

Partial sequences of feline and caprine immunoglobulin epsilon heavy chain cDNA and comparative binding studies of recombinant IgE fragment-specific antibodies across different species

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Abstract

Parts of the feline and caprine IgE epsilon heavy chain cDNA (third and fourth constant domains, rIgEf3/4) were cloned, sequenced, and expressed to raise antibodies (Abs). The DNA and derived protein sequences of the feline recombinant rIgEf (rIgEf) shared high homology with the analogous canine parts (81% at the nucleotide and 71% at the protein levels) and the caprine with the ovine ones (95%/84%), respectively. The polyclonal Abs raised in chickens against the feline and caprine rIgEf3/4 were subjected to a comparative binding study utilizing an ELISA including rIgEf and specific Abs to these rIgEf from dog and horse (rIgEf2 and rIgEf3/4) and sheep (rIgEf3/4). All but the ovine-specific rIgEf3/4 Ab were polyclonal, which had been raised in chickens, and bound to most applied rIgEf; the ovine-specific monoclonal mouse Ab recognized only in addition to ovine rIgEf3/4 the closely related caprine rIgEf3/4. Significant, positive correlations were detected between binding reactions of the polyclonal Abs in ELISA and percentage protein sequence homology ($p < 0.01$). Thus, the newly described feline and caprine IgE nucleotide sequences and corresponding Abs represent useful tools for further species-specific and comparative allergy and disease-associated research. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Recombinant IgE; Cat; Dog; Horse; Goat; Sheep; Sequence; Homology; ELISA

Abbreviations: Ab(s), antibody(ies); DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; rIgEf, recombinant immunoglobulin E fragment; rIgEf2, rIgEf of second constant domain of epsilon heavy chain; rIgEf3/4, rIgEf of third and fourth constant domains of epsilon heavy chain; RNA, ribonucleic acid; OD405, optical density measured at a wavelength of 405 nm

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1. Introduction

Immunoglobulin E (IgE) is known to play a pivotal role in allergy (Ishizaka et al., 1966) and parasitic infections (Capron et al., 1982; Capron and Dessaint, 1992). Several animal species spontaneously develop IgE-mediated diseases that are of interest as comparative animal models (de Weck, 1995) and/or of major clinical or economic importance (Waller, 1994). Specifically, dogs, cats, and horses are affected with presumed IgE-mediated diseases, such as atopic-like dermatitis of dogs (Scott et al., 1995) and cats (Roosje et al., 1998), allergic airway diseases of cats and horses (Halliwell et al., 1993; Padrid et al., 1995), or insect bite dermal hypersensitivity of horses (Marti et al., 1999; van der Haegen et al., 1999). In ruminant livestock, serum total IgE levels represent a marker for resistance against parasites (Baker and Gershwin, 1993; MacDiarmid et al., 1995; Miller et al., 1996; Kooyman et al., 1997). The emerging resistance to anthelmintic therapies and the economic importance in goat industry (Waller, 1994) warrants searches for natural resistance markers such as tests to evaluate total and/or antigen-specific serum IgE levels in addition to genetic markers such as major histocompatibility complex (Obexer-Ruff et al., 1996) and beta-chain of T cell receptor (Obexer-Ruff et al., 1998).

To study IgE-mediated illnesses within or across species, sequence data of IgE and anti-IgE reagents are a prerequisite. The DNA sequences are available for dog (Patel et al., 1995), horse (Marti et al., 1995; Navarro et al., 1995), and sheep (Engwerda et al., 1992) but it is lacking for cat and goat. In cat, there is evidence for existence of IgE (Powell et al., 1980; Bevier, 1990; Baldwin et al., 1993; DeBoer et al., 1993; Roosje et al., 1998) and recently the first antiserum has been raised against a reagin-rich fraction prepared from feline serum (Gilbert and Halliwell, 1998). In goat, one cross-reactive ovine-specific IgE antibody (Ab) has been described (Kooyman et al., 1997). However, for cat, goat, and horse, there are no commercially available IgE-specific Abs.

The study was aimed to clone and sequence feline and caprine IgE fragments of the third and fourth constant domains (rIgEf3/4) and express them to induce specific Abs. Equine rIgEf2 was also cloned and expressed with recombinant technology. The rIgEf and induced rIgEf-specific Abs were subjected to a comparative study across rIgEf from other species, namely canine (Griot-Wenk et al., 1998), equine (Marti et al., 1997), and ovine rIgEf (Kooyman et al., 1997) to assess homologies between DNA and deduced protein sequences and to characterize binding features of rIgEf-specific Ab to various rIgEf in enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

2.1. Cloning and expression of feline and caprine rIgEf3/4 and equine rIgEf2

Techniques established in previous studies were applied for cloning and expression of rIgEf in *Escherichia coli* as histidine-tagged proteins (Marti et al., 1997; Griot-Wenk et al., 1998). For feline rIgEf3/4, RNA isolated from cat spleen was used as template, for caprine rIgEf3/4 from peripheral blood lymphocytes from a 6-year old, free ranging Saanen goat, and for equine rIgEf2 from a mesenteric lymph node (Marti et al., 1995),

Table 1

Primer sequences for PCR amplification of equine IgE heavy chain fragment cDNA (IgEf2) and feline and caprine IgE heavy chain fragments cDNA (IgEf3/4)

Equine IgEf2	Forward	5'-GCA TGC ATC GTG ACC TTC ACC C-3'
	Reverse	5'-TGT GCA CAA GCG GGC CTG G-3'
Feline IgEf3/4	Forward ^a	5'-CGC CCA CGA TCA CCT GTC TG-3'
	Forward ^b	5'-GTG GTG GAC CTG GCC AAC AC-3'
	Reverse	5'-AGG CGG CTG AAG ACG AAG AA-3'
Caprine IgEf3/4	Forward ^a	5'-CGC CCA CGA TCA CCT GTC TG-3'
	Forward ^b	5'-ACC TGT CTG GTG GTG GAC GTG G-3'
	Reverse ^a	5'-AGG CGG CTG AAG ACG AAG AA-3'
	Reverse ^b	5'-CAG GCG ACT GAA GAT GAA GAA GCC-3'

^a First primers applied.

^b Second primers applied.

respectively. Primers for the feline and caprine fragments were designed based on sequences conserved between species (Table 1, two sets of primer sets were used for unidirectional cloning (Footnotes a and b)) and applied in PCR. To amplify equine rIgEf2, primers were constructed based on the published equine IgE sequence corresponding to nucleotides 730–1050 (Navarro et al., 1995). The cloned PCR products were sequenced twice in both directions. DNA and deduced protein sequence analysis was performed with the computer assisted programs Align and Clone Manager (Scientific and Educational Software). The following published sequences were used for comparison — GeneBank Accession Numbers: canine L36872, equine U15150, L31502, and ovine M84356. The histidine-tagged recombinant protein fragments were analyzed by SDS-PAGE, Coomassie staining, and blotting with a nickel-nitrilotriacetic acid alkaline phosphatase (AP)-labeled conjugate as described (Marti et al., 1997; Griot-Wenk et al., 1998).

2.2. Raising and isolation of Ab induced by rIgEf

Immunization of hens with feline or caprine rIgEf3/4 or equine rIgEf2 and isolation of raised Abs from the egg yolk were carried out according to previously established procedures (Marti et al., 1997; Griot-Wenk et al., 1998).

2.3. ELISA for comparative Ab analysis

An established capture ELISA was utilized (Marti et al., 1997; Griot-Wenk et al., 1998). Corresponding rIgEf were coated to the solid-phase (100 ng/ml): canine (Griot-Wenk et al., 1998) and equine rIgEf2 and feline, canine (Griot-Wenk et al., 1998), equine (Marti et al., 1997), ovine, and caprine rIgEf3/4; the ovine rIgEf3/4 and corresponding specific monoclonal mouse Ab were kindly provided by Prof. Dr. H.D.F.H. Schallig (Department of Parasitology and Tropical Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; Kooyman et al., 1997). After coating and blocking the plates,

they were incubated with rIgEf2- or rIgEf3/4-specific Ab (1–100 µg/ml; Marti et al., 1997; Griot-Wenk et al., 1998). All but the ovine rIgEf3/4-specific monoclonal Ab had been raised in chickens. These chicken Abs were not affinity purified except for the equine rIgEf3/4-specific Ab. Binding of chicken Abs were developed by AP-labeled goat anti-chicken IgG Ab (1:1000; Kirkegaard and Perry Laboratories, Gaithersburg, MA, USA) and binding of the mouse monoclonal anti-ovine rIgEf3/4 Ab by AP-labeled goat anti-mouse Ab (1:4000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), respectively. ELISA results were expressed as the mean optical density measured at a wavelength of 405 nm (OD405) of duplicate wells after subtraction of background controls (coated antigen without primary developing Ab) measured after 30 min incubation time. Reactivity was defined as an OD405 of at least three times above background (>0.3).

2.4. Statistical analysis

Statistical analysis was carried out with the statistical software package SPSS SYSTAT for Windows, Version 7.0. To compare percentage protein homologies of the different rIgEf and binding reactivity in ELISA (OD405), Pearson correlation coefficients (r) were calculated for each Ab. The specific rIgEf used to raise the corresponding Ab was set as reference molecule to calculate the respective protein homologies for each correlation coefficient. The level of significance was set at $p < 0.01$.

3. Results

3.1. Cloning and expression of rIgEf and comparative sequence analysis

The cloned feline rIgEf3/4 was 435 bp long. Its nucleotide and deduced amino acid sequence are illustrated in Fig. 1 (GeneBank Accession Number: AF129114). The amplified feline rIgEf3/4 corresponds to nucleotides 711–1145 of the canine IgE heavy chain cDNA (Patel et al., 1995). The analogous feline and canine products share 81% homology at the nucleotide level and 71% at the protein level, respectively. Analysis of the expressed feline rIgEf3/4 by SDS-PAGE, staining by Coomassie blue, and immunoblotting revealed one major protein band at approximately 15.5 kDa (data not shown).

The nucleotide sequence of equine rIgEf2 (321 bp) was consistent with the published sequence (Navarro et al., 1995). Analysis of the expressed protein revealed a 9.8 kDa band (data not shown).

The amplified caprine rIgEf3/4 was 459 bp long and its nucleotide and deduced amino acid sequence are illustrated in Fig. 2 (GeneBank Accession Number: AF140603). Comparison of the sequences to the closely related ovine species resulted in a percentage identity of 95% at the nucleotide level and 84% at the amino acid level, respectively. The caprine nucleotide sequence corresponds to nucleotides 1110–1579 bp of the ovine IgE heavy chain cDNA. The expressed caprine rIgEf resulted in a 14.8 kDa protein-band (data not shown).

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( 1) GTG GTG GAC CTG GCC AAC ACA GAC GGC ATG ATC CTG ACC TGG TCG CGG
      V  V  D  L  A  N  T  D  G  M  I  L  T  W  S  R

( 49) GAG AAT GGG GAG TCT GTG CAC CCA GAC CCG ATG GTC AAG AAG ACT CAG
      E  N  G  E  S  V  H  P  D  P  M  V  K  K  T  Q

( 97) TAC AAC GGG ACA ATC ACC GTC ACT TCC ACC CTG CCT GTG GAT GCC ACT
      Y  N  G  T  I  T  V  T  S  T  L  P  V  D  A  T

(145) GAC TGG GTT GAG GGG GAG ACC TAC CAA TGC AAG GTG ACC CAT CCG GAC
      D  W  V  E  G  E  T  Y  Q  C  K  V  T  H  P  D

(193) CTG CCC AAG GAC ATC GTG CGC TCC ATT GCC AAA GCC CCC GGC CGG CGT
      L  P  K  D  I  V  R  S  I  A  K  A  P  G  R  R

(241) TTC CCC CCG GAG GTG TAC GTG TTC CTG CCG CCC GAG GGG GAG CCG AAG
      F  P  P  E  V  Y  V  F  L  P  P  E  G  E  P  K

(289) ACC AAG GAC AAA GTC ACT CTC ACG TGC CTG ATC CAG AAC TTC TTT CCC
      T  K  D  K  V  T  L  T  C  L  I  Q  N  F  F  P

(337) CCG GAC ATC TCG GTG CAA TGG CTT CAC AAC GAC AGC CCT GTT CGG ACG
      P  D  I  S  V  Q  W  L  H  N  D  S  P  V  R  T

(385) GAA CAG CAG GCC ACC ACG TGG CCC CAC AAG GCC ACC GGC CCC AGC CCA
      E  Q  Q  A  T  T  W  P  H  K  A  T  G  P  S  P

(433) GCC
      A

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Fig. 1. Nucleotide sequence and deduced amino acid sequences of PCR amplified part of feline IgE (sequence available from GeneBank Accession Number: AF129114).

3.2. ELISA reactivity of seven rIgEf-specific Ab to solid-phase bound rIgEf of various species

Results of binding features in ELISA of Abs (100 µg/ml) included in this study are summarized in Table 2. As expected each Ab reacted with its immunogen and also displayed strongest binding to it except for the ovine rIgEf3/4-specific monoclonal mouse Ab. Very narrow binding characteristics were only observed with the monoclonal mouse Ab specific for ovine rIgEf3/4: it bound to ovine rIgEf3/4 and rIgEf3/4 of the closely related goat. All other Abs were polyclonal raised in chickens and thus reacted to most rIgEf across the species examined. Significant, positive correlations between percentage protein homology and binding reaction in ELISA were detected for all polyclonal Abs (Table 2). A summary on percentage homologies between deduced protein sequences of the available rIgEf available is illustrated for each correlation in Table 2. The amino acid sequence alignment for rIgEf3/4 is shown in Fig. 3. The correlations between binding and protein homology ranged from 0.887 to 0.987 ($p < 0.01$). As shown in Fig. 4, the correlations were 0.887 for feline rIgEf3/4-specific Ab (Fig. 4A), 0.941 for caprine rIgEf3/4-specific Ab (Fig. 4B), and 0.987 for equine rIgEf2-specific Ab (Fig. 4C), respectively. None of the preimmune chicken Ab against feline or caprine rIgEf3/4 or

(1) ACC TGT CTG GTG GTG GAC GTG GCC AGC ACG AAT AAC TCG AGC CTG ACC
T C L V V D V A S T N N S S L T

(49) TGG TCG AGG GCA AAC GGG AAA CCT GTG GAC GCA GAC CCC GTC GAC ATC
W S R A N G K P V D A D P V D I

(97) AAG CAT CAG TTC AAC GGG ACG ATC ACC GTC ACG TCG ACC CTG CCG GTG
K H Q F N G T I T V T S T L P V

(145) GAC GTC ATT GAC TGG GTT GAA GGC GAG ACC TAC TAC TGC AAA GTG AGC
D V I D W V E G E T Y Y C K V S

(193) CAC GGC GAC CTG CCC AAG GAT ATC CAG AGA TCC ATC TCC AAG GAT GTT
H G D L P K D I Q R S I S K D V

(241) GGC AAG CGT GTG GCC CCC AAG GTC TAC GTG TTC TGG CCA GAC AGA AAG
G K R V A P K V Y V F W P D R K

(289) GAG CTG GAG GAT CAG GAG GAG CTC ACC CTC ACC TGC CTG ATC CAG TTC
E L E D Q E E L T L T C L I Q F

(337) CCC AAG GAC ATC TCT GTG CAG TGG CTG CGT AAC AAG AAA CCG CGG GAA
P K D I S V Q W L R N K K P R E

(385) GGC CAG CAC ACC ACC CGC AGC CCG ACA GGG CCG ACA ACA ACA GCC TCG
G Q H T T R S P T G P T T T A S

(433) GCT TCT TCA TCT TCA GTC GCC TGT GAA
A S S S S V A C E

Fig. 2. Nucleotide sequence and deduced amino acid sequences of PCR amplified part of caprine IgE (sequence available from GeneBank Accession Number: AF140603).

Table 2
ELISA results of seven antibodies specific to recombinant IgE fragments (rIgEf) of different species against solid phase-bound rIgEf are illustrated^a

rIgEf-specific antibody applied	Solid phase-bound rIgEf (10 ng/well)							Pearson correlation coefficient <i>r</i>
	Feline rIgEf3/4	Canine rIgEf2	Canine rIgEf3/4	Equine rIgEf2	Equine rIgEf3/4	Ovine rIgEf3/4	Caprine rIgEf3/4	
Feline rIgEf3/4	2.1 ^b /100 ^c	0.1/16	0.2/37	0.2/18	1.0/66	0.4/60	0.4/59	0.887
Canine rIgEf2	0.6/21	3.7/100	2.0/30	1.9/60	0.5/22	0.3/22	0.6/21	0.941
Canine rIgEf3/4	2.4/45	1.8/25	2.9/100	1.8/26	1.9/45	1.8/34	1.8/31	0.889
Equine rIgEf2	1.0/22	2.1/60	0.9/27	3.3/100	1.2/22	1.1/21	1.1/19	0.987
Equine rIgEf3/4	2.6/65	0.4/14	1.0/36	0.7/16	3.6/100	0.7/54	1.6/52	0.908
Ovine rIgEf3/4	0.1/56	0.1/13	0.1/26	0.1/16	0.1/51	1.0/100	1.2/81	NA ^d
Caprine rIgEf3/4	3.2/56	0.3/16	0.7/24	0.6/13	1.2/48	3.6/84	3.8/100	0.941

^a Pearson correlation coefficient (*r*) between ELISA results and protein homologies (%) are further shown for each antibody. The rIgEf, against which the antibody was raised, was applied as reference molecule to calculate corresponding protein homologies; all antibodies were raised in chicken except for the ovine rIgEf3/4-specific monoclonal mouse antibody.

^b Optical density measured at a wavelength of 405 nm.

^c Protein homology (%).

^d NA=not applicable, monoclonal antibody.

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feline   ( 1) ---vvdlantdgmiltwsrengesvhpdpmvkktqyngtitvtstlpvda
canine   ( 1) -----
equine   ( 1) tcl.....vq.ls.n....s..plqrhtlatse.f.k.fs.....t
ovine    ( 1) tcl.....s.nnls.....a..kp..a..ldi.h.f.....nv
caprine  ( 1) tcl...v.s.nnss.....a..kp.da..vdi.h.f.....v

feline   ( 48) tdwvegetyqckvthpdlpkdivrsiakapgrfrppevyvflppegepkt
canine   ( 1) -----
equine   ( 51) ...i.....k.t.s....rev.....k.ls.....edqss
ovine    ( 51) i.....y...s.g.....q...s.dv.k.va.k....w.drk.len
caprine  ( 51) i.....y...s.g.....q...s.dv.k.va.k....w.drk.led

feline   ( 98) kdkvtlttcliqnffpdpdisvqwlhndspvrteqqattwphkatgspas--
canine   ( 26) ..r.....a.....r....iq.d.yt..g...vs.sr..ff
equine   (101) .....a.....r.nvliq.d....r.q..n..d.---
ovine    (101) qeel.....k...k....r..r.kelm.eg.ht..q.dr.dnn.l.ff
caprine  (101) qeel.....--..k.....r.kk.-.eg.ht.rs.tgp.ttas.ss

feline   (146) -----
canine   ( 76) ifsrlevsrvdweqknkftcqvvhalsgsrilqkwvsktpgk
equine   (148) -----
ovine    (151) aysrl-----
caprine  (148) ssvace-----

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Fig. 3. Comparative deduced amino acid sequences of recombinant IgE fragments (rIgEf) of the third and fourth constant domains which were applied in the ELISA. Dots indicate identities with amino acids of the feline rIgEf3/4, dashes indicate gaps introduced for maximal alignment, and numbers in parentheses represent counts of amino acids, respectively.

equine rIgEf2 reacted to the corresponding solid-phase bound rIgEf and all binding reactions of postimmune Abs were concentration-dependent (data not shown).

4. Discussion

The sequence information of IgE is known for many species except for cat and goat. In this study, we provide information on partial DNA sequence of the third and fourth constant domains of the IgE heavy chain of these two species, on binding features of chicken Abs induced by these recombinant proteins in capture ELISA compared to rIgEf of dog, horse, and sheep, and on correlations between protein homologies and ELISA reactivity.

Homology of feline rIgEf3/4 was nearest to the analogous IgE part of the closely related dog (81% at the nucleotide and 71% at the amino acid levels); identity shared with the corresponding IgE sequences of other studied species ranged from 70 to 76% for the nucleotide sequence and 57–66% for the deduced protein sequence, respectively (data not shown). Nevertheless, feline rIgEf3/4-specific Ab did not recognize the canine IgEf3/4 (Fig. 3) which is likely due to the fact that the canine IgEf was mainly from the fourth domain (Griot-Wenk et al., 1998); the protein homology between the applied feline and canine rIgEf3/4 was only 37% (Table 2). Analysis between percentage protein homology and binding reactivity in ELISA of the various rIgEf-specific Abs to rIgEf applied in this

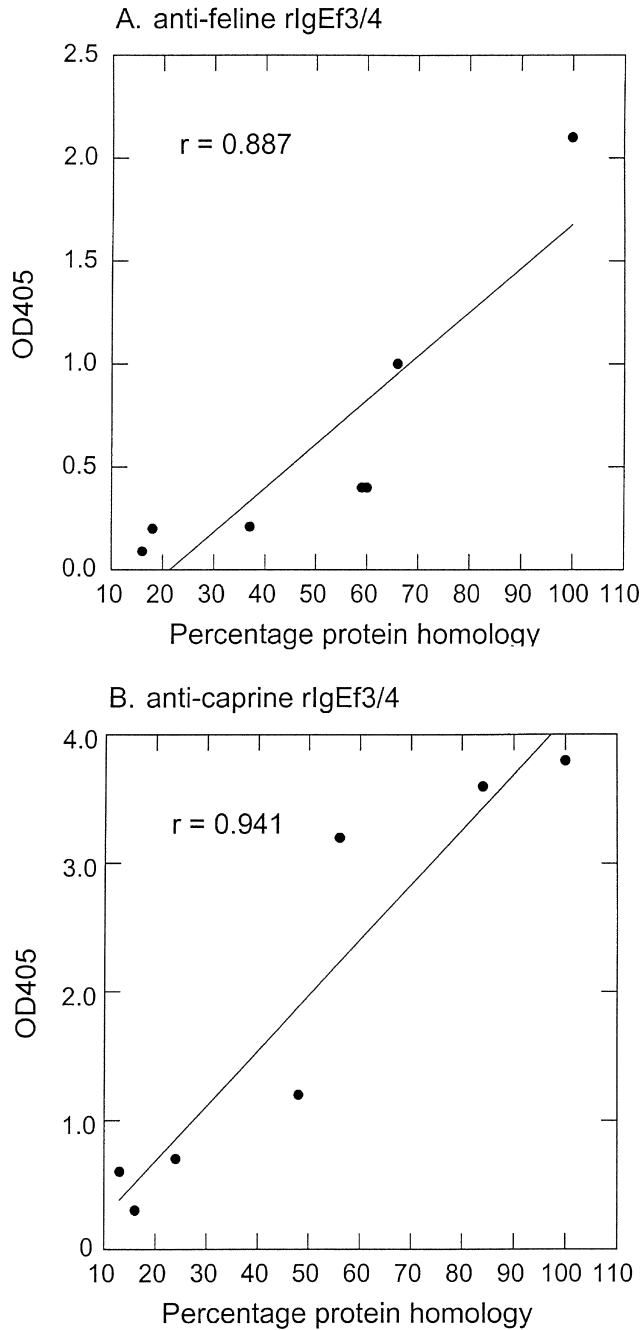


Fig. 4. Pearson correlation coefficients (r , $p < 0.01$) between ELISA results and percentage protein homology of rIgEf are shown for three antibodies: A, B, and C (see below). The rIgEf, against which the antibody was raised, was used as reference molecule to calculate the corresponding protein homologies. Binding of rIgEf-specific antibodies in ELISA to coated rIgEf is expressed as optical density (OD405). A: feline rIgEf3/4-specific Ab, $r = 0.887$; B: caprine rIgEf3/4-specific Ab, $r = 0.941$ and C: equine rIgEf2-specific Ab, $r = 0.987$.

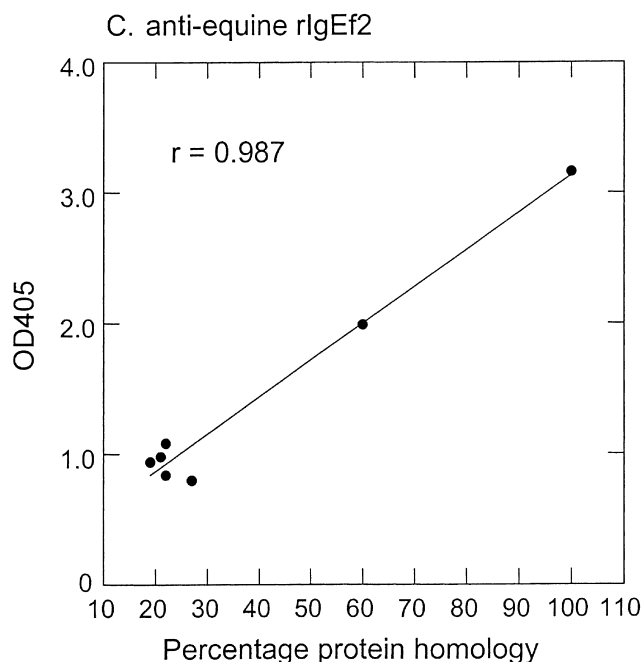


Fig. 4. (Continued).

study revealed significant, positive correlations (Table 2, Fig. 4). Thus, comparative amino acid sequence analysis can to some extent predict trends of binding features of rIgEf-specific Ab to rIgEf (Table 2) which needs to be additionally elucidated for native IgE. Furthermore, positive binding across different rIgEf indicates shared epitopes. The shared amino acid sequence (Fig. 3) in combination with the ELISA data (Table 2) may suggest locations of epitopes which are to be confirmed in future studies by monoclonal Abs. The monoclonal ovine rIgEf3/4-specific mouse Ab recognized its characteristic epitope also on the closely related caprine rIgEf3/4 (Table 2). Thus, this epitope is either only shared between sheep and goat or not accessible or rather different on the other applied rIgEf3/4 and rIgEf2. Similar to our findings, immunoblotting of goat and sheep sera with the same as herein applied monoclonal ovine IgE-specific Ab previously also revealed stronger affinity to caprine IgE (Kooyman et al., 1997).

In conclusion, we provide the partial nucleotide sequence data on the feline and caprine IgE heavy chain and thereby, enable research on IgE-mediated diseases in cat and goat.

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