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Inhibition of passive cutaneous anaphylaxis by synthetic human immunoglobulin E peptide fragments

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In an attempt to determine the regions responsible for type I immediate hypersensitivity, a total of 42 peptide fragments, which cover the CH3–CH4 domains in human immunoglobulin E (IgE), were chemically synthesized. Several peptide fragments located in the amino acid sequences Ala^{329}–Thr^{357} and Arg^{419}–Ala^{463}, inhibited passive cutaneous anaphylaxis (PCA) in vivo. In order to pinpoint the sites responsible for the inhibition of the PCA reaction, various fragment peptides in these two regions were synthesized. As a result, residues Pro^{343}–Leu^{348}, Pro^{426}–Thr^{433}, and Ser^{456}–Thr^{461} were suggested to be involved in type I immediate hypersensitivity.

Immunoglobulin E; Immunoglobulin E peptide fragment; Passive cutaneous anaphylaxis; Immediate hypersensitivity; Fcε receptor.

1. INTRODUCTION

Immunoglobulin E (IgE) is a glycoprotein of molecular weight of approximately 190 kDa which binds reversibly and with high affinity to the specific membrane-bound Fcε receptor on mast cells and basophils [1]. Bridging of the receptor-bound IgE by a specific antigen triggers the secretion of chemical mediators such as histamine, slow-reacting substance of anaphylaxis, and platelet activating factor, which are responsible for type I immediate hypersensitivity [2,3]. By binding to and blocking the Fcε receptor, peptide(s) containing the binding site(s) will inhibit the release of the chemical mediators. To identify such an IgE receptor-binding peptide(s) would lead to the development of an anti-allergic agent for immediate hypersensitivity such as asthma, hay fever and food allergy [4].

It has been suggested by studies with proteolytic peptide fragments of human IgE [5] and its recombinant gene products [6–8], as well as conformational studies [9], that the binding sites in the human IgE molecule are located in the CH3 and/or CH4 domains. The exact binding sites, however, remain unidentified.

In an attempt to precisely locate the binding sites, we chemically synthesized a number of peptide fragments which cover the CH3–CH4 domains and tested their capacity to inhibit the passive cutaneous anaphylaxis (PCA) reaction in vivo.

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Abbreviations: These follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature [(1984) Eur. J. Biochem. 138, 9–39].

2. MATERIALS AND METHODS

2.1. *Peptide synthesis*

All peptides used were synthesized by the stepwise solid-phase method using Fmoc chemistry [10,11] as described previously [12,13]. The purity of each peptide was confirmed by thin-layer chromatography, high-performance liquid chromatography, fast atom bombardment mass spectrometry, sequencing with a gas-phase microsequencer, and amino acid analysis after hydrolyzing in constant-boiling HCl at 110°C for 24 h. The sequences of compounds are shown in Table I.

2.2. *PCA in rats*

The capacity of the synthesized peptides to inhibit degranulation of mast cells in rats in vivo was measured by the PCA test. Diluted mouse anti-egg albumin antiserum [14] (100 μl) was injected intradermally (i.d.) into the back skins of 5 male Wistar rats. After 48 h, 1 ml of a 0.5% Evans' blue solution containing 2 mg of egg albumin was injected intravenously (i.v.). The PCA reaction was determined as a blueing area of the skin 30 min after the antigen challenge. To determine the inhibition of the degranulation by the synthetic peptides, 3 consecutive i.d. injections of each peptide solution (100 μl) were carried out into the same site of the back skin of the rat; 3 and 24 h before the passive sensitization with the antiserum, and at the same time with the antiserum injection. The anti-PCA activity of fragment peptides was expressed as percent inhibition of the blueing area compared with the vehicle control, into which a physiological saline solution was injected three times. Student's *t*-test was used for the statistical analysis.

3. RESULTS

A three-dimensional IgE structure could not be used for designing active peptides, since it has not yet been determined by X-ray crystallography. Therefore, we began with peptides which possibly bind to the Fcε receptor, and finally covered the CH3–CH4 domains

Table I

Effect of the synthetic human IgE peptide fragments on heterologous passive cutaneous anaphylaxis (PCA) in rats

Peptide no.[a]	Compound[b]	Inhibition (%)[c]
1 (329–348)	ADSNPRGVSAYLSRPSPFDL	23.2*
2 (345–356)	PFDLFIRKSPTI	15.9*
3 (349–368)	FIRKSPTITSLVVDLAPSKG[d]	13.2
4 (368–387)	GTVNLTWSRASGKPVNHSTR	8.2
5 (380–399)	KPVNHSTRKEEKQRNGTLTV	9.3
6 (397–416)	LTVTSTLPVGTRDWIEGETY	−6.1
7 (417–437)	QSRVTHPHLPRALMRSTTKTS[d]	20.0*
8 (426–440)	PRALMRSTTKTSGPR	33.2*
9 (438–456)	GPRAAPEVYAFATPEWPGS	25.4*
10 (454–466)	PGSRDKRTLASLI[d]	28.3**
11 (456–470)	SRDKRTLASLIQNFM	25.2**
12 (465–483)	LIQNFMPEDISVQWLHNEV	11.6[e]
13 (479–496)	LHNEVQLPDARHSTTQPR	−2.7
14 (485–504)	LPDARHSTTQPRKTKGSGFF	5.3
15 (497–515)	KTKGSGFFVFSRLEVTRAE	−4.8
16 (514–530)	AEWEQKDEFISRAVHEA	−4.8
17 (531–547)	ASPSQTVQRAVSVNPGK[d]	−2.7
18 (329–357)	ADSNPRGVSAYLSRPSPFDLFIRKSPTIT	32.4*
19 (419–463)	RVTHPHLPRALMRSTTKTSGPRAAPEVYA FATPEWPGSRDKRTLA	32.5*
20 (321–332)	FEDSTKKSADSN[d]	12.5
21 (329–338)	ADSNPRGVSA	2.4
22 (330–334)	DSNPR	−12.2
23 (333–344)	PRGVSAYLSRPS	13.7
24 (339–348)	YLSRPSPFDL	−3.9
25 (343–348)	PSPFDL	17.9*
26 (411–425)	IEGETYQSRVTHPHL[d]	7.0
27 (419–433)	RVTHPHLPRALMRST	−6.2
28 (426–433)	PRALMRST	25.5*
29 (426–434)	PRALMRSTT	9.5
30 (432–438)	STTKTSG	−3.8
31 (434–448)	TKTSGPRAAPEVYAF	−18.9*
32 (441–455)	AAPEVYAFATPEWPG	12.1
33 (449–463)	ATPEWPGSRDKRTLA	5.0
34 (452–461)	EWPGSRDKRT	13.9
35 (453–461)	WPGSRDKRT	1.8
36 (454–461)	PGSRDKRT	−5.8
37 (455–461)	GSRDKRT	16.9**
38 (456–463)	SRDKRTLA	23.0
39 (456–461)	SRDKRT	20.1**
40 (456–460)	SRDKR	15.1
41 (457–461)	RDKRT	23.2
42 (458–461)	DKRT	9.4

[a]Numbers in parentheses indicate the position in human IgE [9].
[b]The single-letter notation for amino acids is used.
[c]Each peptide (100 μg/site) was injected intradermally.
[d]Cys was replaced with Ser.
[e]Percent inhibition of PCA at 30 μg/site i.d.
*$P < 0.05$, **$P < 0.01$, significantly different from the matched vehicle control.

which partially overlap each other. The possible binding regions in the CH3 and CH4 domains for the Fcε receptor were predicted by secondary structure [15], scans for hydrophobic [16] and hydrophilic residues [17], the model of the three-dimensional structure of human IgE constructed by Padlan and Davies [18], and the study

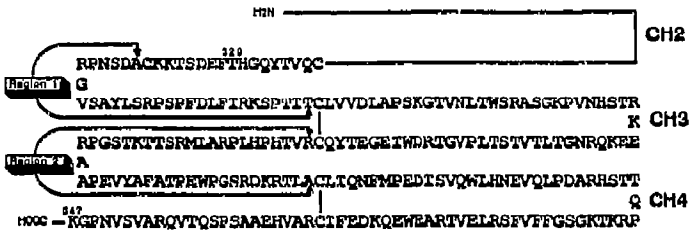

Fig. 1. The amino acid sequence of the CH3 and CH4 domains of the human IgE molecule, and the positions of the two regions (1 and 2) with inhibitory activity on the PCA reaction.

of homologous sequences among immunoglobulins. In the synthesis, the cysteine residues of the disulfide bridges in IgE [19], which are assumed not to be involved in the binding between IgE and its receptor, were replaced by serine residues.

Table I summarizes the results of the inhibitory activity of the synthetic peptides on PCA. Compounds 1, 2, 7, 8, 9, 10 and 11 in a series of compounds 1–17, which cover the CH3–CH4 domains, significantly inhibited the PCA reaction (Table I). These peptides were located in the amino acid sequences Ala^{329}–Thr^{357} (Region 1) and Arg^{419}–Ala^{463} (Region 2), as shown in Fig. 1. Therefore, compounds 18 and 19, which correspond to Regions 1 and 2, respectively, were synthesized and they significantly inhibited the PCA reaction (Table I). In addition, compounds 20–42 in these two regions were synthesized in order to define the sites responsible for the inhibition of PCA. Compounds 25, 28, 37 and 39 inhibited the PCA reaction. Therefore, three sites (Pro^{343}–Leu^{348}, Pro^{426}–Thr^{433}, and Ser^{456}–Thr^{461}) were suggested to be involved in the PCA reaction.

4. DISCUSSION

In the present study, we synthesized a series of 42 peptide fragments of human IgE, which cover the CH3–CH4 domains, and assayed them for their capacity to inhibit the PCA reaction in rats. The peptide fragments which inhibited PCA were located in the sequences Ala^{329}–Thr^{357} in the CH3 domain (Region 1) and Arg^{419}–Ala^{463} in the junction between the CH3 and CH4 domains (Region 2). This finding supported the previous idea that the CH3 and/or CH4 domains played an important role in the binding of IgE to its receptor. Recently, Helm et al. prepared a series of overlapping recombinant Fcε gene products and suggested that the IgE receptor-binding site on human IgE was located within a sequence of 76 amino acids at the CH2/CH3 junction (containing the Gln^{301}–Arg^{376} sequence) [20]. Region 1 is included in Helm's peptide fragment. One of the Fcε receptor binding sites would probably be located in Region 1.

In addition, various fragment peptides in these regions were synthesized in order to locate the sites responsible for PCA inhibition. Residues Pro^{343}–Leu^{348},

Pro^{426}–Thr^{433}, and Ser^{456}–Thr^{461} were identified as being responsible for PCA inhibition in rats. These three sites in human IgE were not homologous with those in human IgA1, IgD, IgG1 and IgM, as determined by sequence homology analysis [21]. Since among human immunoglobulins only human IgE binds to its Fcε receptor with high affinity, the amino acid sequences of the binding sites of IgE should not be homologous with those of other immunoglobulins. On the other hand, these three sites in both human and rodent IgE have a comparatively high degree of homology and the same type of amino acids with regard to charge or hydrophobicity. In general, physiologically important amino acids are conserved. It has also been suggested that human IgE and rodent IgE share common structures in the Fc portion of molecules [22]. The three sites in human IgE were indeed relatively homologous with the corresponding sites in rodent IgE [6,23,24], but not with those in human IgA1, IgD, IgG1 and IgM [21]. In addition, the three active sites were located on the surface of the stereoview model of the Fcε portion of human IgE [18], which was constructed using a known three-dimensional structure of the IgG Fc site. This result indicates that the Fcε receptor binding site(s) is exposed to the surface of the IgE molecule.

From the results, we have tentatively concluded that the three sites (Pro^{343}–Leu^{348}, Pro^{426}–Thr^{433}, and Ser^{456}–Thr^{461}) in the CH3 and CH4 domains are responsible for type I immediate hypersensitivity, and may also contribute to binding of the IgE molecule to its receptor.

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