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(54) Title: IMMUNOMODULATORY PEPTIDE FRAGMENTS OF CD23 AND USES THEREFOR

(57) Abstract: A short oligopeptide derived from the C-terminus of CD23 is capable of blocking the interaction between CD21 and CD23, thereby to down-regulate production of IgE, and is useful in therapy, especially in the treatment of immune-related inflammatory conditions.



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IMMUNOMODULATORY PEPTIDE FRAGMENTS OF CD23 AND USES THEREFOR

INTRODUCTION

The present invention relates to novel peptides and derivatives thereof, to formulations thereof and their use in the treatment and prophylaxis of IgE related conditions.

IgE and two IgE receptors (FcεRI and FcεRII or CD23) are central to allergic disease and potential targets for therapy. Individuals with IgE concentrations above the norm (100 ng/ml serum) have increased risk of developing allergies. The anti-IgE antibody (Omalizumab) has been recently introduced into the clinic to treat moderate to severe asthma and the anti-CD23 antibody (IDEC-152) has shown promising results in clinical trials for asthma, demonstrating that IgE is a viable target. However, antibodies are costly and have limited application. Routinely used drugs for the treatment of allergy act downstream of the IgE-receptor interactions, e.g. anti-histamines, ameliorating immediate hypersensitivity and steroids, allergic inflammation. It is likely that regulating of IgE synthesis may be a much more effective strategy.

CD23 has been shown to regulate a complex network of allergic and inflammatory signaling mechanisms. CD23 functions through interactions with a number of ligands, including IgE, CD21 and several integrins (Gould *et al.*, 2003). The binding of different forms of CD23 to different ligands results in a complex, often seemingly contradictory, role for CD23 in immune regulation.

CD23 exists in multiple active forms, including membrane integrated and soluble, and monomeric and oligomeric, with different functional activities ascribed to the different structures (Sutton and Gould, 1993). The full length human CD23 molecule is 45 kDa type II integral membrane protein, containing an α -helical coiled-coil stalk, responsible for oligomerisation, and a C-type lectin head. A number of soluble fragments of CD23 (37 kDa, 33 kDa, 29 kDa, 25 kDa and 16 kDa) can be detected in B-cell cultures and body fluids in which the coiled-coil stalk has been

proteolytically cleaved in different positions. A matrix metalloprotease is responsible for cleaving CD23 from the membrane (Fourie *et al.*, 2003) but the distinct endogenous proteases responsible for the smaller fragments have not been identified.

The allergenic protease Der p 1 from house dust mite cleaves membrane bound CD23 to a 16 kDa fragment *in vitro* (Schulz *et al.*, 1995; Schulz *et al.*, 1997) and *in vivo* (Shakib *et al.*, 1998). Fragments larger than 29 kDa can trimerise (Beavil *et al.*, 1995) while those smaller than 25kDa contain no α -helical coiled-coil stalk and are generally monomeric. The 16 kDa fragment, termed derCD23, possesses only the lectin-like head, through which all of the ligand binding activities are believed to take place.

CD23 binds IgE with an affinity of about 10^{-7} M and with a 2:1 stoichiometry (Anderson and Spiegelberg, 1981; Shi *et al.*, 1997). The binding site of CD23 on IgE has been shown to be confined to C ϵ 3 (Nissim *et al.*, 1993). The binding site of IgE on CD23 has not been determined, but has been reported to be calcium dependent, suggesting it occurs near one of the conserved calcium binding sites (Richards and Katz, 1990). IgE inhibits the release of soluble CD23 from membrane bound CD23 (Lee *et al.*, 1987). However, modeling suggests that this cannot occur through a direct steric effect on the site of proteolytic cleavage and that IgE binding may stabilise the α -helical coiled-coil stalk (Sutton and Gould, 1993).

Human CD23 has been shown to bind to CD21 (Aubry *et al.*, 1992), which links complement responses to acquired immunity through interaction with C3d (Dempsey *et al.*, 1996). CD21 is a 145 kDa integral membrane glycoprotein composed of 15 or 16 short consensus repeat (SCR) domains. CD23 binds to fragments of CD21 consisting of SCR domains 1-2 (D1-2) and 5-8 (D5-8), through protein-protein and protein-carbohydrate interactions, respectively (Aubry *et al.*, 1994).

CD23 has also been shown to bind CD18/CD11b, CD18/CD11c and the Vitronectin receptor (Aubry *et al.*, 1997; Hermann *et al.*, 1999; Lecoanet-Henchoz *et al.*, 1995) with the interaction mapped to the α -subunit. Additional interactions with

as yet uncharacterised ligands have also been suggested (Kijimoto-Ochiai, 2002; White *et al.*, 1997).

The different forms of CD23 mediate distinct functional activities. Membrane-bound CD23 is thought to contribute to IgE transport across epithelium layers, facilitated antigen presentation, B cell homing and adhesion, and down-regulation of IgE levels in a feedback regulation system (Bonnefoy *et al.*, 1996; Grosjean *et al.*, 1994; Yang *et al.*, 2000). Conversely, soluble CD23 fragments >25 kDa up-regulate IgE expression through ligation to membrane bound CD21 (Bonnefoy *et al.*, 1996), but monomeric fragments inhibit IgE production, possibly through competition with the oligomeric species.

Other cytokine-like activities attributed to CD23, such as rescue of germinal centre B cells from apoptosis, and the growth and differentiation of B cells, T cell and basophils, may be common to the different soluble fragments (Delespesse *et al.*, 1992; Mossalayi *et al.*, 1992).

It has been proposed that CD23 co-ligates membrane IgE and CD21, in a system analogous to the role of complement-mediated stimulation of specific antibody synthesis, resulting in an isotype specific induction of the IgE response (Gould *et al.*, 2003).

CD23 expression is markedly increased in allergic disorders as well as a number of chronic inflammatory conditions such as rheumatoid arthritis and Sjögren's syndrome (Bansal *et al.*, 1992; Ribbens *et al.*, 2000). It plays an important functional role in inflammatory disorders. For example, collagen induced arthritis showed a marked reduction in CD23 knockout mice or in animals treated with monoclonal antibodies against CD23 (Kleinau *et al.*, 1999; Plater-Zyberk and Bonnefoy, 1995). A humanised monoclonal antibody against human CD23 (IDEC-152) has been used in clinical trials for the treatment of allergic asthma (Rosenwasser *et al.*, 2003), allergic rhinitis and chronic lymphocytic leukemia (Mavromatis and Cheson, 2004). These studies highlight the critical regulatory role that CD23 plays in a number of immunological disorders.

The solution structure of derCD23 produced by the dust mite protease Der p 1 has now been solved, by NMR. As noted above, this fragment of CD23 contains the binding sites for both CD21 and IgE, and chemical shift perturbation studies show that these two ligands bind at distinct sites, and that CD23 can bind to both simultaneously.

It has, surprisingly, been found that a single, contiguous peptide sequence in the C-terminus of CD23, when isolated, is capable of blocking binding between CD23 and CD21.

SUMMARY OF THE INVENTION

Thus, in a first aspect, the present invention provides a peptide having at least residues 2 to 7 of SEQ ID NO. 2, or an analogue thereof, and which has no significant affinity for IgE.

Preferably, the peptide comprises amino acids 2 to 7 of SEQ ID NO. 2, or an analogue thereof, and which has no significant affinity for IgE. It will be understood that the residues should be in the order in which they appear in SEQ ID NO.1 and it is, therefore, not envisaged that they should be present in any different order, except where otherwise provided by use of substitution of certain residues, as discussed elsewhere.

Essentially, it has now been established that the hexameric sequence consisting of residues 2 to 7 of SEQ ID NO. 2 is sufficient to block, or interfere with, the interaction between CD23 and CD21. Used *in vivo*, this results in lowering the levels of IgE production, as cross-linking of CD21 molecules is prevented. Accordingly, there is provided a peptide capable of reducing or substantially preventing cross-linking of CD21 molecules.

It is particularly surprising that the region on CD23 is a single, contiguous epitope, especially given that binding regions on IgE and CD21 have been shown to be multiple epitopes. A single epitope is considerably easier to produce, and it is all

the more surprising that such a short epitope is capable of blocking CD23/CD21 binding in its free form.

Without being bound by theory, cross-linking of CD21 is normally believed to occur through 3-way binding with CD21, CD23 and IgE and that, especially when CD23 is in the oligomeric form, the resultant macromolecular complex serves as an efficient stimulus for IgE production.

The same effect may also be achieved by peptide structures comprising a plurality of the epitope defined by amino acid residues 2 to 7 of SEQ ID NO. 2, or analogues thereof, and provides a further aspect of the present invention. Suitable structures are discussed further, hereinbelow, but the provision of multiple epitopes on a larger molecule or oligomer can serve to enable the necessary cross-linking of CD21 molecules *in vivo* to thereby stimulate production of IgE, should this be desirable.

Peptides of the invention have no "significant affinity" for IgE. Naturally occurring CD23 and the derCD23 fragment both possess significant affinity for IgE, while oligomeric CD23 fragments demonstrate a capacity to cross-link CD21 and IgE.

Thus, it is preferred that the peptides of the invention have no binding affinity for IgE greater than general background levels.

Preferably, the peptide is present in concentrations of 100 microM or less when measured under the conditions set out in Example 3 or under or close to standard physiological conditions. These conditions would be readily apparent to the skilled person and may include, for instance, 37 degrees C, pH 7 and standard ionic strength, for instance a salt concentration of about 0.01M to about 0.1M. These conditions may be achieved, for instance, by use of an appropriate buffer such as a saline solution, such as phosphate buffered saline, borate buffered saline, Ringer's solution or a Tris buffer, such as Tris-Cl, Tris buffered saline, or any other physiological buffer.

The affinity of the CD23-CD21 interaction is in the region of 1 microM. The affinity of the peptide for CD21 is, therefore, preferably at least 50 microM, preferably at least 10 microM, or more preferably 1 microM.

It is preferred that the affinity of the peptide is greater than the CD23-CD21 interaction, although this is not essential for the invention to have a positive therapeutic effect. Accordingly, the affinity of the peptide but more preferably 500 nanoM, preferably at least 100 nanoM and more preferably at least 10 nanoM.

More preferably, the peptide need not contain any substantial amount of naturally occurring CD23 N-terminal to the epitope defined by amino acids 2 to 7 of SEQ ID NO. 2, so that there will not generally be any requirement for the peptide to include any of the IgE binding sequence. Given that the simple hexa- and hepta-peptides, or epitopes, of SEQ ID NO. 2 are sufficient to block binding between CD23 and CD21, then longer peptides may only be desirable from the point of view of handling and stability, for example.

BRIEF DESCRIPTION OF THE FIGURES

The invention will now be described by reference to the accompanying figures in which:

Figure 1 shows the effect of the concentration of the CD23 hexapeptide on the binding of derCD23 with CD21. The circular points show the binding of CD21, the triangles show the control.

Figure 2 shows the effect of the CD23 hexapeptide on IgE levels *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "epitope" is a term of convenience pertaining to a short oligopeptide sequence, or its analogue, having a specified activity. The epitope

of the invention may be the hexapeptide defined by amino acids 2 to 7 of SEQ ID NO. 2, for example, or may be a longer peptide or an analogous sequence with similar activity.

The term 'analogue', as used herein, relates to any molecule or substance retaining the blocking activity associated with the epitope, in relation to the binding of CD23 to CD21. Analogues will typically be obtained by modification or variation of the peptide by methods and techniques exemplified and discussed herein, and preferred analogues comprise the epitope together with one or both of an N-terminal and C-terminal blocker, and/or wherein the amino acid residues of the epitope are conservatively substituted, as described hereinbelow. In order to minimise steric hindrance, blockers may, if desired, be separated from the epitope by linker regions, such as one, two, or three, up to about 10 or 15, amino acid residues, especially those characteristic of the original molecule. Such linker regions may also serve as a convenience for processing the peptide of the invention, and may form part of the peptide, even in the absence of a blocker.

Thus, the epitope of the invention comprises at least the amino acid sequence of residues 2 to 7 of SEQ ID NO. 2, or a sequence related thereto in such a manner as to retain blocking activity in relation to the binding of CD23 to CD21.

In the event that it is desired to substitute any of the amino acid residues in the preferred epitope, then it is preferred that any substitutions be conservative in nature, and generally preserve the charge and/or hydrophobicity of the residue. For example, if alanine is substituted, then a suitable alternative amino acid is valine and, slightly less preferred are leucine, isoleucine and glycine. If it is desired to substitute serine, then a suitable substituent is threonine. A glutamic acid residue may suitably be substituted by an aspartic acid residue, while glycine may be substituted by alanine, for example.

In general, substitutions of amino acids are preferred to follow Dayhof's rules for amino acid substitutions (Dayhof, M.D. (1978), Nat. Biomed. Res. Found., Washington D.C., Vol. 5, Supp. 3).

Preferably, only one amino acid substitution is made, although two or more are envisaged. Table 1 below provides an example of suitable analogues based on a single substitution using Dayhof's rules. SEQ ID NO. 4 is the same as residues 2-7 of SEQ ID NO. 2 and is, thus, preferred. In SEQ ID NOS. 5-13, the amino acid residues in bold have been substituted.

Accordingly, it is preferred that the peptide of the invention is any one of, or is selected from the group consisting of, SEQ ID NOS. 4-13.

SEQ ID NO.	POSITION					
	1	2	3	4	5	6
4	S	E	G	S	A	E
5	S	E	G	S	V	E
6	S	E	G	S	L	E
7	S	E	G	S	I	E
8	S	E	G	S	G	E
9	S	E	G	T	A	E
10	T	E	G	S	A	E
11	S	D	G	S	A	E
12	S	E	G	S	A	D
13	S	E	A	S	A	E

Table 1 : analogues of the peptide

The skilled person would be able to provide further analogues of the peptide, including longer peptides and/or peptides with more than substitution, using the above approach and his general knowledge and skill. Such analogues can be easily tested for efficacy using the method set out in Example 3.

In a further aspect, the invention provides polynucleotides encoding the peptides described. It is preferred that the polynucleotide is DNA or RNA, and preferably encodes any of SEQ ID NOS. 1-13. A polynucleotide encoding SEQ ID NO. 4 (or residues 2-7 of SEQ ID NO. 2) is particularly preferred. Where reference is made to a peptide, it will be understood that is also includes reference to a nucleotide encoding it.

Also provided is a pharmaceutical preparation comprising the peptide or a polynucleotide encoding it.

The epitope of the present invention was established using the derCD23 fragment, which has been proteolytically cleaved from the original CD23 (SEQ ID NO. 1), both at the C terminus and the N terminus, and is represented herein as SEQ ID NO. 3. The epitope of the invention can be seen to be the C terminus of derCD23 and, whilst the epitope defined by amino acid residues 2 to 7 of SEQ ID NO. 2 serves to hinder binding between CD23 and CD21, it is probable that one or more naturally occurring amino acids C terminal to the epitope are also involved in binding between CD23 and CD21.

In addition, residue 1 of SEQ ID NO. 2 may also be involved in binding, so that a preferred epitope of the present invention is selected from the entirety of SEQ ID NO. 2 and analogues thereof. More preferred is the heptapeptide of SEQ ID NO. 2.

Where the epitope comprises further, C-terminus amino acid residues, then these are preferably those which are naturally occurring in CD23. These are amino acid residues 299 to 321 in accompanying SEQ ID NO. 1.

While epitopes of the invention may comprise as many as all 23 of the additional C terminus amino acid residues present in CD23, it is generally preferred that they comprise no more than 1 to 12 of these residues. In common with the shorter epitopes referred to above, any of the additional residues may also be conservatively substituted. In addition, as the peptide of SEQ ID NO. 2 already possesses the desired activity, then the additional C terminus sequence is not so restricted in the nature of substitutions, provided that these do not actively interfere with the blocking activity of the epitope. Thus, substitutions by way of deletion, insertion and inversion are also possible, although less preferred, and non-conservative substitutions are also possible, although less preferred.

The peptides of the present invention may be susceptible to *in vivo* enzymic activity, for example. While this may be overcome by the administration of larger

quantities of the peptide in order to achieve the desired activity, it is generally preferred to reduce the susceptibility of the peptide to such attack.

Where the peptides of the present invention are relatively short, then the likelihood of attack by endopeptidases is reduced, and it is often sufficient to block the exposed termini, thereby to substantially reduce peptidase attack.

Suitable N-terminal blocking groups include, but are not limited to: aminobenzoyl; acetyl; 2-aminohexanoic acid; t-butyloxycarbonyl; benzoyl; carboxyphenylpropionyl; 2,4-dinitrophenyl; 5-dimethylaminonaphthyl-1-sulfonyl (dansyl); furylacryloyl; formyl; pyrrolidone carboxyl; (7-methoxy-coumarin-4-yl)acetyl; 7-methoxycoumarin-3-carboxyl; methoxysuccinyl; (4-phenylazo)benzyloxycarbonyl; 3-carboxypropionyl; toluene-p-sulfonyl; and, benzyloxycarbonyl.

Suitable C-terminal blocking groups include, but are not limited to: 7-(4-methyl)coumarylamide; benzylamide; N-(2,4-dinitrophenyl)ethylenediamine; 2-(4-methoxy)naphthylamide; 2,4-dinitroanilinoethylamide; 4-nitroanilide, 2-naphthylamide; nitrobenzylamide; amide; p-aminobenzoate; 4-pyridinium alkoxy; and esters such as ethyl ester, methyl ester, 4-nitrophenyl ester, phenyl ester, and thiobenzyl ester.

Peptides of the invention may also, or in addition, be protected by any other suitable means, such as by substituting elements of the backbone to create a peptidomimetic, by means well known in the art.

The peptides of the invention may be made or used entirely as the peptide which, at its simplest, may be the epitope as defined by residues 2-7 of SEQ ID No. 2, for example, or they may be part of a larger molecule or structure, not necessarily limited to peptides. The terms peptide and protein, and related terms, are used interchangeably herein, and can include both oligopeptides and polypeptides.

Peptides of the invention, especially when limited to oligopeptides of no more than about 50 amino acids, may conveniently be synthesised chemically, by means

well known in the art of organic chemistry, for example. However, it may be desired to incorporate the peptide functionality into larger molecules, such as proteins, including antibodies for example, or it may be desired to produce the peptide biologically as part of, or attached to, a carrier molecule.

Thus, using techniques well known in the art, a suitable vector may be transformed with DNA encoding the epitope, wherein the epitope is part of a larger molecule to be expressed, and the vector expressed in a suitable host. The larger molecule is suitably harvested and processed, if necessary, either to purify it or to separate the desired portion, in any appropriate manner.

Although it is not thought to be essential, it is envisaged that the peptide may also be administered together or alongside other anti-allergy or anti-inflammatory compounds or factors, particularly where effects may be synergistic. These factors may be administered separately or together with the peptide, as appropriate. Potential cooperative effects might allow individual compounds to be administered at lower concentrations to achieve the same effect.

As indicated above, the peptides of the present invention are useful in therapy and, in a further aspect, the present invention provides a peptide as defined above, for use in therapy.

More particularly, the present invention provides the peptides for use in the prophylaxis and treatment of conditions associated with elevated levels of IgE, such as allergic conditions, and especially those associated with autoimmunity, inflammatory disorders or conditions. Exemplary conditions may include, but are not limited to: rheumatoid arthritis; Sjögren's syndrome; allergic asthma; allergic rhinitis; and chronic lymphocytic leukemia.

Also provided is the use of the peptide described above in the manufacture of a medicament for the treatment or prophylaxis of a condition associated with increased IgE levels, as discussed above.

In a further aspect, the invention provides a method of treating a patient with increased IgE levels. The method preferably comprises administering the peptide to a patient in need thereof. Methods of administration are described below, but may be by administration of a pharmaceutically acceptable form of the peptide, for instance orally, intravenously, transdermally, sub-cutaneously, by inhalation or as a suppository.

The patient is preferably selected by an assay for elevated IgE levels, for instance as part of a screening assay.

Methods of administration may also include *genetic engineering techniques*. For instance, the peptide may be encoded by a polynucleotide, preferably under the control of a suitable promoter and/or enhancer, and administered by a gene-gun or by inclusion in a viral vector, such as an attenuated adenovirus, preferably a capsid.

Factors mentioned above, that reduce the levels of CD23 inhibitors may also be administered, either together or alongside the peptide.

Alternatively, or in addition, the method may comprise prophylaxis of a condition associated with increased IgE levels. Preferably, the peptide is administered as part of a daily or weekly regimen for patients with a tendency towards *high IgE levels, in order to keep their IgE levels with acceptable parameters, as would be apparent to the skilled person or the patient's physician.*

Thus, there is also provided, in a further aspect, a kit, comprising a means of administering the peptide, preferably a by self administration, so that the user can keep their IgE levels under control, in much the same way that diabetics do, for instance a small needle connected to a metered supply of the peptide.

Preferably, therefore, the kit also comprises means for testing the levels of IgE in the patient's blood, for instance, associated with an indicator so that the user can identify when administration of the peptide is required and preferably, how much peptide is required. A colour coding or digital readout are examples of such an indicating means.

One advantage of the present peptide is that it is a self peptide and quite small, such that it does not elicit an immune clearance response from the patient.

In general, peptides of the invention will be employed to reduce the stimulus for increased production of IgE although, in certain circumstances, it may be desirable to increase IgE production. In such circumstances, similar considerations apply, as above, regarding the nature of the peptide and its possible inclusion in a larger molecule, or structure, but the peptide will include a plurality of epitopes. In the alternative, there may be a plurality of peptides, each carrying one or more epitopes, each peptide preferably being the same, and wherein the peptides form an oligomeric structure, thereby to offer a plurality of CD21 binding sites to permit CD21 cross-linking and stimulate IgE production.

Peptides of the invention may be administered in any suitable manner effective to allow the peptide to reach the target site. Suitable formulations for small peptides up to large proteins are well known in the art, and form a part of the invention. The preferred small peptides are generally readily soluble, and may be provided in solution form, such as for injection, eyedrops or elixirs, for example, and may be co-formulated with any other suitable ingredients, such as thickeners, antimicrobial agents, flavouring agents, isotonicity modifying agents and other vehicles and excipients.

Peptides of the invention may also be administered as gels and lotions, or in tablet or other suitable form for ingestion, or as pessaries or suppositories, for example.

It is often preferred to localise the peptide in order to ensure site-specific activity, and this may be achieved by administration of a cream, patch or injection, for example. Depending on the nature of the condition to be treated, an injection may be sub-cutaneous, intraperitoneal, intramuscular or intravenous, for example, or the peptide may be administered by a delayed release mechanism, such as a slow-dissolving formulation located subcutaneously, for example.

Other means of administration will be readily evident to those skilled in the art.

The amount of the peptide required for administration will be dependent on the condition it is desired to treat, and whether systemic or local levels of activity are required. Preferred peptides of the invention have a K_i in the micromolar range, as shown in the accompanying Examples, so that any formulation preferably contains sufficient peptide to achieve at least these levels, *in vivo*.

For instance, a concentration of 300 microM, when used under the conditions set out in Example 3, may be appropriate. Concentrations of 10-1,000 microM are preferred, more preferably 100-8,00, more preferably 100-500, more preferably 300-700, more preferably 200-500 microM.

The present invention will be further illustrated by the following, non-limiting Examples.

EXAMPLE 1

EXPERIMENTAL PROCEDURES

Protein expression and purification

Using the numbering from Swiss-Prot accession number P06734, derCD23 is defined by the amino acid sequence from Ser156 to Glu298. The derCD23 construct was subcloned from CD23 cDNA (Ikuta *et al.*, 1987) by PCR. Recombinant derCD23 was expressed in the *E. coli* host strain BL21(DE3), extracted from the cell pellets by the method of Bohmann and Tjian (Bohmann and Tjian, 1989) and refolded by the procedure of Taylor (Taylor *et al.*, 1992). Unlabeled, ^{15}N and ^{13}C -labeled derCD23 were prepared on minimal media or with addition of ^{13}C -glucose/ $^{15}\text{NH}_4\text{Cl}$ to the media. Proteins were purified by hydrophobic interaction chromatography on a phenyl sepharose column (Amersham Pharmacia). Mass spectrometry of these

materials was performed on a Micromass Platform-II ESI mass spectrometer (Waters). Reverse-phase HPLC purified unlabeled, ^{15}N -labeled and ^{13}C , ^{15}N -labeled material had masses of 16143 ± 4 , 16334 ± 3 and 16995 ± 5 Da, respectively, confirming the identity of the material and indicating that that isotope incorporation was highly efficient. Recombinant rCD23, CD21(D1-2), C ϵ 2-4 and C ϵ 3 constructs were prepared as described previously (Beavil *et al.*, 1995; Henry *et al.*, 2000; Szakonyi *et al.*, 2001; Young *et al.*, 1995).

Surface plasmon resonance

All experiments were carried out at 25°C on a BIAcore 2000 instrument (BIAcore AB). A specific binding surface was prepared by coupling derCD23 to a CM5 sensor chip through the amine coupling procedure. Coupling densities of 3000 resonance units (high density) and 400 resonance units (low density) were used. C ϵ 2-4 and CD21(D1-2) in 10mM Hepes pH 7.4, 150mM NaCl, 4mM CaCl₂, 0.005% (v/v) surfactant p20, were injected over the sensor chip at 10 $\mu\text{l min}^{-1}$ with a 3 minute association phase followed by a 15 minute dissociation phase. Double referencing data subtraction methods (Myszka, 1999) were used prior to analysis of rates and equilibrium binding.

NMR spectroscopy

NMR Samples were prepared from concentrated derCD23 dialysed into a buffer containing 25mM Tris, 125mM NaCl and 4mM CaCl₂ at pH 6.8 and placed in a Shigemi NMR tube (Shigemi Corp., Tokyo, Japan). All NMR data were collected at 35°C on homebuilt Omega/GE spectrometers operating at proton frequencies of 500, 600 and 750 MHz. The assignment and structure determination of derCD23 were carried out using standard heteronuclear NMR experiments as described in McDonnell *et al.* (McDonnell *et al.*, 2001). Assignments made use of the following NMR experiments: HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, HNCO, HN(CA)CO, ^{15}N -TOCSY-HSQC (mixing times of 16ms and 25ms) and HCCH-TOCSY (mixing time of 11ms). All triple resonance experiments were collected using trosy-based sequences (Pervushin *et al.*, 1997). Three residues in the N- or C-

termini (residues 156-157, 289) and five residues (residues 252-256) in a central loop were not assigned as well as five other residues in loops and turns (residues 181, 215-216, 249, 263). Several approaches were used to try to help in the assignment of these residues, including changes in temperature, concentration, pH, ionic strength and CaCl_2 concentration, without success. NOE correlations were measured using ^{15}N - and ^{13}C -edited NOESY experiments and a 2D NOESY in D_2O (all mixing times of 125ms). The backbone torsion angles ϕ and ψ were derived for the well defined secondary structure elements using TALOS (Cornilescu *et al.*, 1999). Structure calculations were carried out using the program CNS (v1.1) (Brunger *et al.*, 1998) in an *ab initio* simulated annealing protocol with a two stage torsion angle dynamics and a Cartesian dynamics. From 100 calculations, the 20 structures with the lowest energies were selected and subjected to a four step refinement stage, adjusting for uncertainties in NOE restraints length due to overlap or spectral artefacts.

Backbone ^{15}N relaxation parameters, measuring the rates of ^{15}N longitudinal (R1) and transverse (R2) relaxation and the ^1H - ^{15}N steady-state NOE, were collected using previously described methods (McDonnell *et al.*, 1999). The “model-free” characteristics of the local motions (Lipari, 1982) were derived from these parameters using the program DYNAMICS (Fushman *et al.*, 1997) and hydrodynamic characteristics were calculated using the program ROTDIF (O.Walker, 2004). Fitting the relaxation rate for all structured residues ($S^2 > 0.8$) the following values for overall rotation were calculated, $\tau_c = 9.32 \pm 0.22$ ns, $D_{\parallel}/D_{\perp} = 1.40 \pm 0.06$.

Fast exchanging amide protons were determined using a 2D heteronuclear water exchange filter sequence (WEX II-FHSQC) (Mori *et al.*, 1996). Mixing times of 100 and 500ms for the water magnetisation transfer were set. In the analysis of these data, the intensities of the observed peaks were normalised against a native HSQC and plotted against residue number.

Measurements of translational diffusion coefficients were performed using pulse-field-gradient methods. Sixteen 1-D spectra, with gradient strengths varying from 6.5 to 65 G cm^{-1} , were acquired. Intensities of NMR signals were fitted to standard equations to derive diffusion coefficients (Altieri *et al.*, 1995).

Coordinates

The atomic coordinates of the two derCD23 conformers have been deposited with the Protein Data Bank, accession codes 1T8D (conformation A) and 1T8C (conformation B).

RESULTS

derCD23 interacts with IgE and CD21

CD23 has been shown to interact with multiple ligands, including IgE, CD21 and α M β 2, α X β 2, α v β 3 and α v β 5 integrins; all of these binding events have been attributed to the lectin binding domain of CD23. We used surface plasmon resonance (SPR) methods to characterise the interaction between derCD23 and its main protein ligands, IgE and CD21. Equilibrium constants and interaction kinetics were measured using a BIAcore biosensor 2000 (Biacore AB). For this analysis we used the Fc fragment of IgE (domains C ϵ 2-4), which contains the full binding activity for cellular receptors (Nissim *et al.*, 1993). The CD21 fragment we used was comprised of domains 1 and 2 (D1-2) of the human CD21 protein. Domains 1 and 2 have been implicated in mediating protein-protein interactions with the CD23 receptor, whereas domains 5 to 8 are thought to interact with CD23 through protein-carbohydrate interactions (Aubry *et al.*, 1994).

For the interaction between C ϵ 2-4 and derCD23, we observed a marked change in affinity and off-rates depending on the immobilisation density of derCD23. At a low immobilisation density C ϵ 2-4 and CD21(D1-2) both show fast-on/fast-off kinetics and affinities of 1.7 ± 0.5 and 2.4 ± 1.2 μ M, respectively. At a higher immobilisation density of derCD23, the dissociation rates of C ϵ 2-4 slowed markedly (data not shown). C ϵ 2-4 is a dimer, containing two binding sites for CD23 (Shi *et al.*, 1997), so it is likely that the apparent increase in affinity at higher immobilisation density is a result of a bivalent interaction and the resulting avidity effect. The monovalent CD21(D1-2) construct shows no change in apparent affinity at higher

derCD23 densities (data not shown). The avidity effect is also apparent when the reaction is reversed. Binding of monomeric derCD23 to immobilised Cε2-4 (data not shown) gives similar values to Cε2-4/derCD23 (data not shown) but the binding of a longer CD23 construct (rCD23), that is largely trimeric, shows a significantly higher affinity, with marked differences in the off-rate (data not shown). The interaction kinetics of rCD23 with Cε2-4 are clearly biphasic; this is due the heterogeneity of the oligomerisation state of rCD23, which exists in equilibrium between the monomeric and trimeric states (Beavil *et al.*, 1995). The dissociation curve shows two distinct phases: a minor component that dissociates with a rate of $4 \times 10^{-2} \text{ s}^{-1}$ and a larger component that dissociates at $4 \times 10^{-4} \text{ s}^{-1}$, consistent with the interaction of IgE with a CD23 monomer or the simultaneous interaction with two molecules of a CD23 trimer, respectively.

The binding of IgE and CD21 have been suggested to be calcium dependent (Richards and Katz, 1990). We performed the same binding experiments in the presence of 10mM EDTA, after stripping the derCD23 surface of calcium by overnight incubation in EDTA. The binding of IgE showed a small decrease in affinity, to a K_D value of $15 \pm 4 \text{ } \mu\text{M}$, with the change almost entirely due to changes in the on-rate (data not shown). This is consistent with the idea that the calcium has some effect on the conformation of several loops, as predicted from the structures of other C-type lectins, but that the calcium ions themselves are not an essential part of the binding site. X-ray crystallography of related C-type lectins show up to three calcium binding sites (Drickamer, 1999), all of which are in loops suggested to be involved in IgE binding by chemical shift perturbation studies. The affinity of the CD21(D1-2) binding was largely unaffected by the calcium-free conditions.

The structure of CD23

The three dimensional structure of derCD23 was solved by heteronuclear NMR spectroscopy for use as a framework for studying the interaction of CD23 with CD21 and IgE. Assignment of backbone and side-chains were carried out using both CBCA and NOE-based assignment strategies; trosy-based pulse sequences were employed to improve the efficiency of the triple resonance experiments (Pervushin *et*

al., 1997). Additional data were collected to characterise derCD23 dynamics and hydrodynamics. Analysis of dynamics from ^{15}N relaxation gives a direct measure of backbone flexibility. The derCD23 lectin domain is generally structurally rigid, with highly flexible N- and C-termini and three internal loops ($\alpha 2$ - $\beta 4$, $\beta 4$ - $\beta 5$ and $\beta 5$ - $\beta 6$) showing some degree of flexibility. Five amino acids from a central loop ($\beta 5$ - $\beta 6$) could not be assigned; residues flanking this loop show increased transverse relaxation rates suggesting that this region experiences motions on the ms- μ s time-scale and that loss of peaks are likely due to line broadening.

The structure of derCD23 was based on >2300 NMR-derived structural constraints, including >2100 structurally relevant NOEs, 96 dihedral angle constraints from the program TALOS, and 30 hydrogen bonds, implied by hydrogen-deuterium exchange experiments and local secondary structure. The average density of structural constraints was approximately 25 per residue over the secondary structure elements of the protein.

The three dimensional structure of derCD23 consists of two α -helices which are approximately perpendicular, and eight β -strands forming two anti-parallel β -sheets. Four disulphide bonds contribute to the tertiary structure, while seven tryptophan residues and other conserved residues form a hydrophobic core within the domain. The topology is that of a C-type lectin domain. It shows greatest sequence and structural homology to the lectin domains of DC-SIGN, the H1 subunit of the asialoglycoprotein receptor, and human lung surfactant protein D with sequence identities of 36%, 35% and 26% respectively and root mean squared deviation of 2.7 Å to 2.8 Å over 118 to 126 residues. The global RMSD for the NMR-derived ensemble of 20 structures is 0.26 Å for backbone atoms and 0.8 Å for heavy atoms, in all elements for secondary structure.

derCD23 shows a marked polarity in its electrostatic character. The surface is highly charged but the positive and negative charges are found on opposite faces (data not shown). The highly charged character will affect how CD23 interacts with ligands, particularly in self oligomerisation (discussed further below).

The interaction surface of derCD23 for IgE fragments

CD23 is the low-affinity IgE receptor; the protein is also commonly referred to as FcεRII. In order to investigate the nature of CD23's interaction with IgE, a titration was performed using the Fc region of IgE (Cε2-4) (Young *et al.*, 1995) against ¹⁵N-labeled derCD23. During the titration, most of the peaks in the trosy-HSQC spectrum disappeared due to line broadening. Only peaks mapping to the C-terminus of the derCD23 fragment remained visible (data not shown); the relaxation analysis had indicated that these residues were highly flexible. These observations can be rationalised by assuming a high order oligomerisation involving both Cε2-4 and derCD23. Because Cε2-4 is a homodimeric molecule, and IgE binding has been shown to induce oligomerisation of derCD23 (Shi *et al.*, 1997), the formation of a highly cross-linked oligomer may not be surprising. Analytical ultracentrifuge analysis of this complex shows a heterogeneous mixture of high molecular weight aggregates (data not shown).

To map the ligand binding surfaces of CD23 with IgE we instead titrated ¹⁵N-labeled derCD23 with a monomeric Cε3 domain. It has been reported that the full CD23 binding site is contained within the Cε3 domain (McDonnell *et al.*, 2001). A group of residues were identified by the chemical shift perturbation data revealing a continuous interaction surface for Cε3 on the derCD23 lectin domain. Residues 184, 188-190, 198, 202, 221-222, 224-226, 234-235, 271, 273-274, 276 and 279 showed significant change in chemical shift. These residues form a continuous surface when mapped onto the derCD23 surface (data not shown).

The interaction surface of derCD23 for CD21

We mapped the interaction surface of CD21 on CD23 by titrating an unlabeled fragment of CD21 against a ¹⁵N-labeled derCD23 sample. This CD21 construct is identical to that used in the crystal structure of the CD21 bound to C3d (Szakonyi *et al.*, 2001). Four residues of derCD23 show unambiguous chemical shift perturbation upon addition of CD21(D1-2), all of which are in the C-terminal tail (residues 294, 295, 296, 298). Residue 293 was not assigned, while the peak for residue 297 is in a

region of significant overlap in the ^{15}N -HSQC, making it difficult to identify its shift unambiguously. Residue 292 does not show a change in chemical shift position, therefore we believe the CD21 interaction site involves the C-terminal six residues from this CD23 construct. It is worth noting that derCD23 is proteolytically processed at both the N- and C-termini, and that full-length CD23 has an additional 23 residues at the C-terminus. Consequently, full-length CD23 may have a higher affinity for CD21 than the derCD23 fragment.

Human CD23 binds to CD21, but this interaction does not occur in the murine system (Gould *et al.*, 2003). Although there is very high conservation between the lectin head region of human and mouse derCD23 (62% over 143 residues) the C-terminal region is not conserved in mouse or rat (2 identities over the 10 C-terminal residues). It would seem that region provides the basis of the species-specific recognition of CD21.

The two NMR titration experiments appear to identify independent binding sites for CD21(D1-2) and IgE on CD23. We confirmed the independent nature of the interactions by forming the trimolecular complex [derCD23/C α 3/CD21(D1-2)] through sequential titration experiments and observing an identical set of chemical shift changes seen in the individual binding events.

DerCD23 oligomerisation

In the millimolar concentration range, the derCD23 was observed to exist in monomer-oligomer equilibrium, with an average molecular weight of ~28 kD, estimated from NMR-based translation diffusion measurements. As a result of the change of oligomer state, the chemical shifts of some of the peaks in the HSQC spectrum change as the concentration is reduced, and the equilibrium moves to favour a monomeric state. These residues (211, 212, 213, 229, 231, 232, 240, 241 and 242) map to two distinct regions with opposite charge characteristics, and suggest a possible homo-oligomerisation interaction surface.

X-ray structures of other C-type lectins have been solved in trimeric form, and the arrangement of the subunits is similar for mannose binding protein (MBP),

pulmonary surfactant apoprotein D (SP-D), pulmonary surfactant apoprotein A (SP-A) and tetranectin (Weis and Drickamer, 1994),(Hakansson *et al.*, 1999),(Head *et al.*, 2003),(Nielsen *et al.*, 1997). We predict a different arrangement for the CD23 trimer. MBP, SP-D, SP-A and tetranectin all possess a similar nucleating heptad repeat sequence that allows the formation of a largely hydrophobic interface. Although CD23 has strong homology to these proteins in the lectin-like domain it is not homologous in this interface motif. In addition, an MBP-type arrangement for CD23 would result in an electrostatically repulsive interface. The interface predicted from the NMR chemical shifts produces an electrostatically favourable interface and results in fully solvent exposed interaction sites for IgE and CD21 (data not shown). Interestingly, the distance between two IgE binding sites on the CD23 trimer is approximately 50 Å. This distance is compatible with the simultaneous engagement of the two Cε3 domains from the dimeric IgE Fc and offers an explanation for the affinity difference between monomeric and trimeric CD23 for IgE.

Conclusions

CD23 mediates multiple functions by existing in different states and interacting with several different ligands. Membrane CD23 can be cleaved by different proteases to yield fragments of varying length, which in turn regulates the oligomerisation properties of CD23. Cleavage at the N-terminus controls the oligomerisation state by removing the coiled-coil stalk. These proteolytic events have important regulatory functions, controlling the affinity of CD23 for its ligands. The IgE binding site lies entirely in the lectin head of CD23; consequently proteolytic events leave the binding site intact. Instead, proteolysis regulates the affinity for IgE by controlling the oligomerization state of CD23. The difference in affinity of monomeric CD23 and trimeric CD23 for IgE is approximately 40-fold (K_{DS} of approximately 2μM and 50nM, respectively). Our model of the CD23 trimer suggests an explanation for this through an avidity effect; the distance of the IgE interaction surfaces in the trimer is consistent with the simultaneous engagement of two Cε3 domains from IgE. In contrast, the interaction with CD21(D1-2) appears to be independent of the oligomerisation state of CD23, as would be expected from an interaction with 1:1 stoichiometry, but here proteolytic cleavage might affect CD21

by directly interfering with the binding site. The house dust mite protease Der p 1 cleaves CD23 at both N- and C-termini resulting in a soluble fragment comprised of residues 156 to 298. NMR mapping experiments identify residues 293 to 298 of derCD23 as the binding site for CD21(D1-2). A full-length CD23 molecule would have additional residues at the C-terminus that could potentially interact with CD21. Another naturally occurring proteolytic pathway, using as yet unidentified endogenous proteases, results in a 16 kDa soluble product consisting of residues 150 to 288 (Shi *et al.*, 1997), which lacks the entire CD21 interaction site. This suggests that C-terminal cleavage is a potential mechanism for regulating CD23 interactions with CD21.

EXAMPLE 2

A peptide from the derCD23 C-terminus competitively inhibits the interaction with CD21

Relaxation analysis of derCD23 showed the C-terminus to be highly flexible, and yet this region mediates the interaction with CD21(D1D2). It seemed possible that a short, synthetic peptide would maintain this activity, and a seven amino acid peptide (sequence ASEGSAE) was synthesised and tested for its ability to inhibit derCD23/CD21(D1D2) binding, using a BIAcore biosensor. The small mass of the peptide makes it difficult to monitor direct binding to CD21, so a competition experiment was employed.

CD21(D1D2) was preincubated with various concentrations of the CD23 peptide and allowed to come to equilibrium. This equilibrium mixture was then injected over a derCD23 surface and the amount of unbound CD21 calculated based on the change in the SPR signal. The C-terminal peptide inhibited the derCD23/CD21(D1D2) interaction with an estimated K_D of 31 ± 9 nM. As a control for the specificity of the peptide, it was also assayed for inhibition of Cε2-4 binding. Over the concentration range tested, the C-terminal peptide showed no inhibition of Cε2-4 binding. The results are shown in accompanying Figure 1.

EXAMPLE 3

A peptide from the derCD23 C-terminus inhibits IgE-production in a tonsillar B cell culture

Methods

Cell culture:

Fresh tonsils were obtained from Guy's Hospital (London, UK) from patients undergoing routine tonsillectomy. Tonsil cells were teased from palatine tonsils within 3 hr of surgery, and T cells were removed by rosetting with sheep red blood cells. B cells were collected from the interface of Ficoll-paque and washed twice in phosphate-buffered saline (PBS) to yield a population of $\geq 70\%$ B cells.

IgE production in tonsillar B cell cultures:

B-cell culture systems were set up essentially as previously described (Wheeler *et al.*, 1998). B cells were cultured at 1×10^5 cells/well with interleukin-4 (50 IU/ml) and anti-CD40 (0.5 $\mu\text{g/ml}$), in the absence or presence of the C-terminal CD23 hexapeptide (residues 2-7 of SEQ. ID NO. 2) at a concentration of 300 μM , a concentration significantly higher than its measured K_D . Cell cultures were incubated for 5 days at 37° in a 5% CO₂ incubator. Cell-free supernatants were analyzed for IgE levels by enzyme-linked immunosorbent assay (ELISA).

Results

In the presence of suboptimal levels of IL-4 and anti-CD40, B-cell cultures produced IgE at a level of 27 ng/ml (Figure 2). In this system, IgE production is dependent on soluble CD23 levels, and CD23 inhibitors suppress IgE production (Wheeler *et al.*,

1998). When the C-terminal CD23 peptide was added to the B cell culture, significantly lower levels of IgE were produced (Figure 2). We have shown that this peptide specifically inhibits the interaction between CD23 and CD21 (Figure 1). We suggest that the mechanism of IgE suppression occurs through the inhibition of the CD23/CD21 interaction and that the peptide offers a novel mechanism for down-regulating IgE and controlling allergic disorders.

The results are shown in Fig 2.

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Yang, P. C., Berin, M. C., Yu, L. C., Conrad, D. H., and Perdue, M. H. (2000). Enhanced intestinal transepithelial antigen transport in allergic rats is mediated by IgE and CD23 (FcεRII). *J Clin Invest* 106, 879-886.

Young, R. J., Owens, R. J., Mackay, G. A., Chan, C. M., Shi, J., Hide, M., Francis, D. M., Henry, A. J., Sutton, B. J., and Gould, H. J. (1995). Secretion of recombinant human IgE-Fc by mammalian cells and biological activity of glycosylation site mutants. *Protein Eng* 8, 193-199.

Details of SEQ ID No. 3

derCD23: CD23 cleaved by protease Derp1
AA_SEQUENCE 1.0

Derp1 Length: 143 October 26, 1998 10:45 Type: P Check: 5577 ..

Summary for whole sequence:

Molecular weight = 16144.87 Residues = 143
Average Residue Weight = 112.901 Charged = -4
Isoelectric point = 6.00
Extinction coefficient = 45430

Residue	Number	Mole Percent	..
A = Ala	9	6.294	
B = Asx	0	0.000	
C = Cys	8	5.594	
D = Asp	10	6.993	
E = Glu	9	6.294	
F = Phe	6	4.196	
G = Gly	14	9.790	
H = His	5	3.497	
I = Ile	4	2.797	
K = Lys	7	4.895	
L = Leu	7	4.895	
M = Met	3	2.098	
N = Asn	5	3.497	
P = Pro	6	4.196	
Q = Gln	5	3.497	
R = Arg	8	5.594	
S = Ser	12	8.392	
T = Thr	7	4.895	
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W = Trp	7	4.895	
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Z = Glx	0	0.000	

Claims:

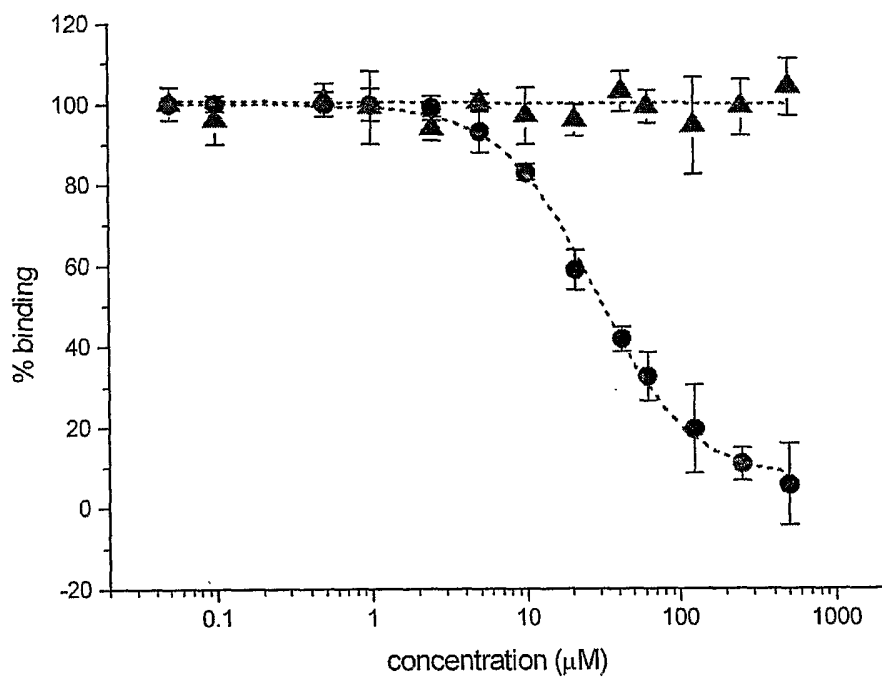
1. A peptide having at least residues 2 to 7 of SEQ ID NO. 2, or an analogue thereof, and which has no significant affinity for IgE.
2. A peptide according to claim 1, having at least residues 1 to 7 of SEQ ID NO. 2.
3. A peptide according to any preceding claim, comprising at least residues 2 to 7 of SEQ ID NO. 2.
4. A peptide according to any preceding claim, comprising at least residues 1 to 7 of SEQ ID NO. 2.
5. A peptide according to any preceding claim, capable of reducing or substantially preventing cross-linking of CD21 molecules.
6. A peptide according to any preceding claim, wherein the affinity of the peptide for CD21 is at least 100 nanoM.
7. A peptide according to any preceding claim, wherein the affinity of the peptide for CD21 is at least 10 nanoM.
8. A peptide according to any of claims 1-2 and 5-7, having a sequence selected from the group consisting of SEQ ID NOS. 4-13.
9. A peptide according to claim 6, having the sequence of SEQ ID NO. 4.
10. A peptide according to any preceding claim, which is blocked at at least one of the N- and C- termini.
11. A peptide according to any preceding claim having at least one, further C-terminus residue, selected from the group consisting of residue 299 of SEQ ID No.1

and sequences commencing with said residue 299 and terminating at or before residue 321 of SEQ ID No.1.

12. A peptide according to any preceding claim, for use in therapy.
13. A peptide according to any of claims 1 to 11, for use in the treatment of an inflammatory condition.
14. A peptide according to claim 13, for use in the treatment or prophylaxis of: rheumatoid arthritis; Sjögren's syndrome; allergic asthma; allergic rhinitis; and chronic lymphocytic leukemia.
15. A polynucleotide encoding a peptide according to any preceding claim.
16. A polynucleotide according to claim 15, encoding the peptide sequence of any of SEQ ID NOS. 1-13.
17. A pharmaceutical preparation comprising the peptide according to any of claim 1-14, or a polynucleotide according to claim 15 or 16.
18. A pharmaceutical preparation according to claim 17, adapted for administration together or alongside anti-allergy or anti-inflammatory compounds.
19. Use of the peptide according to any of claims 1-14 in the manufacture of a medicament for the treatment or prophylaxis of a condition associated with increased IgE levels.
20. A method of treating a patient with increased IgE levels, comprising comprises administering the peptide to a patient in need thereof.
21. A method according to claim 20, comprising administration of a pharmaceutically acceptable form of the peptide to the patient.

22. A method according to claim 20 or 21, wherein the patient is selected by an assay for elevated IgE levels.
23. A method for the prophylaxis of a condition associated with increased IgE levels, comprising administering a peptide according to any of claims 1-14 to the patient.
24. A kit comprising a means of administering a peptide according to any of claims 1-14 and a metered supply of the peptide.
25. A kit, according to claim 24, further comprising means for testing the levels of IgE in the patient's blood.

1/2

**Fig. 1**

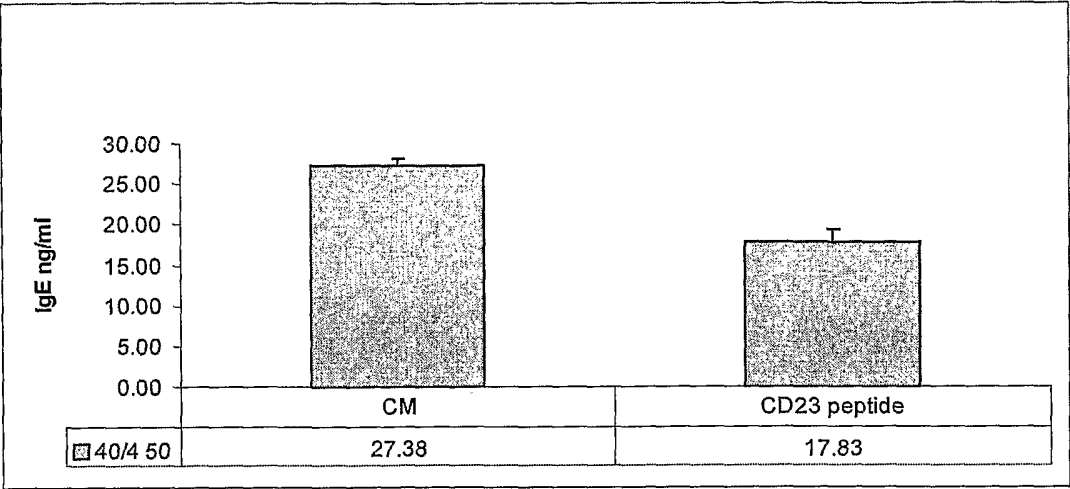


Fig. 2

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INTERNATIONAL SEARCH REPORT

International application No
B2005/004923

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, PAJ, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHULZ OLIVER ET AL: "Cleavage of the low-affinity receptor for human IgE (CD23) by a mite cysteine protease: Nature of the cleaved fragment in relation to the structure and function of CD23" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, no. 3, 1997, pages 584-588, XP009063071 ISSN: 0014-2980 abstract item 3.2 ----- -/-	1-25

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

9 March 2006

Date of mailing of the international search report

30/03/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Griesinger, I

INTERNATIONAL SEARCH REPORT

International application No

PC/GB2005/004923

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HEWITT CR ET AL: "A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 182, November 1995 (1995-11), pages 1537-1544, XP002101668 ISSN: 0022-1007 abstract	1-25
A	SHI JIANGUO ET AL: "Interaction of the low-affinity receptor CD23/Fc-epsilon-RII lectin domain with the Fc-epsilon-3-4 fragment of human immunoglobulin E" BIOCHEMISTRY, vol. 36, no. 8, 1997, pages 2112-2122, XP002371176 ISSN: 0006-2960 abstract	1-25
A	SUTTON B J ET AL: "IGE AND IGE RECEPTORS" ALLERGY AND ALLERGIC DISEASES, BLACKWELL SCIENTIFIC PUBLISHER,, GB, 1997, pages 81-95, XP000783964 page 87, left-hand column, paragraph 1 - page 89, right-hand column, last paragraph page 92, left-hand column, paragraph 2 - right-hand column, paragraph 2	1-25
P,A	HIBBERT RICHARD G ET AL: "The structure of human CD23 and its interactions with IgE and CD21" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 202, no. 6, September 2005 (2005-09), pages 751-760, XP002371177 ISSN: 0022-1007 abstract	1-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/004923

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 20-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.