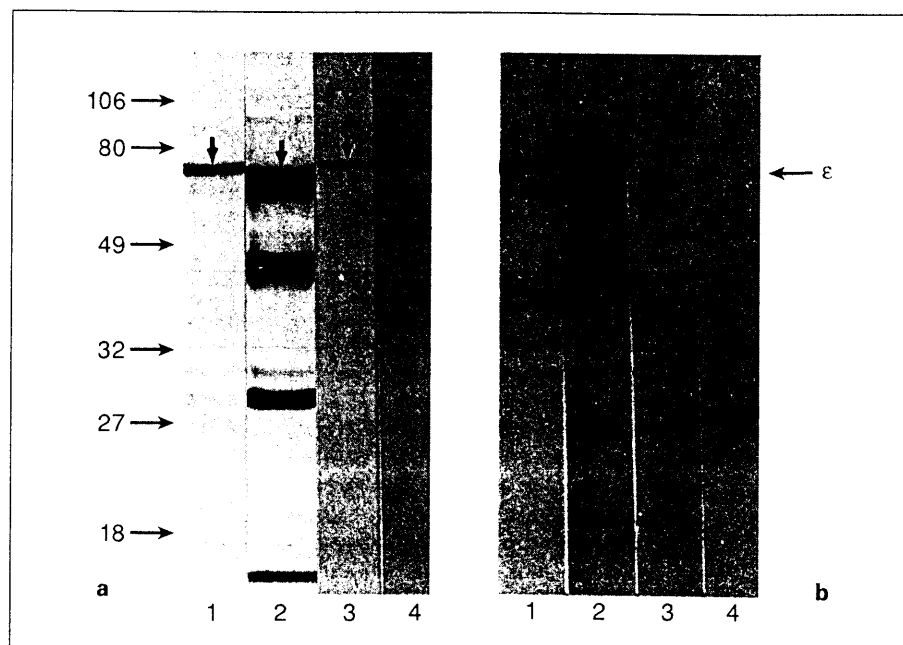


Fig. 2. Cross-reactivities of the ϵ chains among the IgE antibodies of dogs (lane 1), humans (lane 2), rats (lane 3), and mice (lane 4) to rabbit antihuman IgE (a) or mAb antihuman IgE (b) in immunoblot.

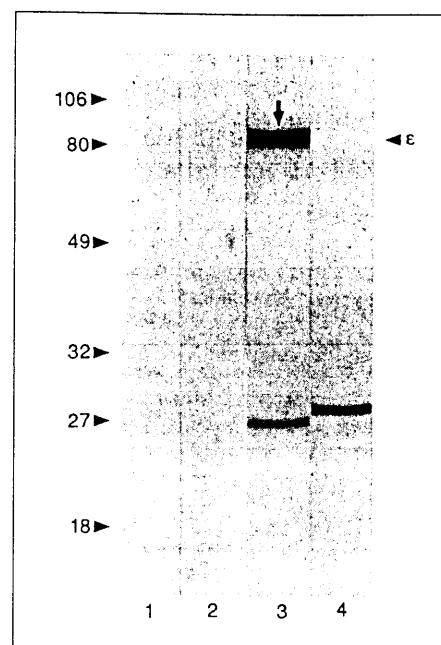


Fig. 3. Cross-reactivities of the ϵ chains among the IgE antibodies of dogs (lane 1), humans (lane 2), rats (lane 3), and mice (lane 4) to mAb mouse antirat IgE in immunoblot.

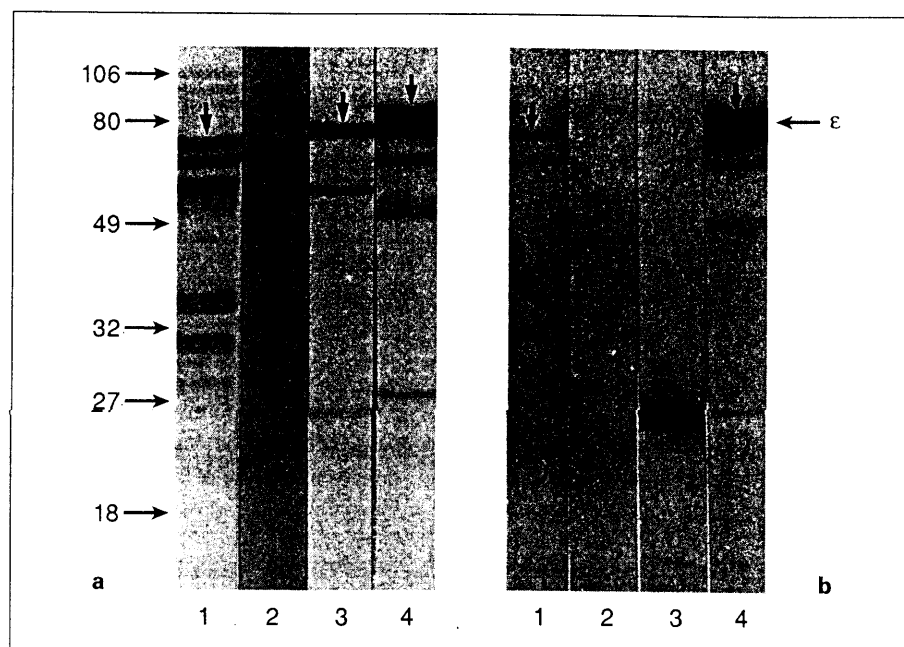


Fig. 4. Cross-reactivities of the ϵ chains among the IgE antibodies of dogs (lane 1), humans (lane 2), rats (lane 3), and mice (lane 4) to goat antimouse IgE (a) or mAb rat antimouse IgE (b) in immunoblot.

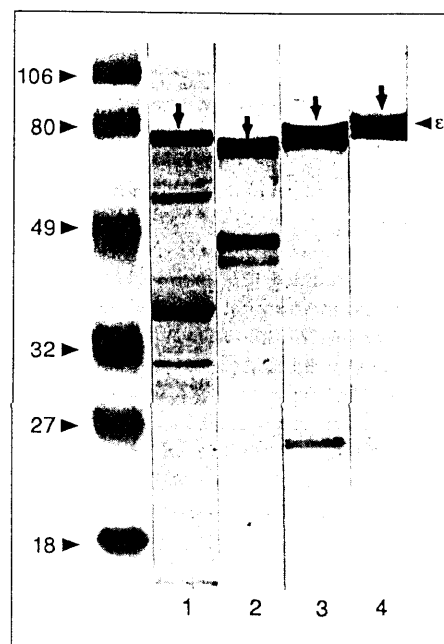


Fig. 5. Comparison of molecular masses of the ϵ chains among the IgE antibodies of dogs (lane 1), humans (lane 2), rats (lane 3), and mice (lane 4) in immunoblot.

antimouse IgE (fig. 4a, lane 2), but not with mAb antimouse (fig. 4a, lane 2) and mAb antirat (fig. 3, lane 2) IgE. Rat immunocytoma IgE cross-reacted with polyclonal antihuman and antimouse IgE (fig. 2, 4a, lane 3), but not with their monoclonal forms (fig. 2, 4b, lane 3) and not with polyclonal antidog IgE (fig. 1, lane 3). Mouse hybridoma IgE slightly cross-reacted with polyclonal antidog IgE (fig. 1, lane 4) and antihuman IgE (fig. 2a, lane 4), but not with mAb antihuman (fig. 2b, lane 4) and rat mAb antimouse IgE (fig. 3, lane 4).

In our study, cross-reactivity of the IgE with the anti-IgE of other species was seen more often when a polyclonal antibody was used than when an mAb was used. Polyclonal antihuman and antimouse IgE cross-reacted with the IgE of the other 3 species, while their monoclonal forms cross-reacted only with dog IgE.

Reverse PCA

Similar to the results of the immunoblot, rabbit antidog IgE induced a positive reverse PCA response at the dilutions of 1:10 and 1:100. Polyclonal antihuman and antimouse IgE elicited a positive response at the dilution of 1:10, while their monoclonal forms did not. Both polyclonal and monoclonal antirat IgE did not induce any response.

Molecular Masses of ϵ chains

The molecular mass of the ϵ chains was 77 kDa for mice, 75 kDa for rats and dogs, and 70 kDa for humans as shown in figure 5.

Discussion

Cross-reactivity between IgE antibodies of various species has been studied using reverse PCA [7–10, 15, 16], PCA inhibition tests [9, 10], histamine release from basophils [11] and mast cells [13, 17], ELISA [12], radioimmunoassay [14], gel diffusion [16] and Western blot [9]. Reverse PCA and PCA tests, and histamine release from basophils and mast cells define the cross-reactivity of the Fc determinants which are involved in binding to the high affinity receptors (Fc ϵ RI). These studies showed that IgE antibodies from various species shared some functional and physicochemical characteristics, and certain antigenic determinants. Comparisons of subunits of the high affinity receptors between rats, mice and humans revealed 68–94% identities at the DNA level and 45–93% identities at the protein level [19]. This explains the existence of cross-reactivity between IgE antibodies of different species.

In our study, cross-reactivity of IgE ϵ chains of the 4 species has been reported by immunoblot analysis. In addition to the ϵ chain, there were some other bands in the immunoblot. These bands were possibly caused by: (1) the contaminating proteins in the IgE materials and the denatured IgE fragments (caused by purification or SDS treatment), (2) inconsistencies in the purity and specificity of the anti-IgE sera, and (3) differences in the specificity of the secondary conjugated antibodies, most of which were against H + L chains as listed in table 1. These will be discussed below in detail.

The dog IgE-enriched fraction contained some other dog immunoglobulins and serum proteins [5]. Also, due to the multiple purification steps, some dog IgE may have been denatured to fragments. This was shown when dog IgE was incubated with mAb antihuman IgE, where several bands below the ϵ chain might represent dog ϵ fragments (fig. 2b, lane 1). On the other hand, although the rabbit antidog IgE antiserum has been purified and extensively adsorbed against normal dog immunoglobulins, there are still some antibodies, e.g. antidog IgG, in the rabbit antidog IgE as were found in our immunoblot study when high concentrations of dog IgG were used [unpubl. data]. Also, we have previously shown that the rabbit antihuman IgE reacted with dog light chains (between 27 and 32 kDa) [5]. For these reasons, some nonspecific bands have appeared in the dog IgE (lane 1).

The purified human myeloma IgE had been stored in our freezer (-70°C) for more than 8 years and had been thawed and refrozen many times. Two denatured human IgE fragments between 32 and 49 kDa were seen when polyclonal and mAb antihuman IgE were used (fig. 2, lane 2). In figure 2a, lane 2, the band between 32 and 27 kDa was due to the cross-reactivity of the conjugate goat antirabbit IgG (H+L) to human IgE light chains and the band below 18 kDa was probably the fragment of human light chains, as these two bands were not seen when monoclonal antihuman IgE was used (fig. 2b, lane 2).

Similarly, due to the cross-reactivity of the conjugated second antibodies to the light chains, bands with different magnitudes between 27 and 32 kDa were found in figure 1 (lane 4), figure 2 (a lane 2, b lanes 3, 4), figure 3 (lanes 3, 4), and figure 4 (a lanes 1–4, b lanes 3, 4). These light chains could be differentiated easily from the ϵ chains by their molecular masses.

The denaturation of some antigenic epitopes on SDS-PAGE and the consequent loss of immunoreactivity by mAb have been previously reported [20]. This may cause some bias, when a monoclonal antibody is used in our study, as monoclonal antibodies bind to a single epitope only.

It would have been better if we had been able to compare monoclonal and polyclonal IgE and both forms of anti-IgE. Only human, rat, and mouse monoclonal IgE and dog polyclonal IgE were available for our use, however. In our study, the polyclonal dog IgE (lane 1 of fig. 1, 2, 4 and 5) seemed to have more bands than the 3 monoclonal IgE. Comparison of monoclonal and polyclonal IgE would be interesting in a future study of IgE antibodies when reagents are available.

For anti-IgE antibodies, we included both polyclonal and monoclonal forms for human and mouse IgE. Rat mAb anti-mouse IgE and mouse mAb anti-rat IgE were the only mAb available for the 2 species, respectively. This choice may affect the possible existence of cross-reactivity between rats and mice. However, we compensated for this shortcoming by the use of polyclonal goat anti-mouse IgE Fc which showed significant cross-reactivity between the 2 species (fig. 4a, lanes 3, 4). For dog IgE, only polyclonal antibodies were available. We did produce a mAb anti-dog IgE, C2, but this mAb does not bind to dog IgE in immunoblotting [unpubl. data]. This suitable comparison of monoclonal and polyclonal forms of anti-IgE was shown in figure 2 in which rabbit anti-human IgE and mouse mAb anti-human IgE were used. Rabbit anti-human IgE bound to all the 4 species IgE, while mouse mAb anti-human IgE bound to dog and human IgE only. This suggests that human IgE is closer to dog IgE than rat and mouse IgE, and that cross-reactivity is better demonstrated using polyclonal antibodies which recognize multiple epitopes of IgE molecules, rather than using monoclonal antibodies.

In one study, incubation with sheep anti-human IgE resulted in a reduction in dog IgE PCA titer. Sheep anti-human IgE was also able to elicit reverse PCA responses in dogs [15]. Our results were consistent with these observations. In our study, not only polyclonal anti-human but also polyclonal anti-mouse IgE bound to dog IgE in immunoblot (fig. 2, 4a, lane 1), and elicited dog reverse PCA responses.

Some disagreements between reverse PCA and immunoblot results were found when mAb anti-IgE was used. Only those anti-IgE antibodies which result in cross-linking of the Fcε receptors on mast cells or basophils are capable of releasing histamine. Monoclonal antibodies react with a single epitope and may therefore fail to cross-link with the receptors. It is not surprising that mAb anti-human IgE does not release histamine in reverse PCA in dogs, but reacted with dog IgE in immunoblot (fig. 2b, lane 1). The same may be true for rat mAb anti-mouse IgE. The cross-reactivity of rabbit anti-human IgE with dog light chains in immunoblot [5] may also cause cross-linking of the Fcε receptors and result in activation of histamine release from mast cells.

It has been reported using PCA inhibition tests that polyclonal anti-human IgE inhibited rat IgE from binding to rat mast cells [10], and that both rat and mouse IgE were able to inhibit the binding of human IgE to the human basophils [11]. Human IgE also bound to rat peritoneal mast cells [13]. These observations suggest that IgE antibodies from humans share common determinants in their binding sites to rat high affinity receptors. In our study, this was supported by the binding of rat IgE to polyclonal anti-human IgE in immunoblot (fig. 2a, lane 3). However, in another study, monoclonal mouse anti-rat IgE failed to bind to human IgE in a solid-phase radioimmunoassay [14]. This is probably due to the use of monoclonal antibody.

Because of the low concentration of polyclonal IgE in serum, knowledge of the IgE structure in humans, mice, and rats has been obtained from monoclonal IgE proteins. As listed in table 2, the molecular mass of the IgE ε chains ranges from 69 to 73 kDa [21–23] in humans, 68 kDa in rats [3], and 75 kDa in mice [24]. The polyclonal IgE ε chains, with a similar molecular mass to those identified from monoclonal IgE antibodies, were recently purified and identified in sheep [6] and dogs [5] (table 2). Because of variations in the methods used, variations among laboratories, and even variations among experiments, it is difficult to make comparisons between the molecular masses of the ε chains from different studies. In our study, we measured the molecular masses of the ε chains of the 4 species in the same gel. Mouse ε chains had the highest molecular mass and human ε chains had the lowest (fig. 5).

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References

- 1 Ishizaka K, Ishizaka T, Hornbrook MM: Physicochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 1966; 97:75-85.
- 2 Johansson SGO, Bennich H: Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967;13:381-394.
- 3 Bazin H, Querinjean P, Becker A, Heremans JF, Dessy F: Transplantable immunoglobulin-secreting tumours in rats. IV. Sixty three IgE-secreting immunocytoma tumours. *Immunology* 1974;26:713-723.
- 4 Böttcher I, Hämmerling G: Continuous production of monoclonal mouse IgE antibodies with known allergenic specificity by a hybrid cell line. *Nature* 1978;275:761-762.
- 5 Peng Z, Arthur G, Kierek-Jaszczek D, Thomas E, Schon AH, Rector ES, Simons FER, Becker AB: Purification and identification of polyclonal IgE antibodies from ragweed-sensitized dog sera. *Int Arch Allergy Immunol* 1993;102:176-184.
- 6 Yilmaz H, Roe JM, Morgan KL: Isolation and preparation of antisera to ovine IgE. *Int Arch Allergy Immunol* 1993;101:369-375.
- 7 Ishizaka K, Ishizaka T: Reversed type allergic skin reactions by anti- τ E-globulin antibodies in humans and monkeys. *J Immunol* 1968;100:554-562.
- 8 Ishizaka K, Ishizaka T, Tada T: Immunoglobulin E in the monkey. *J Immunol* 1969;103:445-453.
- 9 Yilmaz H, Morgan KL, Roe JM: Cross-reactivity of ovine IgE with antihuman IgE. *Int Arch Allergy Immunol* 1994;104:277-280.
- 10 Kanyerezi B, Jatton JC, Bloch KJ: Human and rat τ E: Serologic evidence of homology. *J Immunol* 1971;106:1411-1412.
- 11 Conrad DH, Wingard JR, Ishizaka T: The interaction of human and rodent IgE with the human basophil IgE receptor. *J Immunol* 1983;130:327-333.
- 12 Suter M, Fey H: Further purification and characterisation of horse IgE. *Vet Immunol Immunopathol* 1983;4:545-553.
- 13 Perelmutter L, Khera K: A study on the detection of human reagins with rat peritoneal mast cells. *Int Arch Allergy Immunol* 1970;39:27-44.
- 14 Raybourne R, Conrad DH: Use of monoclonal antirat IgE in a radioimmunoassay for antigen specific rat IgE. *J Immunol Methods* 1983;58:277-283.
- 15 Halliwell REW, Schwartzman RM, Rokey JH: Antigenic relationship between human IgE and canine IgE. *Clin Exp Immunol* 1972;10:399-407.
- 16 Gershwin LJ: Heterologous passive cutaneous anaphylaxis with bovine immunoglobulin E. *Am J Vet Res* 1981;42:1184-1187.
- 17 Nielsen KH: Bovine reaginic antibody III. Cross-reaction of antihuman IgE and antiovine reaginic immunoglobulin antisera with sera from several species of mammals. *Can J Comp Med* 1977;41:345-348.
- 18 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
- 19 Burnette WN: Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulphate polyacrylamide gel to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 1981;112:195-203.
- 20 Ra C, Jouvin ME, Kinet J: Complete structure of the mouse mast cell receptor for IgE (Fc ϵ RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J Biol Chem* 1989;264:15323-15327.
- 21 Bennich H, Johansson SGO: Structure and function of human immunoglobulin E. *Adv Immunol* 1971;13:1-55.
- 22 Kochwa S, Terry WD, Capra JD, Yang NL: Structural studies of immunoglobulin E. I. Physicochemical studies of the IgE molecule. *Ann NY Acad Sci* 1972;189:49-70.
- 23 Ishizaka T: Mechanisms of IgE-mediated hypersensitivity; in Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW (eds): *Allergy Principles and Practice*. St. Louis, CV Mosby, 1988, pp 71-93.
- 24 Bose R, Bundesen PG, Holford-Stevens V, Stefura WP, Kelly KA, Jeffrey JC, Rector ES, Fischer J, Schon AH, Schwenk RJ: Immunochemical properties of some monoclonal IgE antibodies to 4-hydroxy-3-nitrophenylacetyl (NP). *Immunology* 1984;53:801-809.