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(54) Title: IMMUNOGLOBULIN VARIANTS FOR SPECIFIC FC EPSILON RECEPTORS

(57) **Abrégé/Abstract:**

Two classes of polypeptides derived from human IgE are described. One class binds selectively to the high affinity IgE receptor on mast cells and basophils, but not to the low affinity IgE receptor on B-cells, monocytes, eosinophils and platelets. The other class binds to the low affinity receptor, but not the high affinity receptor. The differential binding polypeptides of this invention are useful in diagnostic procedures for IgE receptors or in the therapy of IgE-mediated disorders such as allergies. They also are useful in preparing antibodies capable of binding regions of IgE that participate in receptor binding.



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| <b>(21) International Application Number:</b> <b>PCT/US92/06860</b><br><b>(22) International Filing Date:</b> <b>14 August 1992 (14.08.92)</b><br><br><b>(30) Priority data:</b><br><div style="display: flex; justify-content: space-between;"> <div> <b>744,768</b><br/> <b>879,495</b> </div> <div> <b>14 August 1991 (14.08.91)</b><br/> <b>7 May 1992 (07.05.92)</b> </div> <div> <b>US</b><br/> <b>US</b> </div> </div><br><b>(60) Parent Application or Grant</b><br><b>(63) Related by Continuation</b><br><div style="display: flex; justify-content: space-between;"> <div> <b>US</b><br/> <b>Filed on</b> </div> <div> <b>744,768 (CIP)</b><br/> <b>14 August 1991 (14.08.91)</b> </div> </div><br><b>(71) Applicant (for all designated States except US):</b> <b>GENEN-TECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).</b> |  | <div style="text-align: center; font-size: 2em; font-weight: bold;">2113813</div><br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only) :</b> <b>JARDIEU, Paula, M. [US/US]; 854 Hilldale Avenue, Berkeley, CA 94708 (US). PRESTA, Leonard, G. [US/US]; 1900 Gough Street, San Francisco, CA 94109 (US).</b><br><br><b>(74) Agents:</b> <b>ADLER, Carolyn, R. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).</b><br><br><b>(81) Designated States:</b> <b>AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).</b><br><br><b>Published</b><br><i>With international search report.</i> |                                                                                                                                                |

**(54) Title: IMMUNOGLOBULIN VARIANTS FOR SPECIFIC FC EPSILON RECEPTORS**

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5          β-strand A          loop AB          β-strand B
360  X D S N P R G V S A Y L S R P S P F D X L F I R K S P T I T
                                     1,7                      8

10
          _____ loop BC          β-strand C          loop CD
390  C L V V D L A P S K G T V N L T W S R X A S X X G K P V N H
                        2                      9                      3

15
          β-strand D loop DE          β-strand E          loop EF
420  S T R K E E K O R X N X X G T L T V T S T L P V G T R D W I
                        6                      10                      4

20
          _____ β-strand F          loop FG          β-strand G
450  E G E T Y O C R V T H P H L P R A L X M R S T T K T S G P
                        11                      5                      12

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### (57) Abstract

Two classes of polypeptides derived from human IgE are described. One class binds selectively to the high affinity IgE receptor on mast cells and basophils, but not to the low affinity IgE receptor on B-cells, monocytes, eosinophils and platelets. The other class binds to the low affinity receptor, but not the high affinity receptor. The differential binding polypeptides of this invention are useful in diagnostic procedures for IgE receptors or in the therapy of IgE-mediated disorders such as allergies. They also are useful in preparing antibodies capable of binding regions of IgE that participate in receptor binding.

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## IMMUNOGLOBULIN VARIANTS FOR SPECIFIC FC EPSILON RECEPTORS

Background of the Invention

5 This invention relates to amino acid sequence variant anti-IgE antibodies and to polypeptides containing IgE sequences, especially IgE antagonists and to polypeptides capable of differential binding to FcεRI and FcεRII.

10 IgE is a member of the immunoglobulin family that mediates allergic responses such as asthma, food allergies, type 1 hypersensitivity and the familiar sinus inflammation suffered on a widespread basis. IgE is secreted by, and expressed on the surface of, B-cells. IgE synthesized by B-cells is anchored in the B-cell membrane by a transmembrane domain linked to the mature IgE sequence by a short membrane  
15 binding region. IgE also is bound to B-cells (and monocytes, eosinophils and platelets) through its Fc region to a low affinity IgE receptor (FcεRII, hereafter "FCEL"). Upon exposure of a mammal to an allergen, B-cells are clonally amplified which synthesize IgE that binds the allergen. This  
20 IgE in turn is released into the circulation by the B-cells where it is bound by B-cells (through the FCEL) and by mast cells and basophils through the so-called high affinity receptor (FcεRI, hereinafter "FCEH") found on the surface of the mast cells and basophils. Such mast cells and basophils  
25 are thereby sensitized for allergen. The next exposure to the allergen cross-links the FcεRI on these cells and thus activates their release of histamine and other factors which are responsible for clinical hypersensitivity and anaphylaxis.

30 The art has reported antibodies capable of binding to FCEL-bound IgE but not IgE located on FCEH (see for example WO 89/00138 and US patent 4,940,782). These antibodies are disclosed to be clinically advantageous because they bind to IgE found on B-cells or circulating free in the body, but do  
35 not bind to FCEH and thus will not activate mast cells or basophils. In addition, various amino acid sequence variants of immunoglobulins are known, e.g., "chimeric" and "humanized" antibodies (see, for example, U.S. Patent

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4,816,567; WO 91/09968; EP 452,508; and WO 91/16927). Humanized antibodies are immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub>, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance as will be more further described infra. Also known per se are monovalent and bispecific antibodies.

It is generally understood that FCEH, like FCEL, binds to recognition site(s) in the IgE constant (Fc) domain. The IgE recognition site(s) for the two receptors are poorly defined, despite considerable effort in the past directed to the problem.

Over the past decade several studies have been undertaken to determine which portion of the IgE molecule is involved in binding to FcεRI and FcεRII. Essentially three approaches have been tried. First, peptides corresponding to specific portions of IgE sequence have been used as either competitive inhibitors of IgE-receptor binding (Burt et al., Eur. J. Immun., 17:437-440 [1987]; Helm et al., Nature, 331:180-183 [1988]; Helm et al., Proc. Natl. Acad. Sci., 86:9465-9469 [1989]; Vercelli et al., Nature, 338:649-651 [1989]; Nio et al., Peptide Chemistry, 203-208 [1990]) or to elicit anti-IgE antibodies which would block IgE-receptor interaction (Burt et al., Molec. Immun. 24:379-389 [1987]; Robertson et al., Molec. Immun., 25:103-113 [1988]; Baniyash et al., Molec. Immun. 25:705-711 [1988]). The most effective competitive

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peptide was a sequence that was 1000-fold less active than IgE (Burt et al., Eur. J. Immun., 17:437-440 [1987]).

5 Helm et al., Proc. Natl. Acad. Sci., 86:9465-9469 (1989) found that a peptide corresponding to IgE residues 329-409 blocked in vivo sensitization of human basophil granulocytes with human IgE antibodies. Further studies indicated that residues 395-409 were not essential for binding of the 329-409 peptide to Fc $\epsilon$ RI (Helm et al., Proc. Natl. Acad. Sci., 86:9465-9469 [1989]). Note that the IgE sequence variants 10 described below had the sequence of Padlan et al., Mol. Immun., 23:1063 (1986), but that the immunoglobulin residue numbers used herein are those of Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. 1987).

15 Vercelli et al., Nature, 338:649-651 (1989) used recombinant IgE peptides as well as anti-Fc $\epsilon$  monoclonal antibodies to investigate the B-cell (Fc $\epsilon$ RII) binding site of human IgE. They concluded that the Fc $\epsilon$ RII binding site is in Fc $\epsilon$ 3 near K399-V402.

20 Burt et al., Eur. J. Immun., 17:437-440 (1987) investigated seven peptides for competition against rat IgE in binding to rat mast cells. Their most active peptide, p129, was 1000-fold less active than IgE. p129 corresponds to human sequence 439-453 which includes loop EF. Another of 25 their peptides, p130, corresponding to residues 396-419 in the Fc $\epsilon$ 3 domain, had no activity.

Robertson et al., Molec. Immun., 25:103-113 (1988) assessed IgE binding by sequence-directed antibodies induced by several synthetic peptides. They concluded that the 30 sequence defined by their  $\epsilon$ -peptide-4 (corresponding to residues 446-460), was not significantly involved in receptor binding while the sequence defined by their  $\epsilon$ -peptide-3 (corresponding to residues 387-401), was likely to be proximal to the IgE-receptor recognition site.

35 Nio et al., Peptide Chemistry, 203-208 (1990) evaluated numerous peptides with respect to their ability to inhibit histamine release by human basophils in vitro. Only one peptide (peptide 2, Table 1), exhibited specific inhibition;

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this peptide encompassed residues 376-388. However, a larger peptide which incorporated this sequence (peptide 3, Table 1), had no inhibitory activity.

Second, mutations in IgE have been partially explored. Schwarzbaum et al., Eur. J. Immun., 19:1015-1023 [1989] (supra) found that a point mutant P404H (P442H by the numbering system used herein) had 2-fold reduced affinity for FcεRI on rat basophilic leukemia (RBL) cells, but the interpretation of this finding is controversial (Weetall et al., J. Immunol., 145:3849-3854 [1990]).

Third, chimeric molecules have been constructed. Human IgE does not bind to the murine receptor (Kulczycki Jr., et al., J. Exp. Med., 139:600-616 [1974]) while rodent IgE binds to the human receptor with a reduced affinity (Conrad, et al., J. Immun., 130:327-333 [1983]); human IgG1 does not bind to IgE receptors (Weetall et al., J. Immun., 145:3849-3854 [1990]). Based on these observations, several groups have constructed human-murine chimeras or human IgE-IgG chimeras. Weetall et al., J. Immun., 145:3849-3854 (1990) made a series of human IgG1-murine IgE chimeras and concluded that the Fcε2 and Fcε3 domains are involved in binding murine FcεRI while the Fcε4 domain is unlikely to be involved in binding to murine FcεRI (but may possibly be involved in binding to FcεRII). However, these conclusions are uncertain since they rest primarily on lack of binding by chimeras and three of five chimeras lacked some interchain disulfide bonds.

Nissim et al., EMBO J., 10:101-107 (1991) constructed a series of human-murine IgE chimeras and measured binding to RBL cells and concluded that the portion of IgE which binds with high affinity to the specialized Fcε receptor on RBL cells could be assigned to Fcε3.

The results reported by these authors (e.g. Helm et al. and Burt et al.) are inconsistent. Further, in the case of anti-IgE antibodies it is difficult to eliminate the possibility of nonspecific blocking due to steric hindrance (Schwarzbaum et al., Eur. J. Immun., 19:1015-1023 [1989]). It is apparent that considerable confusion exists in the art as to the domains of IgE Fc which are involved in the binding

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of IgE to FcεH or in the maintenance of IgE conformation responsible for IgE binding to FcεH.

Summary of the invention

5 Broadly, the present invention related to antibodies which are capable of binding to FcεRII bound IgE but which are substantially incapable of binding to FcεRI-bound IgE.

10 According to one aspect, the present invention provides an antibody which is capable of binding to FcεRII-bound IgE but is substantially incapable of binding to FcεRI-bound IgE, comprising a human recipient antibody into which have been substituted at one or more of positions 30, 30b, 30d, 33, 53, 91, 92, 93 and 94 in the light chain and positions 27, 28, 29, 29a, 31, 33, 34, 50, 52, 53, 54, 55, 58, 95, 97, 98, 99, 100 and 101 in the heavy chain, residues from analogous positions in donor antibody MAE11, MAE13 or MAE15 having the light and heavy chain amino acid sequences as set out in SEQ ID NO:2 to 7, or a donor antibody having the characteristics possessed by the MAE11 antibody, in particular in binding soluble IgE, binding IgE-bearing B cells, blocking IgE binding to FcεRI and FcεRII, inhibiting in vitro IgE production and failing to bind to IgE coated basophils.

25 One preferred embodiment is an antibody comprising the heavy and light chain sequences of humaellver.1, 2, 3, 4, 5, 6, 7, 7a, 8, 8a, 8b or 9, wherein humaellver.1 has the heavy and light chain amino acid sequences as set out in SEQ ID NO: 8 and 9, and humaellver.2-9 have the heavy and light chain amino acid sequences of humaellver.1, further incorporating the modifications shown in Table 5.

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Another preferred embodiment is an antibody comprising heavy and light chain amino acid sequences of humaellver.1 as set out in SEQ ID NO: 8 and 9, said heavy chain sequence being substituted at position 60 with asparagine, at position 61 with proline and position 67 with isoleucine.

The differential binding polypeptides of this invention are useful in diagnostic procedures for IgE receptors or in the therapy of IgE-mediated disorders such as allergies. They also are useful in preparing antibodies capable of binding regions of IgE that participate in receptor binding.

In an embodiment of this invention, variant anti-IgE antibodies are provided for use in diagnosis or for the therapy or prophylaxis of allergic and other IgE-mediated disorders. In particular embodiments of this invention anti-IgE variant antibodies are provided in which one or more human (recipient) light chain residues 4, 13, 19, 24, 29, 30, 33, 55, 57, 58, 78, 93, 94, or 104, or heavy chain residues 24, 37, 48, 49, 54, 57, 60, 61, 63, 65, 67, 69, 78, 82, 97 or 100 have been modified, preferably by substitution with the

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residue found in the corresponding position in the donor (generally murine) antibody. In preferred embodiments, the selected residues are light chain 13, 19, 58, 78, or 104, or heavy chain residues 48, 49, 60, 61, 63, 67, 69, 82 or 82c, and most preferably are heavy chain residues 60, 61 or light chain residue 78.

In other embodiments we provide antibodies which are capable of binding FCEL-bound IgE but which are substantially incapable of binding FCEH-bound IgE or inducing histamine release from mast cells or basophils, comprising a human Kabat CDR domain into which has been substituted a positionally analogous residue from a Kabat CDR domain of the murine anti-huIgE antibodies MAE11, MAE13, MAE15 or MAE17. Also provided herein are bispecific antibodies and IgE-monovalent antibodies; and humanized antibodies exhibiting an affinity for IgE which ranges from about 0.1 to 100 times that of MAE11.

#### Brief Description of the Figure

FIG. 1 depicts the sequence of human IgE Fc $\epsilon$ 2 and Fc $\epsilon$ 3 (SEQ. ID. 1). This particular sequence is from Padlan et al., Molec. Immun., 23:1063-1075 (1986). Residues are numbered according to Kabat (supra). "X" residues are included to align the Padlan IgE sequence with the Kabat numbering scheme. Sequences which were altered in preparing various IgE mutants are underlined; bold numbers below the lines denote the mutant number.  $\beta$ -strand residues are overlined; loop residues are defined by all residues intervening between two  $\beta$ -strands.

Fig. 2 depicts light and heavy chain sequences for MAE11 (SEQ.ID. 2 and 3), MAE13 (SEQ.ID. 4 and 5) and MAE15 (SEQ.ID. 6 and 7).

Fig. 3 depicts heavy and light chain sequences for HuMae11V1 (SEQ.ID 8 and 9).

Figs. 4a and 4b depicts the percent inhibition of IgE binding to FCEL and FCEH receptors, respectively, by murine monoclonal antibody Mae11 as well as 3 humanized variants (v1, v8 and v9).

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d

Figs. 5a-5d compare the binding of the MAE11, MAE15 and MAE17 antibodies to various huIgE variants. MAE1 is provided as a control which binds to both B cells and mast cell-bound IgE. The mutants scheduled in the boxes in each figure are identified in Table 11.

#### Detailed Description of the Invention

The IgE analogue polypeptides of this invention contain an amino acid sequence which is homologous to that of a naturally occurring IgE and have the ability to bind specifically or differentially to FcεR1 or FcεR2 but, in varying degree, not to both. The degree of homology of such polypeptides to wild-type IgE is not critical since only enough IgE sequence needs to be retained to enable the IgE to bind differentially or specifically to one of the two receptors. In general, the polypeptides of this invention will be IgE Fc analogues and will be about from 80% to 99% homologous with a polypeptide sequence of a naturally occurring IgE heavy chain Fc region. Homology is determined by conventional methods in which all substitutions are considered to be nonhomologous (whether conservative or nonconservative) and in which the sequences are aligned to achieve maximal homology.

It will be understood that the IgE Fc residue numbers referred to herein are those of Kabat. In applying the residue teachings of this invention to other IgE Fc domains it will be necessary to compare the entire candidate sequence with the Fig. 1 sequence in order to align the residues and correlate the residue numbers. In addition, the identity of certain individual residues at any given Kabat site number may vary from IgE to IgE due to interspecies or allelic divergence. When for example it is stated that substitutions are introduced at residue R383 (human IgE) it will be understood that this means introducing a substitution at the same site in IgE even though this same site (in loop AB) may be located at a different residue number or may be represented in the parental or starting IgE by a residue which is different than that described by Kabat. However, for the sake of clarity and simplicity the residue numbers

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and identities of the Kabat human IgE heavy chain sequences will be used herein. Note that some Kabat residues were deleted in the Padlan sequence, in which case the Kabat numbering system is preserved by insertion of a spacer residue designated "X" (See Fig. 1 ).

Similarly, the Kabat system is used to designate immunoglobulin residues used in the preparation of variant, e.g. humanized, anti-IgE immunoglobulins such as IgG, IgE, IgA or IgD. In preferred embodiments the recipient human immunoglobulin site is numbered in accord with Kabat subgroups III ( $V_H$ ) consensus and  $\kappa$  subgroup I ( $V_L$ ) consensus sequences. In order to determine which donor residues correspond to these Kabat consensus residues the sequences are maximally aligned, introducing gaps as necessary, using the variable domain cysteine residues as principal guideposts. Note that CDRs vary considerably from antibody to antibody (and by definition will not exhibit homology with the Kabat consensus sequences). Maximal alignment of framework residues (particularly the cysteines) frequently will require the insertion of "spacer" residues in the numbering system, to be used for the  $F_v$  region of the donor antibody. For example, the residue "29a" referred to *infra*. This represents an extra residue found in the murine donor antibody  $V_{H1}$  CDR for which a counterpart does not exist in the consensus sequence but whose insertion is needed to obtain maximal alignment of consensus and donor sequences. In practice, then, when a humanized antibody (ver. 1) is prepared from this donor it will contain  $V_{H1}$  with residue 29a.

The differential binding polypeptides of this invention typically contain about from 5 to 250 residues which are homologous to an IgE heavy chain  $F_c$  region, but ordinarily will contain about from 10 to 100 such residues. Usually, the IgE  $Fc3$  and  $Fc4$  regions will be present, with the  $Fc3$  domain providing residues directly involved in receptor binding with  $Fc4$  being present to ensure conformational integrity.

Generally, the IgE is human IgE, although animal IgE such as rat, murine, equine, bovine, feline or porcine IgE is

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included. As noted above, there will be variation in the residue identities and numbers for these IgEs compared to the Fig. 1 sequence.

5 FCEH and FCEL are respectively defined to be the high affinity IgE receptor (Fc $\epsilon$ RI, Ishizaka et al., Immunochimistry, 7:687-702 [1973]) found on mast cells or basophils, and the low affinity receptor (Fc $\epsilon$ RII, or CD23) found on cells involved in inflammation such as monocytes, eosinophils and platelets, as well as B-cells (Capron et al., 10 Immun. Today, 7:15-18 [1986]). FCEH and FCEL include alleles and predetermined amino acid sequence variants thereof which bind IgE. While FCEH contains several polypeptide chains, the binding of candidate polypeptides to its alpha chain is all that needs to be assayed since the alpha chain is the 15 portion of FCEH which binds IgE.

Differential binding means that the polypeptide will bind to one of FCEL or FCEH to the extent of at least about 75% of the degree with which the homologous native IgE binds to that receptor, but will not bind to the other receptor at more 20 than about 20% of the degree that the homologous IgE binds to the other receptor. Binding is determined by the assays of Example 3. Included within this invention are polypeptides that are capable of binding to one of the two receptors to a greater degree than native IgE.

#### 25 FCEL-Specific Polypeptides

These polypeptides preferentially bind to the low affinity receptor. They typically contain Fc $\epsilon$ 3 sequences in which residues within the  $\beta$ -strand D domain or loop EF have been substituted or deleted, and/or an additional residue 30 inserted adjacent to one of such residues. For the purposes herein, the beta strand D domain extends from N418-X431 (Fig. 1, wherein X indicates a residue omitted from U266 IgE but found in the Kabat sequence) and loop EF extends from G444 to T453. A preferred FCEL-specific embodiment is mutant 6 (Table 35 6), in which the substitution of 4 residues within the human IgE heavy chain sequence K423-R428 substantially abolished FCEH binding. Other FCEL-specific embodiments comprising EF loop variants are mutants 85, 89 and the combination of 49,

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51, 52, 83, 86 and 87. These sites (the D and EF domains) are believed to be the principal sites involved in binding IgE to FCEL. However, those skilled in the art will be able to routinely screen for optimal FCEL-specific polypeptides using the methods shown in the examples once it is understood that the beta-strand D and loop EF domains are the principal mutagenesis targets.

The preferred FCEL-specific polypeptide is one in which a residue has been substituted or deleted from within the  $\beta$ -strand D domain or loop EF, or both. For example, four residues were substituted in generating mutation 6, and any one or more of these substitutions may be responsible for the loss in FCEH binding while retaining FCEL binding. As for loop EF, which is involved in both FCEL and FCEH binding, it is desirable to screen both activities in order to select the FCEL-specific IgE variants. For example, mutant 85 (in which 9 IgE residues are substituted by analogously positioned IgG residues) is not detectably capable of binding to FCEH, but does bind to FCEL (see Table 11). On the other hand, conversion of site 444 from Gly to Leu abolishes binding to either receptor, while sites 447 and 452 are involved in binding to both receptors since changes at these locations prevent binding to FCEL but do not abolish FCEH binding.

#### Beta-Strand D Variants for FCEL Specificity

In general, D domain substitutions will be nonconservative, i.e., substituted residues generally will differ substantially from those found within the homologous native IgE in terms of charge, hydrophobicity or bulk. Typically, a maximum of 4 of 14  $\beta$ -strand D domain residues are varied (and are usually residues 423, 424, 426 and/or 428), although typically any 1 to 5 of these residues are suitable for variation. In general, no more than 4 residues need to be varied and optimally only one will be varied.

K423 and/or K426 are substituted with any of a residue selected from the group of Arg, His, Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Ile, Leu, Ser, Thr, Asp, Glu, Gln and Asn, preferably Gly, Pro, Glu, Gln and Asp and most preferably Pro or Gln.

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R424 and/or R425 are substituted with any of a residue selected from Asp, Asn, Gln, His, Lys, Arg, Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser and Thr, preferably Arg, Lys, Pro, Gly and His and most preferably Arg.

R428 and/or R422 are substituted with Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Glu, Asn, Gln, His, and Lys, preferably Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Glu, Asn and Gln, and most preferably Tyr.

T421 is substituted with Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser, Asp, Glu, Asn, Gln, His and Lys, preferably Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Asp, Glu, Asn, Gln, His and Lys, and most preferably Phe, Trp, Pro, Gly, Ala, Val, Leu and Ile.

S420 is substituted with Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu and Ile, and preferably Pro or Gly.

X429 is substituted with any other naturally occurring amino acid residue.

It is likely that optimal differential and FCEL binding activity will be achieved by a combination of mutations. Preferably, FCEH or FCEL binding, as the case may be, will be less than 10% of native homologous IgE, and optionally will range from undetectable to 3% of native homologous IgE, while binding to the other receptor ranges from at least about 75% of native homologous IgE to 90%, and preferably 95% to greater than 100%, e.g. 125%. The mutations should be as conservative as possible, i.e., involve as modest changes in hydrophobicity, charge or bulk as possible, yet still result in a polypeptide exhibiting these differential binding characteristics.

Any one or more of the  $\beta$ -strand D domain residues also may be deleted. Deletion of residues may possess the advantage of not introducing potentially immunogenic sites into the IgE analogue.

Examples of candidate  $\beta$ -strand D domain substitutional or deletional variants are set forth in the following Table 1. To determine the sequence of each variant, identify the

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residue for each variant number under each site. For example, the sequence of compound 19 comprises C388 E389 E390, etc.

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TABLE 1

| 5  | AA <sup>1</sup> | HuIgE Site                                 |                                                             |                                            |                                                                   |                                      |                      |
|----|-----------------|--------------------------------------------|-------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------|--------------------------------------|----------------------|
|    |                 | 423 K                                      | 424 E                                                       | 425 E                                      | 426 K                                                             | 427 Q                                | 428 R                |
|    | C               | 19                                         | 20                                                          |                                            | 37                                                                |                                      | 55                   |
|    | M               | 18                                         | 21                                                          |                                            | 38                                                                |                                      | 56                   |
|    | F               | 8, 80                                      | 22                                                          |                                            | 39                                                                |                                      | 57, 88               |
| 10 | Y               | 7                                          | 23                                                          |                                            | 40                                                                |                                      | 4, 75, 83-84, 89, 97 |
|    | W               | 6                                          | 24                                                          |                                            | 41                                                                |                                      | 58, 85               |
|    | P               | 1, 74, 78-79, 89, 103                      | 25, 97                                                      |                                            | 42                                                                |                                      | 59                   |
|    | G               | 5, 76-77                                   | 26                                                          |                                            | 43                                                                |                                      | 60                   |
|    | A               | 12, 98-99                                  | 27, 98, 100                                                 |                                            | 44, 98, 101                                                       |                                      | 61, 98, 102          |
| 15 | V               | 13, 97                                     | 28                                                          |                                            | 45                                                                |                                      | 62                   |
|    | L               | 14, 81                                     | 29                                                          |                                            | 46                                                                |                                      | 63                   |
|    | I               | 15, 82                                     | 30                                                          |                                            | 47                                                                |                                      | 64                   |
|    | S               | 16                                         | 31                                                          |                                            | 48                                                                |                                      | 65, 103              |
|    | T               | 17                                         | 32                                                          |                                            | 49                                                                |                                      | 66, 104, 105         |
| 20 | D               | 9                                          |                                                             | 79                                         | 50                                                                |                                      | 67, 86               |
|    | E               | 9, 94                                      | 1, 3-19, 37-54, 55-72, 75, 88, 89, 90-93, 99, 101, 102, 105 | 1-72, 74, 76-78, 80-88, 93-94, 99, 100-105 | 51                                                                |                                      | 68, 87               |
|    | N               | 10                                         | 33                                                          |                                            | 52, 79, 84                                                        | 79                                   | 69                   |
|    | Q               | 11                                         | 34                                                          |                                            | 3, 54, 75, 80, 82-83, 85-89, 103-104                              | 1-72, 75, 77, 78, 80-95, 97-103, 105 | 70                   |
|    | H               | 83, 104                                    | 35, 78, 84                                                  |                                            | 53                                                                |                                      | 71                   |
| 25 | K               | 2-4, 20-72, 75, 85-88, 91-93, 100-102, 105 | 36, 77, 79, 94                                              |                                            | 1-2, 5-36, 55-72, 74, 76, 77-90, 91, 93-95, 97, 99, 100, 102, 105 | 104                                  | 72, 79               |

<sup>1</sup>Amino acid residue substituted into the analogue

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|            |            |                                                |        |        |    |                                                                        |
|------------|------------|------------------------------------------------|--------|--------|----|------------------------------------------------------------------------|
| R          | 84         | 2, 74, 76,<br>80, 81 83,<br>85-87, 103-<br>104 | 89     |        |    | 1-3, 5-<br>54, 74,<br>76-78,<br>80-82,<br>90-92,<br>94, 99,<br>100-101 |
| $\Delta^2$ | 90, 95, 96 | 91, 95, 96                                     | 91, 96 | 92, 96 | 96 | 93, 95,<br>96                                                          |

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<sup>2</sup>Signifies a deletion

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Insertion of one or more extraneous residues adjacent to a residue within the  $\beta$ -strand D domain also falls within the scope of this invention. Typically, only one residue will be inserted, although from 2 to 4 or more residues can be inserted adjacent to any one site within the domain. Smaller numbers of inserted residues will be preferred in order to avoid the introduction of immunogenic sites. This, however, is merely a matter of choice. In general, insertions will be made at a single site, although insertions can be made adjacent to any two or more  $\beta$ -strand D domain residues.

Insertions typically are made between the following residues: 422 and 423, 423 and 424, 424 and 425, 425 and 426, 426 and 427, 427 and 428 and/or 428 and 429. The inserted residue or residues generally will exhibit charge, bulk or hydrophobicity character which is distinct from that of the flanking residues. For example, candidate insertions can be selected from the following Table 2.

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TABLE 2

|    | Insertion | $\beta$ -strand D domain site <sup>1</sup> |
|----|-----------|--------------------------------------------|
| 5  | Q         | 1, 2, 3, 4, 5, 7 or 8                      |
|    | D         | 1, 2, 3, 4, 5, 6 or 7                      |
|    | E         | 1, 2, 3, 4, 5, 6 or 7                      |
|    | F         | 1, 2, 3, 4, 5, 6 or 7                      |
|    | W         | 1, 2, 3, 4, 5, 6 or 7                      |
| 10 | P         | 1 or 2                                     |
|    | K         | 2 or 3                                     |
|    | R         | 2 or 3                                     |
|    | EK        | 2 or 7                                     |
|    | ER        | 2 or 7                                     |
| 15 | DK        | 2 or 7                                     |
|    | DR        | 2 or 7                                     |
|    | G         | 1 or 2                                     |
|    | A         | 8                                          |
|    | Y         | 6 or 7                                     |
| 20 | N         | 1, 2, 3, 4, 5, 7 or 8                      |
|    | H         | 1, 2, 3, 4, 5, 7 or 8                      |
|    | I         | 1, 2, 3, 4, 5, 7 or 8                      |

25

<sup>1</sup>422R - site 1 - 423K - site 2 - 424E - site 3 - 3425E - site 4 - 426K - site 5 - 427Q - site 6 - 428R - site 7 - 429X y - site 8. Absence of a site indicates no insertion at that site.

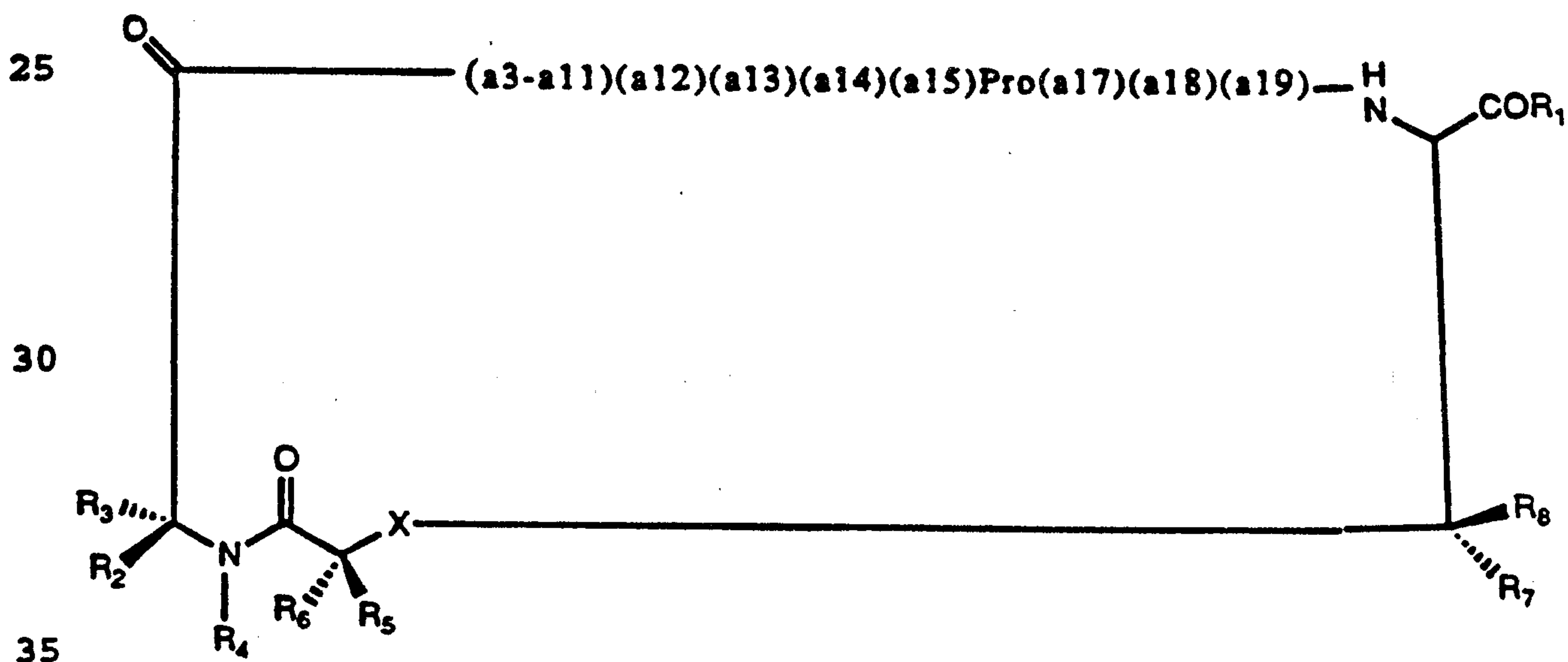
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The FCEL-specific polypeptides need only contain so much of the IgE Fcε AB-B and loop EF domain sequences as are required to substantially achieve FCEL binding. This is readily determinable by preparing polypeptides comprising the AB-B and loop EF domains and incrementally increasing numbers of flanking or normally interposed residues, e.g., β-strand A (N-terminal) or loop BC, β-strand C, loop CD, β-strand D, loop DE, β-strand E, β-strand F, loop EF, loop FG, β-strand G, and Fcε4 (C-terminal). In general, the entire IgE sequence from Fcε3 - Fcε4 is used, although fragments of Fcε3 containing the AB-B domain may be satisfactory, particularly if they contain the AB-B domain, loop EF and intervening sequence, otherwise than as varied according to the teachings herein to achieve specificity for FCEL.

The FCEL-specific polypeptides are provided as linear or conformationally restrained polypeptides. Conformational restraint is accomplished by cross-linking the polypeptide, preferably at the N- and C- termini so as to produce a cyclic structure. In preferred embodiments the cyclic forms have the following structure:

### Formula I



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wherein (a3-a11) is a bond or the sequence -R373 -F381; a12 and a18 are hydrophobic amino acid residues; a13 and a14 are basic amino acid residues; and a15, a17 and a19 are hydrophilic amino acid residues;

5 R<sub>1</sub> is selected from

- (a) hydroxy,
- (b) C<sub>1</sub>-C<sub>8</sub> alkoxy,
- (c) C<sub>3</sub>-C<sub>12</sub> alkenoxy,
- (d) C<sub>6</sub>-C<sub>12</sub> arlyoxy,
- 10 (e) acylamino-C<sub>1</sub>-C<sub>8</sub>-alkoxy
- (f) pivaloyloxyethoxy,

(g) C<sub>6</sub>-C<sub>12</sub> aryl-C<sub>1</sub>-C<sub>8</sub>-alkoxy where the aryl group is unsubstituted or substituted with one or more of the groups nitro, halo, C<sub>1</sub>-C<sub>4</sub>-alkoxy, and amino;

- 15 (h) hydroxy substituted C<sub>2</sub>-C<sub>8</sub> substituted alkoxy; and
- (i) dihydroxy substituted C<sub>3</sub>-C<sub>8</sub> alkoxy;

R<sub>2</sub>, R<sub>3</sub>, R<sub>5</sub>, R<sub>7</sub>, R<sub>8</sub> are the same or different and are selected from

- (a) hydrogen,
- 20 (b) C<sub>6</sub>-C<sub>12</sub> aryl where the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo, C<sub>1</sub>-C<sub>8</sub> alkyl, halo-C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>1</sub>-C<sub>8</sub> alkoxy, amino, phenyl, acetamido, benzamido, di-C<sub>1</sub>-C<sub>8</sub> alkylamino, C<sub>6</sub>-C<sub>12</sub> aroyl, C<sub>1</sub>-C<sub>8</sub> alkanoyl, and hydroxy substituted C<sub>1</sub>-C<sub>8</sub> alkyl,
- 25 (c) C<sub>1</sub>-C<sub>12</sub> alkyl or alkenyl; C<sub>3</sub>-C<sub>10</sub> cycloalkyl or C<sub>3</sub>-C<sub>12</sub> substituted with any of halo, C<sub>1</sub>-C<sub>8</sub> alkoxy, C<sub>6</sub>-C<sub>12</sub> aryloxy, hydroxy, amino, acetamido, C<sub>1</sub>-C<sub>8</sub> alkylamino, carboxy or carboxamide;

30 R<sub>2</sub> and R<sub>3</sub>, R<sub>5</sub> and R<sub>6</sub>, or R<sub>7</sub> and R<sub>8</sub> may optionally and independently be joined together to form a carbocyclic or heterocyclic ring of from four to seven atoms where the heteroatoms are selected from O, S, or NR<sub>10</sub> where R<sub>10</sub> is selected from

- hydrogen, C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>2</sub>-C<sub>8</sub>-alkenyl, C<sub>6</sub>-C<sub>12</sub>-aryl, C<sub>3</sub>-C<sub>10</sub> cycloalkyl, C<sub>6</sub>-C<sub>12</sub>-aryl-C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>1</sub>-C<sub>8</sub>-alkanoyl, and C<sub>6</sub>-C<sub>12</sub> aroyl,
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R<sub>4</sub> is selected from

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hydrogen, C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>2</sub>-C<sub>8</sub>-alkenyl, C<sub>6</sub>-C<sub>12</sub>-aryl, C<sub>3</sub>-C<sub>10</sub> cycloalkyl, C<sub>6</sub>-C<sub>12</sub>-aryl-C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>1</sub>-C<sub>8</sub>-alkanoyl, and C<sub>6</sub>-C<sub>12</sub> aroyl;

R<sub>2</sub> or R<sub>3</sub> may be optionally joined with R<sub>4</sub> to form a piperidine, pyrrolidine or thiazolidine ring;

X is selected from

an O or S atom,

NR<sub>9</sub> wherein R<sub>9</sub> is hydrogen, C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>3</sub>-C<sub>8</sub>-alkenyl, C<sub>3</sub>-C<sub>10</sub> cycloalkyl, C<sub>6</sub>-C<sub>12</sub>-aryl, C<sub>6</sub>-C<sub>12</sub>-aryl-C<sub>1</sub>-C<sub>8</sub>-alkyl, C<sub>1</sub>-C<sub>8</sub>-alkanoyl,

or C<sub>6</sub>-C<sub>12</sub> aroyl;

C<sub>6</sub>-C<sub>12</sub> aryl,

C<sub>1</sub>-C<sub>8</sub> alkanoyl, and

(CH<sub>2</sub>)<sub>k</sub> where k is an integer from 0 to 5; and

pharmaceutically acceptable salts thereof.

As used herein and unless specified otherwise: alkyl and alkenyl denote straight or branched, saturated or unsaturated hydrocarbon chains, respectively; C<sub>6</sub>-C<sub>12</sub> aryl groups denote unsubstituted aromatic rings or fused aromatic rings such as, for example, phenyl or naphthyl; halo denotes F, Cl, Br, or I atoms; alkoxy denotes an alkyl group bonded through O to the indicated site. Examples of C<sub>1</sub>-C<sub>8</sub> alkyl or C<sub>2</sub>-C<sub>8</sub> alkenyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl, isopentyl, hexyl, vinyl, allyl, butenyl and the like; examples of C<sub>3</sub>-C<sub>10</sub>-cycloalkyl groups include cyclopropyl, cyclopentyl, cyclohexyl, and the like; heterocyclic rings include but are not limited to pyridyl, thienyl, furyl, indolyl, benzthienyl, imidazolyl, thiazolyl, quinolinyl and isoquinolinyl. Hydrophobic amino acid residues include naturally occurring or synthetic residues having hydrophobic side chains, e.g. Phe, Leu, Ile, Val, Norleu, and the like. Hydrophilic amino acid residues include naturally occurring or synthetic residues having charged or uncharged hydrophilic side chains, e.g. ornithine, Ser, Thr, Tyr, His, Asp, Glu, Lys and Arg. Preferably a15, a17 and a19 are unchanged and bear normal, secondary or tertiary mono or di-hydroxy substituted alkyl side chains.

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Basic residues have guanidino or amino-substituted side chains for the most part.

The AB-B domain and/or loop EF - containing, FCEL-specific polypeptides of this invention optionally are associated with other substances or are fused to additional polypeptide sequences. The polypeptides generally contain only IgE-homologous sequences, although they also or alternatively are labelled for diagnostic use (employing enzymes, radioisotopes, biotin or avidin, stable free radicals, and chemiluminescent or fluorescent moieties in conventional fashion). Also the polypeptides are fused to non-IgE polypeptides such as cytotoxic or immunosuppressive polypeptides, to other IgE polypeptides (e.g. Fv regions), or to polypeptides capable of binding to a predetermined ligand or antigen.

Cytotoxic polypeptides include IgG Fc effector sequences and polypeptide toxins such as diphtheria toxin or ricin A chain (U.S. Patents 4,714,749 and 4,861,579). A preferred fusion is one in which the FCEL-specific sequence (such as that of the Fc $\epsilon$ 3 - Fc $\epsilon$ 4 sequence of mutant 6) is fused at its N-terminus (i.e., at approximately D360) to the C-terminus of an immunoglobulin, or an immunoglobulin fragment terminating at the C-terminus of IgG Fc $\gamma$ 2 or IgG Fc $\gamma$ 3. Alternatively the FCEL specific polypeptide is fused to an effector IgG sequence in place of one or both of the IgG Fv domains in analogous fashion to known immunoadhesins.

The polypeptides herein optionally are fused to polypeptides which are capable of binding a predetermined antigen or ligand. Generally, these additional polypeptides will be IgE or other immunoglobulin Fv domains, although they optionally are heterologous polypeptides such as receptor extracellular domains (produced in the known fashion of immunoadhesions, e.g. as has been accomplished with CD4). Immunoglobulin sequences fused to the FCEL-specific polypeptides herein include Fc or variable sequences of the heavy chains of IgG1, IgG2, IgG3, IgG4, IgE, IgM, IgD or IgA. Any FCEL-specific heavy chain fusion optionally is disulfide

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bonded in the ordinary fashion to heavy chains having the same specificity (thereby forming homopolymers) or to different heavy chains (thereby forming heteropolymers), including different heavy chains having specificity for a different antigen. Such heteropolymeric heavy chains include heavy chains which are not FCEL-specific, e.g., these comprise native IgE sequences which bind to FCEL and FCEH in the ordinary fashion, or the heavy chains optionally include at least one heavy chain that is FCEL specific and at least one that is FCEH specific. Heteropolymeric heavy chains also may include the heavy chains of non-IgE immunoglobulins, e.g., IgG, IgD, IgM and the like. In addition, the heavy chain hetero- or homopolymers optionally are disulfide bonded to light chains in the fashion of native immunoglobulins so as to cooperatively bind to predetermined antigen in the usual way. Unless the heteropolymeric heavy chains comprise IgM heavy chains they generally will be heterodimeric.

In some embodiments, immunoglobulins comprising a FCEL-specific polypeptide will also comprise an immunoglobulin variable region, preferably (if at all) an IgE Fv domain. The antigenic specificity of the variable region may vary widely, including those which bind haptens, or which bind polypeptides or proteins from human, animal, plant, fungal, bacterial or insect sources. The specificity may be unknown or the variable region may have the ability to bind to a predetermined antigen. If the immunoglobulin is to have a functional variable domain (as opposed to a deleted Fv in the case of Fce3 or Fce4 fragments) it is preferred that it have a known antigenic specificity. Antigenic specificity may include the ability to bind antigens associated with a cytotoxic or immune response, particularly lymphoid cell antigens such as CD3 or CD8, integrins, B-cell surface antigens, helper or suppressor cell surface antigens, or epitopes located in the variable region of effector subtypes of IgG. FCEL-specific Fc domains also are usefully employed in combination with Fv domains capable of binding a particular allergen to which a patient is allergic. These

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generally are human IgEs directed against allergens and which contain an FCEL- specific Fc domain. Alternatively, the immunoglobulin specificity is directed against the Fc region of effector subtypes of IgG, in this case however it being  
5 preferable that the FCEL-specific polypeptide not suppress complement binding or ADCC functions of the IgG.

The polypeptides of this invention that contain antigen or ligand binding capability contain one or more sites capable of binding to the antigen or ligand. For  
10 example, the polypeptides herein comprise one or more IgE or other immunoglobulin Fv domain to produce monovalent or polyvalent immunoglobulins. For the most part such polypeptides will be monovalent for antigen or ligand, as in the case when the immunoglobulin comprises a heavy-light  
15 chain pair that has a deleted or inactivated Fv or CDR so as to not be able to bind to antigen. Alternatively, they will be bivalent in the predominant instance, and will be monospecific or bispecific.

In another embodiment, FCEL-specific polypeptides are  
20 covalently bound to a cytotoxic agent. For example, the polypeptide ricin D toxin isolated from the *Ricinus communis* plant is bound to the carboxy terminus of the Fc domain, either by chemical means or, most preferably, by production of a fusion protein using standard recombinant DNA methods.  
25 This provides a means to selectively deliver the toxin only to cells expressing FCEL on their surfaces.

The FCEL-specific polypeptides need only contain so much of the IgE Fc $\epsilon$  sequence as is required to substantially maintain FCEL binding. This is readily determinable by  
30 synthesizing or expressing the product and determining its activity. In general, the entire IgE sequence extending from Fc $\epsilon$ 2 - Fc $\epsilon$ 4 can be used, although fragments containing only FcE3 and FcE4 are generally satisfactory.

In general the immunoglobulin sequences and the FCEL-  
35 specific sequence will be derived from the same species which is to be treated with the IgE analogue. Preferably, the immunoglobulin sequences are human.

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The FCEL-specific polypeptides of this invention (when employed as such without fusion to non-IgE sequences) exclude the linear polypeptide sequences disclosed by Nio et al. (*supra*), as well as other prior art polypeptides which include the native IgE AB-B domain or loop EF (Burt et al., *supra*).

#### FCEH-Specific Polypeptides

These polypeptides are amino acid sequence variants of IgE or its fragments in which a residue within the AB-B or loop EF domains have been deleted, substituted or another residue inserted so that the AB-B or loop EF domains are no longer capable of binding to FCEL, and which contain sufficient beta strand D sequence and (optionally) loop EF sequence to bind to the high affinity receptor. As disclosed above, the AB-B and loop EF domains have been implicated in binding to FCEL since mutations in these domains have a serious impact on the binding of the IgE variants to the low affinity receptor. In particular, mutations in loop EF or the C-terminal half of the AB loop and in the N-terminal half of beta strand B produce a divergence in IgE FCEL/FCEH specificity wherein the variant continues to bind to the high affinity receptor but largely fails to bind to the low affinity receptor. In addition, we have found that the IgE loop EF and the heavy chain beta strand D domains participate in binding to the high affinity receptor. Therefore, FCEH-specific differential binding polypeptides will comprise at least the FCEH-binding sequence of beta strand D and preferably also will contain a variant AB-B or loop EF domain sequence that binds substantially only to FCEH.

In preferred embodiments amino acid sequence variation is introduced into the low affinity receptor binding functionality of the AB-B or loop EF domains. Preferably, one or more of residues I382, R383, K384, S385, T387, I388, T389, C390, R446, D447, W448, I449, E150, G151, E152 or T153 are varied, although modifications optionally are introduced into loop AB N-terminal to the designated loop AB residues. Only one of R383, K384, S385, T387, T-389, or R446 - T453

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need be mutated, although it is preferable to vary 1, 2 or 3 residues from each domain.

When substituted at all, I382 and/or I388 generally are independently substituted with Asn, Gln, Leu, Val, His, Lys, Arg, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Ser, Thr, Asp or Glu, preferably Trp, Pro, Gly, Ser, Thr, Asp or Glu. Ordinarily these two residues are not modified.

R383 typically is substituted with Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Glu, Asn, Gln, His, or Lys, preferably Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Ser, Thr, Asp, Glu, Asn or Gln and most preferably Ala, Glu, Asp or Ser.

K384 typically is substituted with Arg, His, Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Ile, Leu, Ser, Thr, Asp, Glu, Gln and Asn, preferably Ala, Gly, Pro, Glu, Gln or Asp and most preferably Ala, Glu or Asp.

S385 is substituted with Asp, Asn, Gln, His, Lys, Arg, Cys, Met, Phe, Tyr, Trp, Pro, Gly, Ala, Val, Leu, Ile, Glu and Thr, preferably Ala, Tyr, Val, Ile, Leu, Phe, Arg, Lys and His and most preferably Ala, Val, Ile, Leu, Phe and Tyr.

When substituted, P386 usually is substituted by Gly, Ala, Cys, Val, Leu, Ile, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys, Arg, Phe, Tyr, or Trp, and preferably Gly, Ala, Ser, Thr, Asp, Glu, Asn, Gln, His, Lys, Arg or Trp. Ordinarily, P386 is not modified.

T387 and/or T389 generally are independently substituted by Gly, Ala, Val, Leu, Ile, Ser, Asp, Pro, Glu, Asn, Gln, His, Lys, Arg, Cys, Phe, Tyr and Trp, preferably Gly, Ala, Val, Leu, Ile, Asp, Glu, Asn, Gln, His, Lys, Arg, Phe, Tyr and Trp, and most preferably Ala.

C390 ordinarily is not substituted except when employed as a component of a cyclizing group as shown in Formula I.

The differential FCEH-binding polypeptides of this invention will comprise the sequence of functional FCEH-binding beta strand D and loop EF domains, as defined above. In general, it is expected that the functional domains need not contain all of the beta strand D or loop EF domain

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residues. However, any modifications of the beta strand D domain residues will need to be conservative, if made at all, in order to preserve FCEH binding. Since loop EF is involved in both FCEL and FCEH binding, it likely will be necessary to screen these variants in order to determine their activity as shown in Example 5. However, a number of loop EF mutants already have been identified that substantially abolish FCEL binding without apparently interfering with FCEH binding, e.g. mutants 50 and 52. Thus, loop EF variants may belong in either the FCEL or FCEH specific category, or may equally affect binding to each receptor.

A particularly preferred embodiment of a FCEH-specific polypeptide is one which contains a beta strand D domain together with additional C-terminal sequence. The sequence of this embodiment extends from about T421 to about T440. Generally, the N-terminus of this embodiment is S420 or T421, while the C-terminus is T440, L441 or P442. In addition, one or more residues extraneous to this sequence are fused to its N- or C-termini. These extraneous residues are particularly useful in forming covalent or noncovalent bonds between the N- and C-termini of this polypeptide. The N- and/or C-termini preferably are covalently bonded through a side chain of a residue or through the polypeptide backbone. For example, cysteine residues are fused to the N- and C-termini and, upon oxidation, a polypeptide having a terminal disulfide bond is formed which joins the terminal ends of the polypeptide, thereby conformationally restraining the polypeptide. Alternatively, the alpha amino group of the polypeptide (or that of an extraneous N-terminally located residue) is covalently bonded to the sulfur atom of an extraneous C-terminally located cysteine residue to form thioether cyclic compounds analogous to those depicted in Formula I. Other cyclic compounds are prepared in the same fashion as described elsewhere herein. Also within the scope of this embodiment are amino acid sequence variants of native IgE sequences corresponding to the sequence of this embodiment. Beta strand D variants are selected to enhance

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binding to FCEH, while the sequence outside of the beta strand D domain need only retain sufficient conformational structure to properly juxtapose the N- and C-termini in substantially the same position as is the case with the native IgE sequence.

The FCEH-specific polypeptides herein optionally comprise non-IgE polypeptides exactly as described above for the FCEL-specific polypeptides, except that it is not preferred that the FCEH-specific polypeptides comprise cytotoxic functionalities. In addition, conformationally restrained (typically cyclic) polypeptides comprising the FCEH-binding sequence of the beta strand D domain are included within the scope hereof. Such polypeptides are identical to those shown in Formula I above except that the FCEH-binding beta strand D domain replaces the (a3)-(a19) moiety. Exemplary replacement moieties include S420-R428, T421-N430, S420-G433 and R422-R428 (note that sequences such as T421-N430 from U266 that omit a residue from the Kabat sequence can contain a residue at that site or may have a deletion at the same location, in the latter case here the Asn residue would occupy site 429).

Any one or more of the AB-B domain residues also may be deleted in order to substantially reduce or eliminate FCEL binding. Residue deletion may be preferred for the same reason noted above with respect to the beta strand D domain.

Examples of candidate AB-B domain substitutional or deletional variants are set forth in the following Table 3. To determine the sequence of each variant, identify the residue for each variant number under each site. For example, the sequence of compound 98 comprises A383 A384 A385, and represents the class of mutations to which mutant 7 belongs.

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TABLE 3

|    | AA <sup>1</sup> | HuIgE Site                       |                                                |                                            |                                                          |
|----|-----------------|----------------------------------|------------------------------------------------|--------------------------------------------|----------------------------------------------------------|
|    |                 | 350 I                            | 351 R                                          | 352 K                                      | 353 S                                                    |
| 5  | C               |                                  | 55                                             | 19                                         | 37                                                       |
|    | M               |                                  | 56                                             | 18                                         | 38                                                       |
|    | F               |                                  | 57, 88                                         | 8, 80                                      | 39                                                       |
|    | Y               |                                  | 4, 75, 83-84, 89, 97                           | 7, 73                                      | 40                                                       |
|    | W               |                                  | 58, 85                                         | 6                                          | 41                                                       |
| 10 | P               |                                  | 59                                             | 1, 74, 78-79                               | 42                                                       |
|    | G               |                                  | 60, 73                                         | 5, 76-77                                   | 43                                                       |
|    | A               |                                  | 61, 98, 102                                    | 12, 98-99                                  | 44, 98, 101                                              |
|    | V               | 72                               | 62                                             | 13, 97                                     | 45                                                       |
|    | L               | 73                               | 63                                             | 14, 81                                     | 46                                                       |
| 15 | I               | 75                               | 64                                             | 15, 82                                     | 47                                                       |
|    | S               |                                  | 65, 103                                        | 16                                         | 1-2, 5-36, 55-72, 74, 76-91, 93-95, 97, 99-100, 102, 105 |
|    | T               |                                  | 66, 104, 105                                   | 17                                         | 49                                                       |
|    | D               |                                  | 67, 86                                         | 9                                          | 50                                                       |
|    | E               |                                  | 68, 87                                         | 89, 94                                     | 51                                                       |
| 20 | N               | 79                               | 69                                             | 10                                         | 52, 79, 84                                               |
|    | Q               | 1-71, 77, 78, 80-95, 97-103, 105 | 70                                             | 11, 103                                    | 3, 54, 75, 80, 82-83, 85-89, 103-104                     |
|    | H               |                                  | 71                                             | 83, 104                                    | 4, 53                                                    |
|    | K               | 104                              | 72, 79                                         | 2-4, 20-72, 75, 85-88, 91-93, 100-102, 105 | 48                                                       |
|    | R               |                                  | 1-3, 5-54, 74, 76-78, 80-82, 90-92, 94, 99-101 | 84                                         | 73                                                       |
| 25 | Δ <sup>2</sup>  | 96                               | 93, 95, 96                                     | 90, 95, 96                                 | 92, 96                                                   |

<sup>1</sup>Amino acid residue substituted into the analogue<sup>2</sup>Signifies a deletion

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Insertion of one or more extraneous residues adjacent to a residue within the AB-B domain also falls within the scope of this invention, although substitutions or deletions are preferred. Typically, only one residue will be inserted, although from 2 to 4 or more residues can be inserted adjacent to any one site within the AB-B domain. Smaller numbers of inserted residues will be preferred in order to avoid the introduction of immunogenic sites. This, however, is merely a matter of choice. In general, insertions will be made at a single site, although insertions can be made adjacent to any two or more AB-B domain residues.

Insertions typically are made between the following residues: S385 and P386, P386 and T387, T387 and I388, and I388 and T389. The inserted residue or residues generally will exhibit charge, bulk or hydrophobicity character which is distinct from that of the flanking residues. For example, candidate insertions can be selected from the following Table 4.

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TABLE 4

|    | Insertion | AB-B domain site <sup>1</sup> |
|----|-----------|-------------------------------|
| 5  | Q         | 1, 2, 3, 4 or 5               |
|    | D         | 1, 2, 3, 4 or 5               |
|    | E         | 1, 2, 3, 4 or 5               |
|    | F         | 1, 2, 3, 4 or 5               |
|    | W         | 1, 2, 3, 4 or 5               |
| 10 | P         | 1 or 2                        |
|    | K         | 2 or 3                        |
|    | R         | 2 or 3                        |
|    | T         | 3 or 4                        |
|    | EK        | 2 or 4                        |
| 15 | ER        | 2 or 4                        |
|    | DK        | 2 or 4                        |
|    | DR        | 2 or 4                        |
|    | G         | 1 or 2                        |
|    | A         | 5                             |
| 20 | Y         | 3 or 4                        |
|    | N         | 1, 2, 3, 4 or 5               |
|    | H         | 1, 2, 3, 4 or 5               |
|    | I         | 1, 2, 3, 4 or 5               |

25 <sup>1</sup>I382 - site 1 - R383 - site 2 - K384 - site 3 - S385 - site 4 - P386 - site 5 - T387. Absence of a site indicates no insertion at that site.

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One or more of the AB-B domain residues are substituted or deleted, or additional residues inserted adjacent to such residues. In general, no more than 4 residues or sites are varied and optimally only one will be varied. Variations  
5 herein include combinations of insertions, deletions or substitutions. Excluded from the scope of FCEH specific polypeptides are the linear IgE polypeptide fragments disclosed by Nio et al. (or the naturally occurring sequence  
10 variants of such fragments, e.g. alleles and the like), together with any other such fragments disclosed by the prior art.

#### Loop EF Variants

Loop EF is defined above. Loop EF variants not described in the examples may require screening against both  
15 FCEH and FCEL assays since loop EF is involved in both FCEL and FCEH binding. However, this screening will be routine and well within the ordinary skill when following the directions and principles herein. In general, FCEH or FCEL-binding differential polypeptides will comprise substitutions  
20 or deletions of (or insertions adjacent to) one or more of residues 446, 447, 448, 449, 450, 452 and 453. It should be noted that sites such as 446 and 447, while shown in the case of Ala substitution to lead to loss of FCEL binding (Example 5), also serve as sites for selecting variants which bind  
25 FCEL to a greater degree than native IgE. For the most part, however, sites 446 and 447 are not preferred for introducing variants in which the objective is FCEL binding. For this, one should focus on the region extending from residue 448 to 453, and preferably residues 450, 452 and 453. In general,  
30 loop EF variants are employed with variants introduced into loop AB - beta strand B or beta strand D or both.

R446 typically is substituted by Gly, Ala, Val, Leu, Ile, Ser, His, Lys, Met, Thr, Asp, Pro, Glu, Asn, Gln, Cys, Phe, Tyr or Trp, preferably Ala for FCEH specificity.

35 D447 generally is substituted by Gly, Ala, Val, Leu, Ile, Met, Cys, Ser, Thr, Pro, Glu, Asn, Gln, His, Lys, Arg, Phe, Tyr or Trp, preferably Ala for FCEH specificity.

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W448 also generally is not substituted, but if so then Gly, Ala, Val, Leu, Ile, Met, Cys, Ser, Thr, Pro, Glu, Asn, Asp, Gln, His, Lys, Arg, Phe or Tyr are employed.

5 I449 likewise generally is not substituted, but if so then Gly, Ala, Val, Leu, Met, Cys, Ser, Thr, Pro, Glu, Asn, Asp, Gln, His, Lys, Arg, Phe, Tyr or Trp are employed.

E450 typically is substituted with Gly, Ala, Val, Ile, Leu, Met, Cys, Ser, Thr, Pro, Gln, Asn, Asp, His, Lys, Arg, Phe, Tyr or Trp, preferably Ala for FCEH specificity.

10 G151 generally is not substituted, but if so then Ala, Val, Leu, Met, Cys, Ser, Thr, Pro, Glu, Asn, Ile, Asp, Gln, His, Lys, Arg, Phe, Tyr or Trp are employed.

E452 also generally is substituted with Ala, Val, Leu, Met, Cys, Ser, Thr, Pro, Gly, Asn, Ile, Asp, Gln, His, 15 Lys, Arg, Phe, Tyr or Trp.

T453 typically is substituted with Ala, Val, Leu, Met, Cys, Ser, Pro, Gly, Asn, Glu, Ile, Asp, Gln, His, Lys, Arg, Phe, Tyr, or Trp.

20 Exemplary IgE variants are set forth in Table 5 It will be understood that this table may contain variants that bind to both receptors, differentially to one or the other, or to neither receptor.

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TABLE 5

| AA <sup>1</sup> |                                                              | HuIgE Site                                     |                                                                                                       |                                                                                           |                                                                                 |
|-----------------|--------------------------------------------------------------|------------------------------------------------|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
|                 | 446 R                                                        | 447 D                                          | 450 E                                                                                                 | 452 E                                                                                     | 453 T                                                                           |
| 5               | C 47                                                         | 46                                             | 45                                                                                                    | 44                                                                                        | 43                                                                              |
|                 | M 34                                                         |                                                |                                                                                                       |                                                                                           |                                                                                 |
|                 | F 33                                                         | 25                                             |                                                                                                       |                                                                                           |                                                                                 |
|                 | Y 41                                                         |                                                |                                                                                                       |                                                                                           | 30                                                                              |
|                 | W                                                            | 26                                             | 36, 38                                                                                                |                                                                                           | 36, 38                                                                          |
| 10              | P                                                            |                                                | 49                                                                                                    |                                                                                           |                                                                                 |
|                 | G                                                            |                                                |                                                                                                       |                                                                                           |                                                                                 |
|                 | A 13, 17                                                     | 16                                             | 12, 15                                                                                                | 12, 14                                                                                    | 12                                                                              |
|                 | V                                                            |                                                |                                                                                                       |                                                                                           | 31                                                                              |
|                 | L                                                            |                                                |                                                                                                       |                                                                                           | 40                                                                              |
| 15              | I                                                            | 48                                             |                                                                                                       |                                                                                           |                                                                                 |
|                 | S                                                            |                                                |                                                                                                       |                                                                                           | 29                                                                              |
|                 | T 43                                                         |                                                |                                                                                                       |                                                                                           | 1-3, 5-7, 9,<br>10, 13-17,<br>24-26, 28,<br>33, 34, 37,<br>39, 44-48,<br>50, 51 |
|                 | D 39                                                         | 1, 2, 4-15,<br>17-23, 31-<br>45, 47, 49-<br>52 | 5, 8, 11, 18,<br>23, 27, 32,<br>33, 35, 40,<br>42, 52                                                 | 1, 29, 30,<br>34, 50                                                                      | 42                                                                              |
|                 | E 9, 20                                                      | 24, 29, 30                                     | 1-5, 7, 9, 10,<br>13, 14, 16,<br>17, 24-28, 30,<br>31, 34, 37,<br>39, 43, 44,<br>46, 47, 48, 51<br>52 | 3, 4, 6, 7,<br>9, 10, 13,<br>15-17, 24-<br>26, 28, 31-<br>33, 37, 39,<br>43, 45-49,<br>52 | 8, 11, 18-<br>23, 27, 35                                                        |
| 20              | N 19, 22, 40                                                 | 3                                              | 50                                                                                                    | 51                                                                                        |                                                                                 |
|                 | Q 10, 11, 23,<br>35, 36, 42                                  |                                                |                                                                                                       | 2                                                                                         | 52                                                                              |
|                 | H 21, 30                                                     | 27                                             |                                                                                                       | 36                                                                                        |                                                                                 |
|                 | K 18, 28, 29,<br>52                                          | 28                                             |                                                                                                       | 8, 11, 18-<br>23, 27, 35,<br>40, 42                                                       | 32                                                                              |
|                 | R 1-8, 12, 14-7<br>16, 24-27,<br>31, 32, 38,<br>44-46, 48-51 |                                                | 6                                                                                                     | 5                                                                                         | 4                                                                               |
| 25              | Δ <sup>2</sup> 37                                            |                                                |                                                                                                       | 38                                                                                        |                                                                                 |

<sup>1</sup>amino acid residue substituted into the variant<sup>2</sup>signifies a deletion

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Variant Anti-huIgE antibodies

Variant anti-huIgE antibodies were produced by first obtaining a group of murine monoclonal antibodies which were capable of binding to FCEL but not to FCEH. 8 such murine monoclonal antibodies, designated MAE10, MAE11, MAE12, MAE13, MAE14, MAE15, MAE16 and MAE17, were obtained by conventional methods involving immunizing mice with human IgE or a polypeptide consisting of residues 315-547 of huIgE and screening for anti-IgE activity.

MAE11/15 and MAE13 recognize different epitopes. It appears that the MAE13 epitope is located three-dimensionally adjacent to a key component of the FCEH binding site of IgE (but does not directly occupy that site) since a slight amount of histamine release will occur at high concentrations of MAE 13 suggesting that some limited antibody mediated crosslinking of FCEH occurs with MAE 13. MAE17 was most effective in suppressing B-cell IgE synthesis despite the fact that MAE11 and MAE13 exhibited greater IgE affinity. This may be attributed to its ability to mediate complement fixation (it possessed an IgG2a isotope, thus containing an Fc capable of eliciting effector function).

MAE11 and MAE15 are believed to recognize the same IgE epitope. Each antibody shared certain unusual features in its amino acid sequence. For example, CDR1 of the light chain of each contained 3 aspartic acid residues. CDR3 of the heavy chains of MAE11 and MAE15 contained 3 histidine residues (and contained two arginine residues, respectively).

Antibodies such as the foregoing having desired IgE binding characteristics may be further modified. Such modifications fall into two general classes. In the first class the antibodies are modified so that they are monovalent for IgE. This means that only one "arm" of the antibody, i.e., one light-heavy chain fork of the antibody, shall be capable of binding IgE. The remaining Fv "arm" of the antibody (or arms in the case of IgM) is specific for a second (non-IgE) antigen, is not capable of binding any

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antigen, or is deleted entirely. Thus, the term IgE monovalent covers polyvalent antibodies that are monovalent for IgE. The best results may be obtained with the second alternative, since this would preserve the structure of the antibody most faithfully and would likely confer the longest circulating half-life on the antibody. IgE-monovalent antibodies specific for FCEL bound IgE optimally will comprise sufficient fc domains of the heavy chains to be capable of complement binding and Ig effector functions.

10 The second antigen recognized by one embodiment of IgE monovalent antibody is one which, when indirectly cross-linked to FCEL by the antibody herein, will not produce any toxic or deleterious response, i.e. the second antigen is not FCEH, and generally is one which is not found in the animal  
15 to be treated (in order to avoid undesired absorption of the antibody onto tissues or proteins within the body). Thus, the second antigen ordinarily will not (but may be) FCEL. However, in some circumstances the second antigen will be a protein present in the patient to be treated, e.g. where such  
20 proteins are to serve as carriers or depot releases for the therapeutic antibodies herein.

Such IgE-monovalent antibodies are made by methods known per se. For example, DNA encoding the anti-IgE Fv heavy and light chains is ligated to DNA encoding the Fc of a human  
25 recipient antibody. In addition, DNA is provided that encodes heavy and light chains for an antibody capable of binding second antigen or an unidentified antigen, or that encodes heavy and light chain having sufficient residues deleted from the CDRs that non-IgE antigen binding no longer  
30 can occur. A conventional recombinant host is transformed with all four DNAs and the products recovered. Assuming random chain assortment, a subpopulation of antibody products will contain one arm with anti-IgE heavy and light chain and at least another arm having specificity for second antigen or  
35 no antigen. The desired subpopulation then is purified by conventional methods, e.g., immunoaffinity absorption or by molecular sieving. These antibodies also can be made by reduction of the starting antibodies followed by oxidative

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chain recombination, as has heretofore been employed in the preparation of monovalent antibodies (see for example Glennie et al., Nature 295:712 [1982]).

In addition to IgE-monovalency, in other embodiments the antibodies are modified so that they contain a maximum proportion of human sequence (commensurate with retention of required or desired activity), i.e., they are converted to chimeras or are humanized. In both instances the functional effect is to place the anti-IgE binding capability of the murine or other donor antibody into a human background to make it as non-immunogenic as possible. General methods are known for making chimeras and for humanizing antibodies (as noted above). A minimal amount of non-human antibody sequence is substituted into the recipient human antibody. Typically, the non-human residues are substituted into the  $V_H$ ,  $V_L$ ,  $V_H$ - $V_L$  interface or framework of the recipient human antibody. Generally, the Kabat CDR's of the humanized antibodies are about 80% and more typically about 90% homologous with the non-human donor CDR's. The  $V_H$ - $V_L$  interface and framework residues of the humanized antibody, on the other hand, are about 80%, ordinarily 90% and preferably about 95% homologous with the recipient human antibody. Homology is determined by maximal alignment of identical residues. The resulting antibody is (a) less immunogenic in humans than a murine antibody and (b) capable of binding to FCEL-bound huIgE but substantially incapable of binding to FCEH-bound huIgE. Such antibodies typically comprise a human antibody which is substituted by an amino acid residue from a complementarity determining region (CDR),  $V_L$ - $V_H$  interface or a framework region of a non-human anti-IgE antibody which is capable of binding. One or more, and preferably all, of the nonhuman CDR's L1, L2, L3, H1, H2 or H3 are substituted into the human antibody recipient.

The characteristics possessed by the MAE11 antibody were preferred for therapeutic use. Since MAE11 bound to soluble IgE, bound to IgE bearing B cells, blocked IgE binding to the low and high affinity IgE receptor, inhibited *in vitro* IgE production and failed to bond to IgE coated basophils, it

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was chosen as the donor antibody for humanization. The recipient antibody was Kabat human kappa (light) subgroup I and human subgroup III heavy chain, although it will be understood that any other human antibody can be suitably employed. Surprisingly, optimal results were not obtained by simply substituting the murine CDRs in place of the CDRs in a recipient human antibody (Fig. 3; Table 8 *infra*). Instead, it was necessary to restore donor framework hydrophobic residues such as V<sub>H</sub> 78, 48, 49, 63, 67, 69; 82 or 82c, or V<sub>L</sub> 13, 19, 58, 78 or 104, in order to achieve a degree of inhibition of IgE binding similar to that of the donor antibody. While these residues function to establish the conformation of CDRs, they generally are not exposed to the exterior of the antibody so use of the murine residues should not exert a significant impact on immunogenicity. Other non-CDR residues exerting an effect on binding included V<sub>H</sub>60, 61, 37, 24, and V<sub>H</sub>50, 52, 58 and 95 (non-CDR by Chothia), and V<sub>L</sub>4, V<sub>L</sub>33 (non-CDR by Chothia) and V<sub>L</sub>53 (non-CDR by Chothia). The human framework hydrophobic residues generally are substituted with other hydrophobic residues (especially those from the donor antibody) such as valine, isoleucine, leucine, phenylalanine or methionine. The remaining non-CDR residues are substituted with any other amino acid residue, but again preferably the murine residue found at the analogous site.

In general, the character of the anti-IgE antibody is improved by substituting, deleting or inserting a residue at or adjacent to V, sites 30, 30b, 30d, 33, 55, 57, 58, 78, 93, 94, or 104 (where sites 30, 30a, 30b, 30c, 30d are identified with reference to the sequence DYDGD in the light chain sequence depicted in Figure 3).

Position V<sub>H</sub>-78 is most preferably substituted with phenylalanine. However, it also is substituted with leucine, valine, isoleucine, methionine, alanine or any other residue which results in an improvement in the characteristics of the antibody (see *infra*).

Position V<sub>H</sub>-60 is most preferably substituted with asparagines, although substitution with glutamine, histidine, lysine, arginine or any other residue which improves the

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characteristics of the antibody shall fall within the scope of this invention.

Position  $V_H$ -61 is most preferably substituted with proline, although glycine, alanine, valine, leucine, isoleucine or any other residue which results in an improvement in the characteristics of the antibody also is suitable.

CDR residues were imported from the donor MaE11. These included four inserts in  $V_L$  30a-30d as well as 91-94 ( $V_L$ ),  $V_H$  27-29, 29a, 31, 33 and 34,  $V_H$ 53-55, and  $V_H$ 97-101.  $V_L$  30, 30b or 30d as well as  $V_H$ 97, 100a or 100c, are important in conferring on the CDR ability to bind IgE.

$V_H$  positions 97, 100a and 100c in humaell (humanized Mae11) are all histidine, and 2 are arginine in Mae15. These residues are important in IgE binding. One, two or three of these are modified by substitution with basic residues, particularly lysine or arginine, but also with alanine, glycine, valine, isoleucine, serine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan or proline.

$V_L$  positions 30, 30b and 30d of humaell also are important for IgE binding. In humaell each of these positions are occupied by the acidic residue, aspartic acid. They are substituted in other embodiments by glutamic acid, but also may be substituted with alanine, glycine, valine, isoleucine, serine, threonine, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan or proline. It is within the scope of this invention to reverse the charges on positions  $V_L$  30, 30b and 30d with those on  $V_H$  97, 100a and 100c, e.g. by employing aspartic acid residues in the three  $V_H$  sites (2 in the case of humanized Mae15) and histidine in the three  $V_L$  sites.

Residues also may be inserted adjacent to  $V_H$  positions 97, 100a, 100c, 60 or 61, or  $V_L$  residues at positions 30, 30b, 30d or 78. Inserted residues generally will be of like kind, e.g. an acid residue would be inserted adjacent to  $V_L$ -30, 30b or 30d, while a basic residue is inserted adjacent to

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V<sub>H</sub>-97, 100 or 100c. The residues at these sites also may be deleted.

Humanized IgE-monovalent antibodies also are included within the scope of this invention. In this instance  
5 humanization extends to the anti-IgE arm as well, if necessary, to the remaining arm(s). Non-IgE binding arms of course can originate from human antibodies and in such case will not require humanization.

The foregoing variations are made by introducing  
10 mutations into the DNA encoding the precursor form of the antibody and expressing the DNA in recombinant cell culture or the like. This is accomplished by conventional methods of site directed mutagenesis. The variants then are screened for the desired character in assays conventional per se. In  
15 the case of anti-huIgE, desired character includes increasing the antibody affinity for huIgE, increasing its capacity and specificity for FCEL bound IgE, increasing the concentration of antibody required to stimulate histamine release from mast cells or basophils, reducing immunogenicity in humans, and  
20 other improvements apparent to the ordinary artisan. Optimizing these characteristics frequently will require balancing one improvement against another and therefore is a matter of judgment and is dependent upon the performance parameters dictated by the use intended for the antibody.

25 It is preferable to use a human IgG1 (or other complement fixing antibody) as the recipient immunoglobulin for humanization, although hu IgG2, IgG3, IgG4, IgE, IgM, IgD or IgA also can be used as recipient. Preferably the recipient is a complement fixing IgG antibody or an IgG antibody  
30 capable of participating in ADCC.

#### Therapeutic, Diagnostic and Preparatory Uses

The anti-IgE antibodies herein are useful in identifying IgE amino acid sequence variants in which the FCEL or FCEH-binding domains have been modified. Candidate FCEL or FCEH-  
35 specific polypeptides are incubated with these antibodies, and analogues to which these antibodies fail to bind are selected for further evaluation, e.g., determination, respectively of their FCEH and FCEL receptor binding

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characteristics. Any antibody, whether of murine, human, or another animal species in origin, or a variant thereof such as the humanized immunoglobulins described above, which has the epitopic specificity of any of antibodies MAE10 - MAE17 (especially MAE11/15, MAE13 or MAE17) will be equally acceptable. Such antibodies are easily identified by immunizing a suitable animal or using an in vitro Fv selection system, e.g. phagemid, with IgE of the appropriate animal origin and screening the animals or products for antibodies having the ability to compete for IgE with MAE11/15, 13, 17 or other antibodies which map to substantially the same epitopic site(s) as those described herein. As noted, the antibodies desirably are monovalent for FCEL- bound IgE when employed therapeutically. They may be bivalent and/or bispecific when used to purify IgE from plasma, serum or recombinant cell culture.

The FCEH and FCEL-specific, differential binding polypeptides are useful for diagnostics and therapeutics. In in vitro diagnostic assays they are employed as specific binding reagents in assays for FCεRI or FCεRII, respectively. The polypeptides of this invention are labelled with a detectable substance such as an enzyme, fluorescent or chemiluminescent group, radioisotope or a specific binding moiety that binds to a detectable substance (such as an enzyme). A typical specific binding moiety is an immunoglobulin variable domain which is capable of binding to the detectable substance. FCEL and FCEH specific polypeptides comprising immunoglobulin variable domains are described in more detail above.

Assay systems that employ the FCEL or FCEH specific polypeptides of this invention are analogous to the sandwich-type systems heretofore generally used in the immunoassay field. Here, the specific polypeptide is employed in the same fashion as labelled antibodies directed against antigen (the FCEL or FCEH receptor) or as an absorption agent insolubilized on a matrix for the isolation of receptor from test sample. Redox, proteolytic, esterolytic or other

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conventional enzyme labels are conjugated to the polypeptides of this invention for use in conventional assay systems.

The differential binding polypeptides of this invention also are useful for the isolation of FCEL or FCEH from cell culture in preparing FCEL or FCEH for therapeutic or research purposes. The polypeptide is covalently bonded or noncovalently adsorbed to a matrix such as an ion exchange resin, an immunoaffinity column (containing an antibody capable of binding a polypeptide fused to the FCEH or FCEL-specific polypeptide), an immobilized antigen (where the FCEH or FCEL-specific polypeptide comprises an immunoglobulin variable region capable of binding to the antigen) or a cyanogen bromide activated polysaccharide. The immobilized FCEH or FCEL-specific polypeptide then is contacted with the receptor preparation under conditions such that the receptor is bound to the FCEH or FCEL-specific polypeptide. The receptor then is eluted by changing the pH or ionic conditions and separating the polypeptide preparation from the receptor.

The differential binding polypeptides herein are useful in preparing antibodies specific to the FCEH or FCEL-binding domain of IgE. For example, antibodies capable of binding specifically to the FCEH or FCEL-binding domains of IgE are selected by first immunizing a subject with IgE. Monoclonal antibodies then are selected in the ordinary way for native IgE binding, and the monoclonal antibodies then screened to identify those that bind to a FCEH or FCEL-specific polypeptide of this invention. Preferably the FCEH or FCEL-specific polypeptide will be identical in sequence to the corresponding sequence of the IgE used as immunogen except, of course, for the minimal mutations need to confer FCEH or FCEL differential binding specificity. For example, the IgE monoclonal antibodies can be selected for their inability to bind to mutation 6. If they are unable to bind to mutation 6 one can conclude that they bind to the FCEH-binding site and are therefore promising for use in diagnostic or therapeutic procedures that depend upon an antibody that fails to bind to FCEH-bound IgE but which binds to FCEL-bound

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IgE. Confirmation is obtained by determining that the antibody selected in fact binds to IgE bound to FCEL. Since the selected antibody is highly specific for the key site(s) involved in receptor binding it is then possible to reduce the size of the antibody; the bulk of the antibody is not needed for steric hinderance of the IgE-receptor interaction. Thus, it becomes feasible in allergy therapy to use anti-IgE monovalent antibodies or other anti-IgE fragments such as Fab, Fab' and the like.

Similarly, the FCEL or FCEH-specific polypeptides are useful as immunogens for raising antibodies capable of cross-reacting with native IgE only at epitopic sites outside of the domains varied in creating the FCEH or FCEL-specific polypeptides. For example, mutations 6 and 7 are useful for raising antibodies specific for IgE epitopes except for the mutated AB-B or beta strand B domains as the case may be.

The FCEH and FCEL-specific polypeptides and anti-IgE antibodies (especially those with reduced immunogenicity) are useful in therapies for the treatment or prophylaxis of allergies, although the FCEH specific polypeptide subgroup which bears cytotoxic functionalities is not considered suitable for therapy since it could lead to degranulation of mast cells and basophils. Otherwise, the polypeptides typically are administered to a patient who is known to be sensitized to an allergen, preferably prior to an acute allergic response. The dosages and administration route will depend upon the accessory functionalities accompanying the polypeptides (e.g. cytotoxic agents, immunoglobulin effector functions, etc.), the condition of the patient (including the population of B cells or mast cells and basophils), the half-life of the polypeptide, the affinity of the polypeptide for its receptor and other parameters known to the clinician. As a general guide in the case of FCEH-specific polypeptide, one will determine from blood tests the amount of target cells circulating in the patient and determine the amount of polypeptide to displace or effectively compete with endogenous IgE taking into account the population of FCEH receptors as well as the half life and affinity of the

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polypeptide for FCEH. An excess of polypeptide calculated to be necessary to substantially displace native FCEH-bound IgE over a reasonable therapeutic interval will then be administered. Similar analysis used to determine the dosage of anti-IgE antibody or FCEL polypeptide.

Therapeutic polypeptides are administered by intravenous intrapulmonary, intraperitoneal subcutaneous or other suitable routes. Preferably the polypeptides are administered s.c. or i.v. over a period of about from 1 to 14 days as required. In the case of FCEL-specific polypeptide or anti-FCEL-bound IgE one would determine the amount needed to inhibit, suppress or kill a substantial portion of the IgE-secreting B cell population. Inhibition or suppression of the B cell population includes either or both of reductions in IgE secretion and attenuation of the total number of IgE secreting B cells. Candidate doses are readily determined by the use of *in vitro* cell cultures or animal models.

Therapy of allergic disorders with anti-FCEL bound IgE and FCEL or FCEH polypeptides optionally is accomplished with other known therapies for allergies. These include administration of gamma interferon, allergen desensitization, reduction in exposure to allergen, treatment with anti-histamines and the like.

#### Preparation of FCEH- and FCEL-Specific Polypeptides

The FCEH- or FCEL-specific polypeptides of this invention are made in conventional fashion, i.e., modifications of amino acid sequence are accomplished by commonly available DNA mutagenesis methods such as PCR amplification using primers bearing the mutants, or by M13 mutagenesis, followed by expression of the mutated DNA in recombinant host cells. The polypeptides also can be made by Merrifield or other *in vitro* methods of synthesis if they are sufficiently small (generally, under about 100 residues). However, the polypeptides preferably are made by recombinant methods. Selection of recombinant host cells, vectors, culture conditions and other parameters are not believed to be critical. In general, hosts, vectors and methods heretofore

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used in the recombinant expression of immunoglobulins (generally, IgGs) are also useful for the preparation of the polypeptide sequences of this invention. Preferably, mammalian cells such as myelomas, CHO, Cos, 293s and the like are employed as hosts, and the vectors are constructed for secretory expression of the polypeptide. Recombinant expression systems facilitate the preparation of functional immunoglobulin variants containing FCEL- or FCEH-specific sequences since the host cells can be transformed with DNA encoding one heavy chain containing the FCEL- or FCEH-specific sequences and one light chain, each of which contains a variable domain for binding a first antigen, and an immunoglobulin that binds antigen and FCEL or FCEH recovered. Similarly, the same process is used with DNA encoding in addition another heavy chain containing the FCEL- or FCEH-specific domain and another light chain, each of which contain a variable domain for binding a second antigen, and a bivalent immunoglobulin recovered. Properly assembled immunoglobulin analogues are recovered by affinity chromatography on a matrix containing the two antigen(s).

The polypeptides of this invention are recovered from lysed recombinant cell culture or (when secreted) the culture supernatant. Substantial purification is achieved by passing cell free extracts which contain the polypeptides over an immobilized FCEL or FCEH receptor affinity matrix. Other methods heretofore used to purify IgE or other appropriate immunoglobulins are equally acceptable here, including immunoaffinity and (when appropriate) absorption on immobilized antigen.

Polypeptides of this invention which contain short sequences preferably are prepared using solid-phase synthesis, e.g. the method of Merrifield, J. Am. Chem. Soc., 85:2149 (1963). However, other equivalent chemical syntheses known in the art are acceptable. The recombinant or in vitro synthesized polypeptides then are cross-linked to matrices (for use in diagnostic or preparatory procedures) or are placed into conformationally restrained structures. Known cyclizing procedures such as those described in PCT 90/01331

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or Lys/Asp cyclization using N $\alpha$ -Boc-amino acids on solid-phase support with Fmoc/9-fluorenylmethyl (Ofm) side-chain protection for Lys/Asp, followed by piperidine treatment and cyclization, are useful. Methods which depend upon cross-linking or cyclization through residue side chains may require that an extraneous residue be inserted at the C and/or N terminus of the AB-B or beta strand D domains, as the case may be, to provide a suitable cyclizing or cross-linking site.

10 Glu and Lys side chains also have been crosslinked in preparing cyclic or bicyclic peptides: the peptide is synthesized by solid phase chemistry on a p-methylbenzhydrylamine resin, the peptide is cleaved from the resin and deprotected. The cyclic peptide is formed using 15 diphenylphosphorylazide in diluted methylformamide. For an alternative procedure, see Schiller et al., Peptide Protein Res. 25:171-77 (1985). See also U.S. Patent 4,547,489.

Disulfide crosslinked or cyclized peptides are generated by conventional methods. The method of Pelton et al., J. Med 20 Chem., 29:2370-2375 (1986) is suitable. Also useful are thiomethylene bridges (Tetrahedron Letters 25:2067-2068 (1984)). See also Cody et al., J. Med Chem.: 28:583 (1985). The C390 residue found in the C-terminal sequence of the AB-B domain is useful in cross-linking or cyclizing this domain.

25 Typically, extraneous residues which are to participate in cyclization or cross-linking are inserted at the N- and C-termini of the chosen AB-B or beta strand D sequence as part of the synthesis of the polypeptide precursor to be employed in the procedure. The desired cyclic or cross-linked 30 peptides are purified by gel filtration followed by reversed-phase high pressure liquid chromatography or other conventional procedures. The peptides are sterilized by 0.2  $\mu$ m filtration and formulated into conventional pharmacologically acceptable vehicles.

35 The compounds described in this invention may be the free acid or base or converted to salts of various inorganic and organic acids and bases. Such salts are within the scope of this invention. Examples of such salts include ammonium,

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metal salts like sodium, potassium, calcium and magnesium; salts with organic bases like dicyclohexylamineN-methyl-D-glucamine and the like; and salts with amino acids such as arginine or lysine. Salts with inorganic and organic acids  
5 may be like prepared, for example, using hydrochloric, hydrobromic, sulfuric, phosphoric, trifluoroacetic, methanesulfonic, maleic, fumaric and the like. Non-toxic and physiologically compatible salts are particularly useful although other less desirable salts may have use in the  
10 processes of isolation and purification.

A number of methods are useful for the preparation of the salts described above and are known to those skilled in the art. For example, reaction of the free acid or free base form of a compound of Formula I with one or more molar  
15 equivalents of the desired acid or base in a solvent or solvent mixture in which the salt is insoluble; or in a solvent like water after which the solvent is removed by evaporation, distillation or freeze drying. Alternatively, the free acid or base form of the product may be passed over  
20 an ion exchange resin to form the desired salt, or one salt form of the product may be converted to another using the same general process.

Additional pharmaceutical methods may be employed, to control the duration of action of the polypeptides of this  
25 invention. Controlled release preparations are achieved through the use of polymers which complex with or absorb the subject polypeptides. Controlled delivery is achieved by formulating the polypeptides into appropriate macromolecular articles (for example, those prepared from polyesters,  
30 polyamino acids, polyvinyl, polypyrrolidone, ethylene vinyl acetate, methylcellulose, carboxymethylcellulose, or polyamine sulfate).

Alternatively, instead of entrapping the polypeptides in polymeric matrices, it is possible to entrap these materials  
35 in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization. Hydroxymethylcellulose or gelatin microcapsules and poly-(methacrylate) microcapsules, respectively, are useful,

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as are in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules). See Remington's Pharmaceutical Sciences (1980).

5

**EXAMPLE 1****Preparation of monoclonal antibodies to IgE**

Eight monoclonal antibodies with the ability to block the binding of IgE to the FCEH were used. These monoclonal antibodies, referred to as MAE10 - MAE17, were made in the following manner. Purified human IgE was prepared from supernatants of U266B1 cells (ATCC TIB 196) using affinity chromatography on a previously isolated anti-IgE antibody (Genentech MAE1, although other anti-huIgE antibodies are equally useful). For MAE12, five BALB/c female mice, age six weeks, were immunized in their foot pads with 10  $\mu$ g of the purified IgE in Ribi's adjuvant. Subsequent injections were done in the same manner one and three weeks after the initial immunizations. Three days after the final injection, the inguinal and popliteal lymph nodes were removed and pooled, and a single cell suspension was made by passing the tissue through steel gauze. For MAE14, MAE15, and MAE13 the immunizations were done in a similar manner except that for MAE13 30  $\mu$ g of IgE per injection were used and IgE 315-547 was used as a prefusion boost; for MAE14 and MAE15 five injections of 50  $\mu$ g each were used; and the IgE immunogen for MAE17 was IgE 315-547. For MAE10 and MAE11, injections were given subcutaneously in two doses of 100  $\mu$ g and a final booster of 50  $\mu$ g, and spleen cells were used for the fusions. The cells were fused at a 4:1 ratio with mouse myeloma P3X63-Ag8.653 (ATCC CRL 1580) in high glucose (DMEM) containing 50% w/v polyethylene glycol 4000.

Fused cells were plated at a density of  $2 \times 10^5$  per well in 96 well tissue culture plates. After 24 hours HAT selective medium (hypoxanthine/aminopterin/thymidine, Sigma Chemical Company, # H0262) was added. Of 1440 wells plated, 365 contained growing cells after HAT selection.

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Fifteen days after the fusion, supernatants were tested for the presence of antibodies specific for human IgE using an enzyme-linked immunosorbent assay (ELISA). The ELISA was performed as follows, with all incubations done at room temperature. Test plates (Nunc Immunoplate) were coated for 2 hours with rat anti-mouse IgG (Boehringer Mannheim, # 605-500) at 1  $\mu$ g/ml in 50 Mm sodium carbonate buffer, Ph 9.6, then blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS) for 30 minutes, then washed four times with PBS containing 0.05% Tween\*20 (PBST). Test supernatants were added and incubated two hours with shaking, then washed four times with PBST. Human IgE (purified from U266 cells as described above) was added at 0.5  $\mu$ g/ml and incubated for one hour with shaking, then washed four times in PBST. Horseradish peroxidase conjugated goat anti-human IgE (Kirkegaard & Perry Labs, # 14-10-04, 0.5 mg/ml) was added at a 1:2500 dilution and incubated for one hour, then washed four times with PBST. The plates were developed by adding 100  $\mu$ l/well of a solution containing 10 mg. of o-phenylenediamine dihydrochloride (Sigma Chemical Company # P8287) and 10  $\mu$ l of a 30% hydrogen peroxide solution in 25 ml of phosphate citrate buffer Ph 5.0, and incubating for 15 minutes. The reaction was stopped by adding 100  $\mu$ l/well of 2.5 M sulfuric acid. Data was obtained by reading the plates in an automated ELISA plate reader at an absorbance of 490 nm. For MAE12, 365 supernatants were tested and 100 were specific for human IgE. Similar frequencies of IgE specificity were obtained when screening for the other antibodies. All of the monoclonal antibodies described herein were of the IgG1 isotype except for MAE17, which was IgG2b, and MAE14, which was IgG2a.

Each of the IgE specific antibodies was further tested in cell-based and plate assays to select for antibodies which bound to IgE in such a way as to inhibit IgE binding to FCEH and which are not capable of binding to FCEH-bound IgE. The results of these assays are set forth in Table 5a and Table 5b below.

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**TABLE 5**  
**SUMMARY OF MURINE Anti-Hu IgE mAb CHARACTERISTICS**

| mAb    | Immunogen               | Schedule/<br>Dose ( $\mu$ g) | B-cell<br>source | Isotype | † Binding<br>FCBH-bound<br>IgE <sup>1</sup> | PBL<br>Histamine<br>Release <sup>2</sup><br>(EC50) | Amount<br>blocking<br>FCBH <sup>3</sup><br>(EC50) |
|--------|-------------------------|------------------------------|------------------|---------|---------------------------------------------|----------------------------------------------------|---------------------------------------------------|
| MaE 1  | PS IgE                  | 3x50                         | Lymph<br>Node    | IgG1    | .05 $\mu$ g/ml                              | 1 $\mu$ g/ml                                       | 0.3 $\mu$ g                                       |
| MaE 10 | U266 IgE                | 2x100,<br>1x50               | Spleen           | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 2.5 $\mu$ g                                       |
| MaE 11 | U266 IgE                | 2x100,<br>1x50               | Spleen           | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 0.6 $\mu$ g                                       |
| MaE 12 | U266 IgE                | 3x30                         | Lymph<br>Node    | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 0.8 $\mu$ g                                       |
| MaE 13 | U266 IgE                | 3x30                         | Lymph<br>Node    | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >10<br>$\mu$ g/ml                                  | 0.6 $\mu$ g                                       |
| MaE 14 | U266 IgE                | 5x50                         | Lymph<br>Node    | IgG2a   | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 2.5 $\mu$ g                                       |
| MaE 15 | U266 IgE                | 5x50                         | Lymph<br>Node    | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 0.6 $\mu$ g                                       |
| MaE 16 | rHIGE<br>aa 315-<br>547 | 5x1                          | Lymph<br>Node    | IgG1    | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | 0.7 $\mu$ g                                       |
| MaE 17 | rHIGE<br>aa 315-<br>547 | 5x1                          | Lymph<br>Node    | IgG2b   | No binding<br>at 10 $\mu$ g/ml              | >100<br>$\mu$ g/ml                                 | >5.0<br>$\mu$ g                                   |

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Table 5a. Summary of murine Anti-Hu IgE mAb (continued)

| mAb    | * Binding to Membrane IgE on U2668L (EC50) <sup>4</sup> | * Binding of IgE on FcERII (CD23) IM9 (EC50) <sup>5</sup> | Blocks 1µg IgE binding to FcER II (EC 50) <sup>6</sup> | Inhibition of <u>in-vitro</u> IgE synthesis <sup>7</sup> | Affinity constant for IgE <sup>8</sup> (Kd) |
|--------|---------------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------------|----------------------------------------------------------|---------------------------------------------|
| MaE 1  | 0.4 µg/ml                                               | .05µg/ml                                                  | >100µg                                                 | (-)                                                      | 5.4x10 <sup>-4</sup>                        |
| MaE 10 | 0.5 µg/ml                                               | No binding at 10µg/ml                                     | 2.5µg                                                  | (-)                                                      | 7x10 <sup>-9</sup>                          |
| MaE 11 | 0.15µg/ml                                               | No binding at 10µg/ml                                     | 0.6µg                                                  | (+)                                                      | 3x10 <sup>-4</sup>                          |
| MaE12  | >10µg/ml                                                | 1µg/ml                                                    | 5.0µg                                                  | (-)                                                      | 4x10 <sup>-7</sup>                          |
| MaE 13 | 1µg/ml                                                  | No binding at 10µg/ml                                     | 0.7µg                                                  | (++)                                                     | 5x10 <sup>-3</sup>                          |
| MaE 14 | 6µg/ml                                                  | No binding at 10µg/ml                                     | 2.5µg                                                  | (±)                                                      | 1.4x10 <sup>-3</sup>                        |
| MaE 15 | 6µg/ml                                                  | No binding at 10µg/ml                                     | 0.6µg                                                  | (±)                                                      | 7x10 <sup>-3</sup>                          |
| MaE 16 | 10µg/ml                                                 | <.05µg/ml                                                 | 5µg                                                    | (+)                                                      | ND                                          |
| MaE 17 | 10µg/ml                                                 | No binding at 10µg/ml                                     | 5µg                                                    | (++)                                                     | ND                                          |

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1. FACS based assays for analysis of murine anti-human IgE monoclonals. Screen of murine anti-human IgE monoclonal binding to IgE on CHO 3D10 (FcERI alpha +)

5 a. CHO 3D10 cells (FcERI alpha chain stable transfectant; Hakimi et al., J. Biol. Chem. 265:22079) at  $5 \times 10^5$  cells per sample are incubated with U266 IgE standard (lot no. 13068-46) at  $10 \mu\text{g/ml}$  in  $100 \mu\text{l}$  FACS buffer (0.1% BSA 10mM sodium azide in PBS pH 7.4) for 30 minutes at  $4^\circ\text{C}$  followed by one wash with FACS buffer. The amount of IgE  
10 binding is determined by incubating an aliquot of IgE loaded cells with a polyclonal FITC conjugated rabbit anti-human IgG (Accurate Chem. Co. AXL-475F, lot no 16) at  $50 \mu\text{g/ml}$  for 30 minutes at  $4^\circ\text{C}$  followed by three washes with FACS buffer.

15 b. IgE loaded cells are incubated with  $100 \mu\text{l}$  of murine anti-human IgE hybridoma supernatant (murine IgG concentration ranging from 1 to  $20 \mu\text{g/ml}$ ) for 30 min. at  $4^\circ\text{C}$  followed by one wash with FACS buffer. A Genentech monoclonal anti-human IgE (MAE1) at  $10 \mu\text{g/ml}$  is used as a positive control for binding. Genentech monoclonal (MAD 6P)  
20 which does not recognize IgE is used at  $10 \mu\text{g/ml}$  as a negative control.

c. Monoclonal binding to human IgE on CHO cells is detected by incubating cells with  $20 \mu\text{g/ml}$  FITC-conjugated, affinity purified F(ab) 2 Goat anti-mouse IgG (Organon  
25 Teknica cat. no. 10711-0081) for 30 minutes at  $4^\circ\text{C}$  followed by three washes with FACS buffer. Cells are added to  $400 \mu\text{l}$  buffer contain  $2 \mu\text{g/ml}$  propidium iodide (Sigma cat no. P4170) to stain dead cells.

d. Cells are analyzed on a Becton Dickinson FACSCAN  
30 flow cytometer. Forward light scatter and 90 degree side scatter gates are set to analyze a homogeneous population of cells. Dead cells which stain with propidium iodide are excluded from analysis. Hybridoma supernatants which do not bind IgE on CHO 3D10 cells were considered candidates for  
35 further screening.

2. Histamine release from peripheral blood basophils: Heparinized blood was obtained from normal donors and diluted 1:4 in a modified Tyrodes buffer (25mM tris, 150mM NaCl, 10mM

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CaCl<sub>2</sub>, MgCl<sub>2</sub>, 0.3 mg/ml HSA, pH 7.35) then incubated with 1nM human IgE (ND) at 4°C for 60 minutes. Cells were then added to Tyrodes buffer containing either the murine monoclonal anti-IgE Abs (10 mg/ml) or a polyclonal anti-human antiserum as the positive control, and incubated at 37°C for 30 minutes. Cells were pelleted, histamine in supernatants was acetylated and histamine content was determined using an RIA kit (AMAC, Inc. Westbrook, Main). Total histamine was determined from cells subjected to several rounds of freeze thawing. Percent histamine release was calculated as nM histamine content in supernatant - nM histamine spontaneously released divided by nM total histamine in the sample.

3. Blocking of Fitc conjugated IgE binding to FcERI alpha chain.

The effect of the antibodies on IgE binding was studied by preincubating Fitc labelled IgE with the various Mae antibodies at 37° C for 30 minutes in PBS containing 0.1% BSA and 10mM Sodium Azide pH 7.4, then incubating the complex with 5 x 10<sup>5</sup> 3D10 cells at 4°C for 30 minutes. The cells were then washed three times and mean channel fluorescence at 475 nM was measured. A murine anti-human IgE mAb (Mae1) which does not block IgE binding to the FcERI alpha chain was used as a control.

4. Analysis of murine anti-human IgE binding to membrane IgE positive B cell U266

a. U266 B1 cells (membrane IgE +) are cultured in base medium supplemented with 15% head inactivated fetal calf serum (Hyclone\* cat no. A-1111-L), penicillin, streptomycin (100 units/ml) and L-glutamine (2mM).

b. Cells (5x10<sup>5</sup>/aliquot) are incubated in 100μl FACS buffer containing murine anti-Human IgE monoclonals at 10, 5, 1, 0.5, and 0.1μg/ml for 30 minutes on ice in 96 well round bottom microtiter plates followed by two washes with FACS buffer. The Genentech monoclonal MAE1 is used as a positive control.

c. Cells are incubated in 100μl FACS buffer containing 50μg/ml (1:20 stock) FITC conjugated F(ab')<sub>2</sub> affinity purified goat anti-mouse IgG (Organon Teknika Cat. no. 1711-

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0084) for 30 minutes on ice followed by three washes with FACS buffer. Cells are added to 400  $\mu$ l FACS buffer containing propidium iodide at 2  $\mu$ g/ml to stain dead cells.

5. FACS based binding assays to FcERII(CD23+) B cell IM9

5 a. FACS analysis of IgE binding to FcERII(CD23) (+) B cell line IM9. The IM9 human B cell myeloma ATCC CCL 159. (Ann. N.Y. Acad. Sci., 190:221-234 [1972]) was maintained in GIF base medium with 10% heat inactivated fetal bovine serum, penicillin, streptomycin (100 units/ml) and L-glutamine  
10 (2mM).

b. Cells ( $5 \times 10^5$  aliquot) were incubated in 100 $\mu$ l of FACS buffer containing U266 IgE standard at 2 $\mu$ g/ml for 30 minutes at 4°C in 96 well microtiter plates followed by 2 washes with FACS buffer. As a control, cells were incubated  
15 in buffer alone or buffer containing 2 $\mu$ g/ml human IgG1 (Behring Diagnostics Cat. no. 400112, lot no. 801024).

c. Cells were then incubated with murine anti-human IgE monoclonals at 0.1 to 10 $\mu$ g/ml for 30 minutes on ice. Genentech monoclonal MAE1 was used as a positive control.

20 d. Cells were incubated in 100 $\mu$ l FACS buffer containing FITC conjugated F(ab')<sub>2</sub> goat anti-mouse IgG at 50  $\mu$ g/ml (Organon Teknika Ca #1711-0084) for 30 minutes at 4°C followed by 3 washes with FACS buffer.

e. Cells were added to 400 $\mu$ l buffer containing  
25 propidium iodide at 2 $\mu$ g/ml to stain dead cells.

f. Cells were analyzed on a Becton Dickinson FACSCAN flow cytometer. Forward light scatter and 90 degree side scatter gates were set to analyze a homogeneous population of cells and dead cells which stained with propidium iodide were  
30 excluded from analysis. FITC positive cells (IgE binding) were analyzed relative to cells stained with FITC rabbit anti-Human IgE alone.

g. As a positive control to determine the level of CD 23 on the surface of IM9 cells in each experiment, an aliquot  
35 of cells was stained with Becton Dickinson murine monoclonal Leu 20 (anti-CD23) at 10 $\mu$ g/ml for 30 minutes at 4°C followed by 2 washes. The cells were then incubated with FITC

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conjugated f(ab')<sub>2</sub> affinity purified goat anti-murine IgG at 50 µg/ml.

6. Antibody blocking of Fitc conjugated IgE binding to the low affinity IgE receptor.

5        The binding of 40 nM FITC labelled IgE to the low affinity IgE receptor (CD23) expressed on the B lymphoblast cell IM-9 was analyzed by flow cytometry on a FACSCAN flow cytometer. The effect of the antibodies on Fitc IgE binding was studied by preincubating Fitc IgE with the murine anti-  
10 human antibodies at 0.1 to 10 µg/ml. chimera at 37°C for 30 minutes in PBS containing 0.1% BSA and 10mM Sodium Azide pH 7.4, then incubating the complex with  $5 \times 10^5$  cells at 4°C for 30 minutes. The cells were then washed three times and mean channel fluorescence at 475 nM was measured.

15    7. IgE In Vitro Assay Protocol

a. Peripheral blood mononuclear cells were separated from normal donors.

b. Cells were washed extensively with phosphate buffered saline to remove as many platelets as possible.

20    c. Mononuclear cells were counted and resuspend in media at  $1 \times 10^6$  cells/ml. (Media=DMEM + pen/strep + 15% horse serum + IL-2 (25U/ml) + IL-4 (20ng/ml)).

d. Antibodies were added at appropriate concentrations on day 0, 5, and 8.

25    e. Cultures were incubated in 24 well Falcon tissue culture plates for 14 days.

f. On day 14 supernatants were removed and assayed for IgE concentrations by an IgE specific ELISA protocol.

30    8. Affinity constant (kd) of murine mAb for human IgE was determined by equilibrium binding (Scatchard analysis as follows:

a. IgE (ND and PS allotypes were iodinated by the chloramine T method and separated from free <sup>125</sup>I Na with a PD10 sephadex\*G25 column (Pharmacia cat. no. 17-0851-01) in  
35 RIA buffer:PBS, 0.5% bovine serum albumin (Sigma cat. no. A-7888), 0.05% Tween 20 (Sigma cat. no. P-1379), 0.01% thimerosal (Sigma cat. no. T-5125), pH 7.4. Approximately 78-95% of the post column counts were precipitated with 50%

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trichloroacetic acid and specific activity of iodinated IgE preparations ranged from 1.6 to 13  $\mu\text{Ci}/\mu\text{g}$  assuming 70% counting efficiency.

b. A fixed concentration of  $^{125}\text{I}$  IgE (approximately  $5 \times 10^4$  cpm) was added to varying concentrations of unlabelled IgE (1 to 200 nM) in a final volume of 0.1ml RIA buffer in 12x75mm polypropylene test tubes. Murine anti-human IgE mABs (20nM final concentration) in 0.1 ml RIA buffer were then added for a final assay volume of 0.2ml.

c. Samples were incubated 16-18 hours at  $25^\circ\text{C}$  with continuous agitation.

d. Bound and free  $^{125}\text{I}$  IgE was separated by the addition of a 0.3 ml mixture of affinity purified goat anti-mouse IgG (Boehringer Mannheim cat. no. 605 208) coupled to CN Br activated Sepharose\* 4B (cat no. 17-0430-01) and carrier protein A sepharose (Repligen cat. no. IPA 300) in RIA buffer and incubated 1 to 2 hours at  $25^\circ\text{C}$  with continuous agitation. RIA buffer (1ml) was then added, and tubes were centrifuged 5 min. 400 xg. Samples were counted to determine total counts. Supernatants were aspirated with a finely drawn pasteur pipet, samples were recounted and bound versus free counts were calculated.

e. Scatchard analysis was performed utilizing a Fortran program (scanplot) based on the Ligand program written by P. Munson at NIH. Scatplot uses a mass action equation fitting bound as a function of total using the Rodbard type regression analysis.

## EXAMPLE 2

### Preparation of Variant IgE

Based on the model of IgE Fc by Padlan & Davies (Mol. Immunol. 23:1063 (1986), which is based on the crystal structure of human IgG1 Fc (Deisenhofer, Biochem. 20:2361-2370 [1981]), a series of mutants were designed which could be used to test the binding of human IgE to its receptors. These mutants are designated Emut 1-13, and are listed in Table 6 below. The Fc $\epsilon$ 3 domain is comprised of seven  $\beta$ -strands which form a  $\beta$ -sheet structure representative of all immunoglobulin domains; there are six loops which connect

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these seven  $\beta$ -strands. We refer to these loops by the 2  $\beta$ -strands they connect, e.g. loop AB connects  $\beta$ -stands A and B. We have constructed mutants of human IgE in which we have substituted five of the Fc $\epsilon$ 3 domain loops with their counterparts from human IgG1 (Table 6, 1-5). The sixth loop contains the glycosylation site in both IgE and IgG and hence was not altered. One mutant, (Table 6, 6), was made by exchanging human Fc $\epsilon$ 3  $\beta$ -strand D with its human IgG1 Fc $\gamma$ 2 counterpart. Seven additional mutants, (Table 6, 7-13), consisted of the substitution of Ala residues into Fc $\epsilon$ 3  $\beta$ -strands and a loop in Fc $\epsilon$ 2.

A human IgE gene was cloned from U266, a publicly available cell line. The gene was cloned into a previously described phagemid vector containing the human cytomegalovirus enhancer and promoter, a 5' intron and sv40 polyadenylation signal (Gorman et al., DNA and Prot. Eng. Techn., 2:3-10 [1990]). Mutagenesis was performed by the Kunkel method (T.A. Kunkel, Proc. Natl. Acad. Sci. USA, 82:488-492 (1985) using buffers and enzymes supplied with the BioRad Muta-gene phagemid in vitro mutagenesis kit, together with oligonucleotides encoding the human IgG1 sequences shown in Table 6 below. Sequences of the mutant IgE DNAs were checked only at the site of mutation using <sup>35</sup>S dideoxy sequencing

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TABLE 6

| Mutant | Kabat<br>Residue No.<br>(Structure) <sup>1</sup> | Human IgE<br>Fcε3 Seq.         | Human IgG1<br>Fcγ2 Seq.        |                                              |
|--------|--------------------------------------------------|--------------------------------|--------------------------------|----------------------------------------------|
| 5      | 1                                                | FDLFIRKS<br>(SEQ.ID NO. 10)    | KDTLMISRT<br>(SEQ.ID NO. 11)   |                                              |
|        | 2                                                | APSKGT<br>(SEQ.ID NO. 12)      | SHEDPQ<br>(SEQ.ID NO. 13)      |                                              |
|        | 3                                                | SRASGKPVNHS<br>(SEQ.ID NO. 14) | YVDGVQVHNAK<br>(SEQ.ID NO. 15) |                                              |
|        | 4                                                | GTRDWIEGET<br>(SEQ.ID NO. 16)  | LHQDWLDGKE<br>(SEQ.ID NO 17)   |                                              |
|        | 5                                                | 465-469 (1FG)                  | RALM<br>(SEQ.ID NO. 18)        | APIE<br>(SEQ.ID NO. 19)                      |
| 10     | 6                                                | 423-428 (βD)                   | KEEKQR<br>(SEQ.ID NO. 20)      | PREQQY<br>(SEQ.ID NO. 21)                    |
|        | 7                                                | 383-385 (1AB)                  | RKS                            | [AAA] <sup>2</sup>                           |
|        | 8                                                | 387, 389 (βB)                  | T(I)T                          | [A(I)A] <sup>2</sup>                         |
|        | 9                                                | 403, 405 (βC)                  | N(L)T                          | [A(L)A] <sup>2</sup>                         |
| 15     | 10                                               | 438-440 (βE)                   | T(S)T                          | [A(S)A] <sup>2</sup>                         |
|        | 11                                               | 455, 457, 459<br>(βF)          | Q(C)R(V)T<br>(SEQ.ID NO. 22)   | [A(C)A(V)A] <sup>2</sup><br>(SEQ.ID NO. 23)  |
|        | 12                                               | 471, 473 (βG)                  | S(T)T                          | [A(T)A] <sup>2</sup>                         |
|        | 13                                               | 329-331, 334-<br>336           | QKH(WL)SDR<br>(SEQ.ID NO. 24)  | [AAA(WL)AAA] <sup>2</sup><br>(SEQ.ID NO. 25) |

<sup>1</sup>loop = 1 B-strand = β

20      <sup>2</sup>Sequences in brackets are from mutants in which alanine residues rather than IgG sequences were used to replace the IgE target sequence. Residues in parentheses were not altered in these mutants.

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The mutant IgEs were transiently expressed in human embryonic kidney 293 cells (Gorman et al., supra), purified on a mouse anti-human IgE antibody affinity column and samples run using SDS-PAGE to ascertain that the mutant proteins were of the proper molecular weight.

### EXAMPLE 3

#### Soluble FCEH binding assay

This assay is a sequential inhibition ELISA which measures binding to the FCEH only. In this assay, a monoclonal antibody against the FCEH is coated onto ELISA plates at a concentration of 1  $\mu$ g/ml in 50 mM sodium carbonate pH 9.6 for two hours at room temperature, and blocked for two hours with PBS containing 0.5% bovine serum albumin (PBSA), then washed three times with ELISA wash buffer (0.05% Tween 20 in PBS). Recombinantly produced soluble FCEH is added at a concentration of 50 units/ml and incubated for one hour, then washed five times in ELISA wash buffer. Mutant IgE samples are then added to the wells and incubated for one to two hours. The excess mutant IgE is removed by aspiration, and biotinylated IgE is then added at 50 ng/ml for 15 minutes followed by five washes with ELISA wash buffer. Streptavidin conjugated to horseradish peroxidase (Sigma Chemical Company #S5512) was added at a 1:5000 dilution for 15 minutes, then washed three times with ELISA wash buffer. Color was developed with a tetramethyl benzidine peroxidase substrate system (Kirkegaard & Perry Labs # 50-76-00, Lot. no. NA 18) for seven minutes at 25° C. The reaction was stopped by the addition of 1 M HCl. The ability of the mutant IgE to bind the FCEH is assessed by the degree to which the biotinylated IgE is prevented from binding. This assay is designed to test for any FCEH binding by the mutant IgE and is not meant to determine the affinity of the mutant for the FCEH relative to native IgE.

#### FACS based binding assays for U266 IgE mutants

Tissue culture supernatants from 293s cells transfected with U266 IgE cDNA were harvested at either 48 or 96 hours post transfection. Tissue culture supernatants were

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concentrated 5-X with Amicon Centriprep 30<sup>®</sup> centrifugal concentrators (30,000 MW cutoff). Concentrated supernatants were passed through a mouse monoclonal anti-U266 IgE affinity column (Genentech MAE1 coupled to CnBr-Sepharose). U266 IgE was eluted from the column with 3.0 M potassium cyanate in 50 mM tris buffer Ph 7.8. Eluate fractions containing protein as determined by 0.D.280 nm were pooled and placed in Amicon Centricon 30<sup>®</sup> concentrators. Eluate buffer was exchanged for PBS by passing multiple volumes of PBS through the concentrator. The final volume of affinity purified supernatant ranged from 0.5-1 ml. Structural integrity of recombinant IgE mutants was analyzed on 1-12% SDS PAGE gels and compared with U266 IgE standard obtained from the U266 cell line. Mutants were also analyzed for the ability to bind to a series of monoclonal and IgE antibodies to further ascertain proper folding and structural identity with native IgE. The concentration of immunoreactive IgE for each IgE mutant was determined by a human IgE capture ELISA as follows. Nunc Immunoplate Maxisorp<sup>®</sup> plates (Nunc # 4-39451) were coated overnight at 4° C with a Genentech murine IgG1 anti-U266 IgE (MAE1) at 1 µg/ml in coat buffer (50 mM sodium carbonate buffer pH 9.6 ). Coat antibody was removed by three washes with ELISA wash buffer (0.05% Tween 20 (US Biochemical Corporation # 20605) in PBS). Non-specific sites were blocked with ELISA diluent buffer (50 mM tris buffered saline containing 0.5% BSA (Sigma Chemical Company # A-7888), 0.05% Tween 20 and 2 mM EDTA) for two hours at 25° C on an orbital shaker. Diluent buffer was removed with 3 washes of ELISA wash buffer. Serial two-fold dilutions of IgE mutants in ELISA diluent buffer were added to the plate. U266 IgE standard (lot 13068-46) was added at 1000, 500, 250, 125, 62.5, 31.3, and 15.6 ng/ml in duplicate as standards. Samples and standard were incubated two hours at 25° C followed by three washes with ELISA wash buffer. IgE was detected with HRP conjugated Sheep anti-human IgE (ICN # N060-050-1) at 1:8000 in ELISA diluent buffer for 90 min. at 25° C followed by 3 washes with ELISA wash buffer. HRP

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conjugate was developed with a tetramethyl benzidine peroxidase substrate system (Kirkegaard & Perry Labs. # 50-76-00, Lot. no. NA 18) for 7 minutes at 25° C. The reaction was stopped by the addition of 1 M HCl. The reaction product was analyzed with a dual wavelength spectrophotometer at 450 nm minus absorption at 570nm. The U266 IgE standards were used to generate a standard curve and IgE concentrations of the sample were extrapolated by non-parametric linear regression analysis.

10 FcERI alpha (+) CHO 3D10 (FCEH expressing) and FcERII(CD23) (+) IM9 (FCEL expressing) B cell lines were used for the binding assays. The stably transfected CHO (duk -) cell clone 3D10 (JBC 265, 22079-22081, 1990) was maintained in Iscove's modified Dulbecco's media supplemented  
15 with 10% heat inactivated fetal calf serum, 80 µg/ml gentamicin sulfate and  $5 \times 10^{-7}$  M methotrexate. The IM9 human B cell myeloma ATCC CCL 159. (Ann. N.Y. Acad. Sci. 190:221-234, 1972) was maintained in GIF base medium with 10% heat inactivated fetal bovine serum, penicillin, streptomycin (100  
20 units/ml) and L-glutamine (2mM). As a positive control to determine the level of CD23 on the surface of IM9 cells in each experiment, an aliquot of cells was stained with Becton Dickinson murine monoclonal Leu 20 (anti-CD23) at 10 µg/ml for 30 minutes at 4° C followed by two washes in FACS buffer.  
25 The cells were then incubated with FITC conjugated F(ab')<sub>2</sub> affinity purified goat anti-murine IgG at 5 µg/ml. Adherent CHO3D10 cells were removed from tissue culture dishes by incubation with 10 mM EDTA in PBS for 2 minutes at 37°C. Cells were counted, then resuspended in FACS buffer (0.1%  
30 BSA, 10 mM Na azide in PBS pH 7.4 ) at a concentration of  $5 \times 10^6$ /ml. CHO3D10 and Im9 cells ( $5 \times 10^5$ /aliquot) were incubated in 100 µl of FACS buffer containing U266 IgE standard or IgE mutants at 2µg/ml for 30 minutes at 4° C in 96 well microtiter plates followed by two washes with FACS  
35 buffer. As a control, cells were incubated in buffer alone or buffer containing 2 µg/ml human IgG1 (Behring Diagnostics # 400112, lot no. 801024). Cells were then incubated in 100

5  $\mu$ l FACS buffer containing FITC conjugated rabbit anti-human IgE at 20  $\mu$ g/ml (Accurate Chem. Co. # AXL 475F, lot.no. 040A) for 30 minutes at 4° C followed by 3 washes with FACS buffer. 400  $\mu$ l of buffer containing propidium iodide at 2 $\mu$ g/ml was added to the cell suspension to stain dead cells. Cells were analyzed on a Becton Dickinson FACSCAN flow cytometer. Forward light scatter and 90 degree side scatter gates were set to analyze a homogeneous population of cells and dead cells which stained with propidium iodide were excluded from analysis. FITC positive cells (IgE binding) were analyzed relative to cells stained with FITC rabbit anti-H IgE alone.

10 The foregoing assays were used to determine the ability of the example 2 IgE analogues to bind to FCEH and FCEL. The results are set forth in Table 7.

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TABLE 7

## BINDING OF IGE AND IGE ANALOGUES TO FCEH AND FCEL

| Sample/Mutant               | Conc.<br>(ug/ml)  | FCEH alpha<br>% CHO<br>3D10(+) | FCEL (CD23)<br>% IM9 (+) |
|-----------------------------|-------------------|--------------------------------|--------------------------|
| U266 IgE                    | 10                | 90.3                           | 92.5                     |
| U266 IgE                    | 5                 | 89.9                           | 82.6                     |
| U266 IgE                    | 0.5               | 59.6                           | 4.6                      |
| U266 IgE                    | 0.1               | 15.8                           | 1.7                      |
| 1                           | 1.65 <sup>1</sup> | 1.7                            | 4.3                      |
| 2                           | 1.65              | 34.3                           | 48.9                     |
| 3                           | 1.65              | 32.3                           | 1.2                      |
| 4                           | 1.65              | 4.9                            | 9.2                      |
| 5                           | 1.65              | 60.5                           | 73.9                     |
| 6                           | 1.65              | 1.4                            | 71.6                     |
| 7                           | 1.65              | 76.4                           | 4.6                      |
| 8                           | 1.65              | 70.3                           | 16.3                     |
| 9                           | 1.65              | 84.2                           | 94.3                     |
| 10                          | 1.65              | 67.5                           | 84.8                     |
| 11                          | 1.65              | 70.8                           | 61.5                     |
| 12                          | 1.65              | 84.7                           | 90.3                     |
| 13                          | 1.65              | 85.7                           | 96.1                     |
| dh 184 (+)                  | 1.65              | 83.8                           | 21.1                     |
| PA13 <sup>2</sup> (control) | 10                | 1.3                            |                          |

<sup>1</sup>Values based on quantitative Elisa. U266 was used as the standard and murine anti-F<sub>c</sub> monoclonal antibody to capture.

<sup>2</sup>A CDR grafted human IgG.

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Three mutant IgEs exhibited complete loss of binding to the FCEH receptor: mutants 1, 4 and 6. Mutant 6 altered  $\beta$ -strand D at the end of Fc $\epsilon$ 3 close to the Fc $\epsilon$ 2 domain. Mutants 1 and 4 involved alteration of two Fc $\epsilon$ 3 loops which are adjacent and near the Fc $\epsilon$ 4 domain. Note that mutant 7 is subset of mutant 1 in which the three C-terminal residues of loop AB have been changed to alanines (Table 6, 1 vs. 7). However, mutant 7 does not affect binding to FCEH. We interpret this to mean that either 1) Fc $\epsilon$ RI binds at least one of IgE residues 377-381 or 2) the extra residue in IgG1 loop AB (9 residues) substituted for IgE loop AB (8 residues) effected deformation of some adjacent binding determinant, possibly loop EF. That mutants 8 and 10 had no affect on Fc $\epsilon$ RI binding most likely means that the FCEH receptor does not protrude into the cavity bounded by loop AB and  $\beta$ -strand D.

Although mutant 4 had a Leu replacing Gly444 (Table 6), this should not affect the conformation of loop EF. Residue 444 is prior to the N-terminus of this  $\alpha$ -helix. In addition, murine IgE has a Val at position 444 and rat IgE has an Asp. The two buried hydrophobic residues in the middle of the  $\alpha$ -helix, W448 and I449, are retained in the substituted IgG1 loop (W448, L449) as is G451 which terminated the  $\alpha$ -helix. Hence the conformation of loop EF should be similar in IgE and IgG1.

Mutants 2 and 3 exhibited decreased binding to FCEH. Since loop BC lies near  $\beta$ -strand D and loop CD is in the vicinity of loop EF, it is conceivable that one or two residues in loops BC and CD contact FCEH.

Five mutant IgEs exhibited loss of binding to the FCEL receptor: mutants 1, 3 4, 7 and 8. Mutants 1 and 4 were discussed above. Mutant 3 involved alteration of loop CD; in contrast to FCEH, loop CD evidently plays a major role in FCEL binding. Mutant 7, a subset of mutant 1 as discussed above, comprises the C-terminal portion of loop AB and is proximal to loop EF. Additionally, mutant 8 consists of replacement of two Thr residues (387,389) with Ala; these two

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residues are part of  $\beta$ -strand B which is at the bottom of the aforementioned cavity bounded by loop AB and  $\beta$ -strand D. Mutant 10 comprised a different two residues in this cavity (438,440) on  $\beta$ -strand E, which is adjacent to  $\beta$ -strand B. Since mutant 10 did not affect FCEL binding, we conclude that the FCEL receptor should have only a minimal incursion into cavity while the high affinity receptor does not intrude into the cavity.

In addition to a glycosylation site at Asn430 which corresponds to the glycosylation site in IgG Fc, human IgE contains another glycosylation site at Asn403. Mutant 9 converted Asn403 and Thr405 to alanines (Table 6). Loss of carbohydrate did not affect binding to either receptor.

Based on the information from mutants 1-13, we propose that FCEH and FCEL have binding sites on IgE Fc which are distinct but overlap. The low affinity receptor seems to interact with a relatively smaller portion of the IgE Fc $\epsilon$ 3 domain involving three adjacent loops: AB, CD and EF. In contrast, the high affinity receptor interacts with a larger portion of IgE Fc $\epsilon$ 3, which spans loop EF,  $\beta$ -strand D and, possibly, the N-terminal portion of loop AB. Portions of loops BC and CD in the vicinity of loop EF and  $\beta$ -strand D may also interact with FCEH. In addition, FCEL may protrude into the cavity bounded by loop AB and  $\beta$ -strand D whereas FCEH does not do so. Since we have not evaluated any mutants in Fc $\epsilon$ 4 and only one in Fc $\epsilon$ 2 (mutant 13), it is possible that portions of these two domains play a role in IgE-receptor binding.

#### EXAMPLE 4

##### Preparation of Humanized MaE11

Residues were selected from MaE11 and inserted or substituted into a human Fab antibody background ( $V_H$  region Kabat subgroup III and  $V_L$  region kappa subgroup I). A first version, humaellv1 or version 1, is described in Table

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**TABLE 8. Changes in V<sub>H</sub> human subgroup III and V<sub>L</sub> x subgroup I (Kabat) consensus sequences for humanized MaE11 Version 1**

|    | Domain         | hu<br>Residue | Residue<br>No. | V.1                         | CDR<br>by Kabat | CDR<br>by Chothia |
|----|----------------|---------------|----------------|-----------------------------|-----------------|-------------------|
| 5  | V <sub>L</sub> |               |                |                             |                 |                   |
|    |                | M             | 4              | L                           |                 |                   |
|    |                | insert        | 30abcd         | YDGD<br>(SEQ.ID.<br>NO. 26) | L1              | L1                |
|    |                | L*            | 33             | M                           | L1              |                   |
|    |                | S             | 53             | Y                           | L2              |                   |
| 10 |                | Y             | 91             | S                           | L3              | L3                |
|    |                | N             | 92             | H                           | L3              | L3                |
|    |                | S             | 93             | E                           | L3              | L3                |
|    |                | L             | 94             | D                           | L3              | L3                |
|    | V <sub>H</sub> |               |                |                             |                 |                   |
| 15 |                | A             | 24             | V                           |                 |                   |
|    |                | F*            | 27             | Y                           | H1              | H1                |
|    |                | T             | 28             | S                           | H1              | H1                |
|    |                | F*            | 29             | I                           | H1              | H1                |
|    |                | insert        | 29a            | T                           | H1              | H1                |
| 20 |                | D             | 31             | G                           | H1              | H1                |
|    |                | A             | 33             | S                           | H1              | H1                |
|    |                | M*            | 34             | W                           | H1              | H1                |
|    |                | V             | 37             | I                           |                 |                   |
|    |                | V             | 50             | S                           | H2              |                   |
| 25 |                | S             | 52             | T                           | H2              |                   |
|    |                | N             | 53             | Y                           | H2              | H2                |
|    |                | G             | 54             | D                           | H2              | H2                |
|    |                | S             | 55             | G                           | H2              | H2                |
|    |                | Y             | 58             | N                           | H2              |                   |

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|  |   |        |                                        |    |    |
|--|---|--------|----------------------------------------|----|----|
|  | L | 78     | F                                      |    |    |
|  | D | 95     | G                                      | H3 |    |
|  |   | 97-101 | All<br>Changed<br>to MaE11<br>Sequence | H3 | H3 |

5       \*       These residues typically do not vary despite their  
position within CDRs. The remaining residues found in the KI  
and III CDR sequences (particularly the CDRs by Chothia  
10 structural analysis), will vary widely among recipient human  
antibodies.

15       The affinity of version 1 was assayed and found to be  
about 100 times lower than that of the donor antibody Mae11  
(see Figs. 4a and 4b). Therefore, further modifications in  
the sequence of version 1 were made as shown in Table 9.  
Determination was made of the ability of these further  
modifications to inhibit the binding of labelled huIgE to  
FCEH.

20       The 50% inhibition assays whose results are shown in  
Table 9 were conducted as follows:

A 96-well assay plate (Manufn Nunc.) was coated with  
0.05 ml of the FcεRI alpha chain IgG1 chimeric receptor in  
1 µg/ml coating buffer (50nmol carbonate/bicarbonate, pH  
9.6). Assay was done for 12 hours at 4-8° C. The wells  
25 were aspirated and 250 µl blocking buffer (PBS--1% BSA pH  
7.2) was added and incubated for one hour at 4°C. In a  
separate assay plate the samples and reference murine Mae11  
antibody were titrated from 200 µg/ml by 1 to 10-fold  
dilution with assay buffer (0.5% BSA, 0.05% Tween 20, PBS,  
30 pH 7.2) and an equal volume of 10ng/ml biotinylated IgE at  
10ng/ml was added and the plate incubated for 2-3 hours at  
25°C. The FcεRI-coated wells were washed three times with  
PBS-0.05% Tween20, and then 50 µl from the sample wells  
were transferred and incubated with agitation for 30  
35 minutes at 25°C. 50 µl/well of streptavidin-HRP diluted  
1:5000 in assay buffer was incubated for 15 minutes with  
agitation and then the plate was washed as before. 50

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5  $\mu$ l/well of Microwell peroxidase substrate (Kirkgaard & Parry Laboratories) was added and color was developed for 30 minutes. The reaction was stopped by adding an equal volume of 1 normal HCl and the adsorbance measured at 450nm. The concentration for 50% inhibition was calculated by plotting percent inhibition versus concentration of blocking antibody with a nonlinear 4-parameter curve-fit for each antibody using INPLOT.

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TABLE 9

## Humanized MaE11 Variants

|    |                          |                                      |                                                                                             |                                                                                      |                                          |                              |                          |
|----|--------------------------|--------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------|------------------------------|--------------------------|
| 5  | Version<br>[F(ab)-<br>X] | Domain                               | Changes from<br>F(ab)-Version 1                                                             | Purpose                                                                              | Conc. at<br>50% inh.<br>(ng/ml)*<br>Mean | S.D.<br>for<br>prev.<br>col. | F(ab)-X<br>——<br>F(ab)-1 |
|    | 1                        | -                                    | -                                                                                           | -                                                                                    | 6083                                     | 1279                         | 1.0                      |
|    | 2                        | V <sub>L</sub>                       | L4M<br>M33L                                                                                 | Packing; CDR-L1                                                                      | 9439                                     | 508                          | 1.6                      |
| 10 | 3                        | V <sub>L</sub>                       | E55G<br>G57E                                                                                | Sequence usually<br>E55-X-G57                                                        | 5799                                     | 523                          | 1.0                      |
|    | 4                        | V <sub>H</sub>                       | I37V                                                                                        | VL-VH interface                                                                      | 8622                                     | 107                          | 1.4                      |
|    | 5                        | V <sub>H</sub>                       | V24A                                                                                        | Packing; CDR-H1                                                                      | 9387                                     | 733                          | 1.6                      |
|    | 6                        | V <sub>H</sub>                       | F78L                                                                                        | Packing; CDR-<br>H1,H2                                                               | 17537                                    | 4372                         | 2.9                      |
|    | 7                        | V <sub>L</sub><br><br>V <sub>H</sub> | L4M<br>R24K<br>E55G<br>G57E<br>V24A<br>I37V<br>T57S<br>A60N<br>D61P<br>V63L<br>G65N<br>F78L | remake version 1<br>to accomplish a<br>direct exchange<br>of CDR residues            | > 100000                                 |                              | > 16.0#                  |
| 15 | 7a                       | V <sub>H</sub>                       | As V.7 except<br>V <sub>H</sub> L78 is F                                                    |                                                                                      | 98000                                    |                              | 16.0                     |
|    | 8                        | V <sub>H</sub>                       | A60N<br>D61P                                                                                | Extended Kabat<br>CDR-H2 & A60N<br>is at V <sub>L</sub> -V <sub>H</sub><br>interface | 1224                                     | 102                          | 0.20                     |
|    | 8a                       | V <sub>H</sub>                       | As V.8 except<br>V <sub>H</sub> V63 is L and<br>F67 is I                                    | CDR-H2; packing<br>of L63 and I67                                                    | 416                                      | 66                           | 0.07                     |
|    | 8b                       | V <sub>H</sub>                       | As V.8 except<br>F67 is I                                                                   | CDR-H2; packing<br>of V63 and I67                                                    | 501                                      | 84                           | 0.08                     |

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| Version<br>[F(ab)-<br>X] | Domain                               | Changes from<br>F(ab)-Version 1                                                                        | Purpose                                            | Conc. at<br>50% inh.<br>(ng/ml)*<br>Mean | S.D.<br>for<br>prev.<br>col. | F(ab)-X<br>——<br>F(ab)-1 |
|--------------------------|--------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------|------------------------------------------|------------------------------|--------------------------|
| 1                        | -                                    | -                                                                                                      | -                                                  | 6083                                     | 1279                         | 1.0                      |
| 9                        | V <sub>L</sub><br><br>V <sub>H</sub> | A13V<br>V19A<br>V58I<br>L78V<br>V104L<br>V48M<br>A49G<br>A60N<br>V63L<br>F67I<br>I69V<br>M82L<br>L82cA | Repack Version<br>1 interior as in<br>murine MaE11 | 842                                      | 130                          | 0.14                     |
| 23                       | V <sub>L</sub>                       | L4M                                                                                                    | Packing; CDR-L1                                    | 6770                                     | 349                          | 1.1                      |
| 10                       | V <sub>L</sub>                       | D30A<br>D30bA<br>D30dA                                                                                 | CDR-L1<br>modification                             | >100000                                  |                              | >16.0                    |
| 11                       | V <sub>L</sub>                       | E93A<br>D94A                                                                                           | CDR-L3<br>modification                             | 17456                                    | 7115                         | 2.9                      |
| 12                       | V <sub>H</sub>                       | D54A                                                                                                   | CDR-H2<br>modification                             | 2066                                     | 174                          | 0.34                     |
| 13                       | V <sub>H</sub>                       | H97A<br>H100aA<br>H100cA                                                                               | CDR-H3<br>modification                             | >100000                                  |                              | > 16.0                   |
| 14                       | V <sub>L</sub>                       | D30A                                                                                                   | CDR-L1<br>modification                             | 3452                                     | 183                          | 0.57                     |
| 15                       | V <sub>L</sub>                       | D30bA                                                                                                  | CDR-L1<br>modification                             | 6384                                     | 367                          | 1.0                      |
| 16                       | V <sub>L</sub>                       | D30dA                                                                                                  | CDR-L1<br>modification                             | >100000                                  |                              | > 16.0                   |
| 17                       | V <sub>H</sub>                       | H97A                                                                                                   | CDR-H3<br>modification                             | 19427                                    | 8360                         | 3.2                      |
| 18                       | V <sub>H</sub>                       | H100aA                                                                                                 | CDR-H3<br>modification                             | 2713                                     | 174                          | 0.45                     |
| 19                       | V <sub>H</sub>                       | H100cA                                                                                                 | CDR-H3<br>modification                             | 15846                                    | 8128                         | 2.6                      |

15 \* Inhibition of fitc-IgE binding to FcεR1 (FcεRI). Full  
length antibody and humanized fragment versions: mean and  
standard deviation of three assays.

20 # A F(ab)-X / F(ab)-1 ratio of > 16 means that this  
variant exhibited no binding even at the highest F(ab)  
concentrations used.

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As can be seen from Table 9 and Figs. 4a and 4b, version 8 (in which human residues of version 1 at sites 60 and 61 in the light chain were replaced by their Mae11 counterparts) demonstrated substantially increased affinity. Further increases in affinity are seen in versions 8a and 8b, where one or two murine residues replaced human residues. Other increases, at least virtually to the level of Mae11, were accomplished by replacing hydrophobic human residues found in the interior of  $V_H$  and  $V_{H1}$  with their MaE11 counterparts, resulting in the variant designated version 9 (see Table 9 and Figs. 4a and 4b). Accordingly, the humanized antibodies of this invention will possess affinities ranging about from 0.1 to 100 times that of MAE11.

Table 10 explores the effects on FCEH affinity of various combinations of humanized maE11 IgG1 variants.

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Table 10. Humanized MaE11 IgG1 Variants

| Variant | Conc. at 50%<br>inh. (ng/ml)<br>Mean* | S.D. from<br>previous<br>column* | Var. X<br>-----<br>IgL1H1 | Var. X<br>-----<br>MaE11 |
|---------|---------------------------------------|----------------------------------|---------------------------|--------------------------|
| IgL1H1  | 7569                                  | 1042                             | 1.0                       | 16.9                     |
| IgL1H8  | 3493                                  | 1264                             | 0.46                      | 7.8                      |
| IgL9H9  | 1118                                  | 172                              | 0.15                      | 2.5                      |
| IgL1H9  | 608                                   | 364                              | 0.08                      | 1.4                      |
| IgL9H1  | 5273                                  | 2326                             | 0.70                      | 11.7                     |
| IgL1H8b | 1449                                  | 226                              | 0.19                      | 3.2                      |
| MaE11   | 449                                   | 53                               | 0.06                      | 1.0                      |

\* L1 = V<sub>L</sub> as in F(ab)-1 (human buried residues--not exposed to solvent); L9 = V<sub>L</sub> as in F(ab)-9 (murine buried residues); H1 = V<sub>H</sub> as in F(ab)-1 (human buried residues); H8 = V<sub>H</sub> as in F(ab)-8 (F(ab)-1 with AlaH60Asn, AspH61Pro); H9 = V<sub>H</sub> as in F(ab)-9 (murine buried residues); H8b = V<sub>H</sub> as in F(ab)-8b (F(ab)-8 with PheH67Ile).

**EXAMPLE 5****Creation of IgE Mutants**

IgE mutants (Table 11) were prepared to evaluate their effect on binding to anti-IgE, especially MaE11, and to FcεRI and FcεRII. Some of the mutants were designed to substitute for a specific amino acid residue another residue with either similar or very different charge or size. The impact of these changes on receptor binding is reflected in the table below.

The receptor assays are performed substantially as follows:

A 96-well assay plate (Manufn Nunc.) was coated with 0.05 ml of FcεRI or RII IgG1 chimeric receptor in 1 μg/ml coating buffer (50nmol carbonate/bicarbonate, pH 9.6).

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Assay was done for 12 hours at 4-8° C. The wells were aspirated and 250  $\mu$ l blocking buffer (PBS--1% BSA pH 7.2) was added and incubated for one hour at 4°C. In a separate assay plate the samples and reference murine MaE11 antibody were titered from 200  $\mu$ g/ml by 1 to 10-fold dilution with assay buffer (0.5% BSA, 0.05% Tween 20, PBS, pH 7.2) and an equal volume of 10ng/ml biotinylated IgE at 10ng/ml was added and the plate incubated for 2-3 hours at 25°C. The Fc $\epsilon$ RI-coated wells were washed three times with PBS-0.05% Tween20, and then 50  $\mu$ l from the sample wells were transferred and incubated with agitation for 30 minutes at 25°C. 50  $\mu$ l/well of streptavidin-HRP diluted 1:5000 in assay buffer was incubated for 15 minutes with agitation and then the plate was washed as before. 50  $\mu$ l/well of Microwell peroxidase substrate (Kirkgaard & Parry Laboratories) was added and color was developed for 30 minutes. The reaction was stopped by adding an equal volume of 1 normal HCl and the adsorbance measured at 450nm. The absorbance was plotted versus concentration of blocking antibody MaE11 and an inhibition standard curve was generated using INPLOT.

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Table 11. Amino acid sequences of IgE mutants

|    | Mutant     | Kabat residue # | Human IgE Fcε3 seq.        | Mutant seq.                | Fcε-RI*   | FcεRII* |
|----|------------|-----------------|----------------------------|----------------------------|-----------|---------|
| 5  | Loop AB    |                 |                            |                            |           |         |
|    | 1          | 377-385         | FDLFIRKS<br>(SEQ.ID.27)    | KDTLMISRT<br>(SEQ.ID.28)   | -         | -       |
|    | 7          | 383-385         | RKS                        | AAA                        | +/-, -    | +, -    |
| 10 | 21         | 377, 381        | F(DL)F<br>(SEQ.ID.29)      | Q(DL)H<br>(SEQ.ID.30)      | +         | +       |
|    | 66         | 382             | I                          | A                          | +         | +       |
|    | 67         | 383             | R                          | A                          | +         | +/-     |
|    | 68         | 384             | K                          | A                          | +         | +       |
|    | 69         | 385             | S                          | A                          |           |         |
| 15 | 102        | 383, 384        | RK                         | DD                         |           |         |
|    | β-strand B |                 |                            |                            |           |         |
|    | 8          | 387, 389        | T(I)T                      | A(I)A                      | +/-, +    | -       |
| 20 | 70         | 387             | T                          | A                          | +         | +/-, +  |
|    | 71         | 389             | T                          | A                          | +         | +       |
|    | Loop BC    |                 |                            |                            |           |         |
|    | 2          | 396-401         | APSKGT<br>(SEQ.ID.31)      | SHEDPQ<br>(SEQ.ID.32)      |           |         |
|    | β-strand C |                 |                            |                            |           |         |
|    | 9          | 403, 405        | N(L)T                      | A(L)A                      | +         | +       |
| 25 | Loop CD    |                 |                            |                            |           |         |
|    | 3          | 407-420         | SRASGKPVNHS<br>(SEQ.ID.33) | YVDGVQVHNAK<br>(SEQ.ID.34) | +/-       | -       |
|    | 55         | 407-415         | SR(A)S(G)K<br>(SEQ.ID.35)  | AA(A)A(G)A<br>(SEQ.ID.36)  | +/-       | +       |
| 30 | 59         | 407             | S                          | A                          | +         | +       |
|    | 60         | 408             | R                          | A                          | +         | -       |
|    | 61         | 411             | S                          | A                          | +         | +       |
|    | 62         | 415             | K                          | A                          | +         | -       |
|    | 63         | 418             | N                          | A                          | +/-       | +       |
| 35 | 64         | 419             | H                          | A                          | +         | +       |
|    | 65         | 420             | S                          | A                          | +/-       | +       |
|    | 100        | 408             | R                          | E                          |           |         |
|    | 101        | 415             | K                          | D                          |           |         |
| 40 | β-strand D |                 |                            |                            |           |         |
|    | 6          | 423-428         | KEEKQR<br>(SEQ.ID.37)      | PREQQY<br>(SEQ.ID.38)      | +         | +       |
|    | 35         | 422             | R                          | A                          | +         | +       |
|    | 36         | 4423            | K                          | A                          | +         | +       |
| 45 | 37         | 424             | E                          | A                          | +         | +       |
|    | 38         | 425             | E                          | A                          | +         | +       |
|    | 39         | 426             | K                          | A                          | +         |         |
|    | 40         | 427             | Q                          | A                          | -, +/-    | +       |
|    | 41         | 428             | R                          | A                          | +         | +       |
|    | 75         | 423-425         | KEE                        | AAA                        | -, +/-, + | +       |
| 50 | 76         | 426-428         | KQR                        | AAA                        |           |         |
|    | 79         | 423, 425, 427   | KEEKQR<br>(SEQ.ID.39)      | AEAKAR<br>(SEQ.ID.40)      |           |         |
|    | 80         | 424, 426, 428   | KEEKQR<br>(SEQ.ID.41)      | KAEAQA<br>(SEQ.ID.42)      |           |         |
| 55 | 81         | 28              | K                          | P                          |           |         |
|    | 82         | 423, 423-427    | KEEKQR<br>(SEQ.ID.43)      | AAEAQA<br>(SEQ.ID.44)      |           |         |

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|    |                         |                     |                           |                           |        |      |
|----|-------------------------|---------------------|---------------------------|---------------------------|--------|------|
|    | <b>β-strand E</b><br>10 | 438,440             | T(S)T                     | A(S)A                     | +      | +    |
| 5  | <b>Loop EF</b><br>4     | 444-453             | GTRDWIEGET<br>(SEQ.ID.45) | LHQDWLDGKE<br>(SEQ.ID.46) | -      | -    |
|    | 49                      | 445                 | T                         | A                         | +      | +    |
|    | 50                      | 336                 | R                         | A                         | +      | -    |
|    | 51                      | 337                 | D                         | A                         | +      | +, - |
| 10 | 52                      | 450                 | E                         | A                         | +/-    | -    |
|    | 53                      | 452                 | E                         | A                         | +      | +    |
|    | 77                      | 445,446             | TR                        | AA                        | +      | +/-  |
| 15 | 78                      | 450,452,4<br>53     | E(G)ET<br>(SEQ.ID.47)     | A(G)AA<br>(SEQ.ID.48)     | -      | -    |
|    | 83                      |                     | G                         | L                         | +      | +    |
|    | 84                      | 444                 | G                         | A                         | +      | +    |
|    | 85                      | 444                 | TRDWIEGET<br>(SEQ.ID.49)  | HQDWLDGKE<br>(SEQ.ID.50)  | -      | +    |
| 20 | 86                      | 445-453             | T                         | H                         | +      | +    |
|    | 87                      | 445                 | TR                        | HQ                        | +/-, + | +    |
|    | 88                      | 445,446             | R                         | E                         | -      | +    |
|    | 89                      | 446                 | E(G)ET<br>(SEQ.ID.51)     | D(G)KE<br>(SEQ.ID.52)     | +/-, - | +/-  |
| 25 | 93                      | 450,452,4<br>53     | D                         | R                         | +      | +    |
|    | 94                      |                     | E                         | R                         | +/-, - | +    |
|    | 95                      | 447                 | E                         | R                         | +      | +    |
|    | 96                      | 450                 | T                         | R                         | +      | +    |
| 30 | 97                      | 452                 | D                         | N                         | +      | +    |
|    | 98                      | 453                 | E                         | Q                         | +      | +    |
|    | 99                      | 447                 | E                         | D                         | +      | +    |
|    |                         | 452                 |                           |                           |        |      |
|    |                         | 452                 |                           |                           |        |      |
|    | <b>β-strand F</b><br>11 | 445,457,4<br>59     | Q(C)R(V)T<br>(SEQ.ID.53)  | A(C)A(V)A<br>(SEQ.ID.54)  |        |      |
| 35 | <b>Loop FG</b><br>5     | 465-469             | RALM<br>(SEQ.ID.55)       | APIE<br>(SEQ.ID.56)       |        |      |
|    | <b>β-strand G</b><br>12 | 471,473             | S(T)T                     | A(T)A                     | +, +   |      |
|    | <b>Fcε2</b><br>13       | 329-331,<br>334-336 | QKH(WL)SDR<br>(SEQ.ID.57) | AAA(WL)AAA<br>(SEQ.ID.58) | +, +   |      |
| 40 | <b>Fcε4</b><br>72       | 498-501             | PRAA<br>(SEQ.ID.59)       | QPRE<br>(SEQ.ID.60)       |        |      |
|    | 73                      | 594-599             | ASPSQT<br>(SEQ.ID.61)     | LHNHY<br>(SEQ.ID.62)      |        |      |
| 45 | 74                      | 595-599             | S(P)SQT<br>(SEQ.ID.63)    | A(P)AAA<br>(SEQ.ID.64)    |        |      |

\* Positive receptor binding indicated by "+", no binding by "-", and positive binding but less than unaltered is shown by "+/-". Where more than one assay was performed, results are separated by commas.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Jardieu, Paula M.  
Presta, Leonard G.
- (ii) TITLE OF INVENTION: Immunoglobulin Variants
- 10 (iii) NUMBER OF SEQUENCES: 64
- (iv) CORRESPONDENCE ADDRESS:  
 15 (A) ADDRESSEE: Genentech, Inc.  
 (B) STREET: 460 Point San Bruno Blvd  
 (C) CITY: South San Francisco  
 (D) STATE: California  
 (E) COUNTRY: USA  
 (F) ZIP: 94080
- 20 (v) COMPUTER READABLE FORM:  
 (A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk  
 (B) COMPUTER: IBM PC compatible  
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
 (D) SOFTWARE: patin (Genentech)
- 25 (vi) CURRENT APPLICATION DATA:  
 (A) APPLICATION NUMBER:  
 (B) FILING DATE: 14-AUG-1992  
 (C) CLASSIFICATION:
- 30 (vii) PRIOR APPLICATION DATA:  
 (A) APPLICATION NUMBER: 07/879495  
 (B) APPLICATION DATE: 07-MAY-1992
- 35 (vii) PRIOR APPLICATION DATA:  
 (A) APPLICATION NUMBER: 07/744768  
 (B) APPLICATION DATE: 14-AUG-1991
- 40 (viii) ATTORNEY/AGENT INFORMATION:  
 (A) NAME: Adler, Carolyn R.  
 (B) REGISTRATION NUMBER: 32,324  
 (C) REFERENCE/DOCKET NUMBER: 718P2
- 45 (ix) TELECOMMUNICATION INFORMATION:  
 (A) TELEPHONE: 415/225-2614  
 (B) TELEFAX: 415/952-9881  
 (C) TELEX: 910/371-7168

## (2) INFORMATION FOR SEQ ID NO:1:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 118 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Asp | Ser | Asn | Pro | Arg | Gly | Val | Ser | Ala | Tyr | Leu | Ser | Arg | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
| Ser | Pro | Phe | Asp | Xaa | Leu | Phe | Ile | Arg | Lys | Ser | Pro | Thr | Ile | Thr |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |
| Cys | Leu | Val | Val | Asp | Leu | Ala | Pro | Ser | Lys | Gly | Thr | Val | Asn | Leu |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |
| Thr | Trp | Ser | Arg | Xaa | Ala | Ser | Xaa | Xaa | Gly | Lys | Pro | Val | Asn | His |

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50 55 60  
 Ser Thr Arg Lys Glu Glu Lys Gln Arg Xaa Asn Xaa Xaa Gly Thr  
 65 70 75  
 5  
 Leu Thr Val Thr Ser Thr Leu Pro Val Gly Thr Arg Asp Trp Ile  
 80 85 90  
 10  
 Glu Gly Glu Thr Gln Cys Arg Val Thr His Pro His Leu Pro Arg  
 95 100 105  
 Ala Leu Xaa Met Arg Ser Thr Thr Lys Thr Ser Gly Pro  
 110 115 118

## 15 (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 111 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu  
 1 5 10 15  
 Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp  
 20 25 30  
 30 Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly  
 35 40 45  
 Gln Pro Pro Ile Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Gly Ser  
 50 55 60  
 35 Glu Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 65 70 75  
 40 Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Phe  
 80 85 90  
 Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Ala Gly  
 95 100 105  
 45 Thr Lys Leu Glu Ile Lys  
 110 111

## (2) INFORMATION FOR SEQ ID NO:3:

- 50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 134 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser  
 1 5 10 15  
 60 Gln Ser Leu Ser Leu Ala Cys Ser Val Thr Gly Tyr Ser Ile Thr  
 20 25 30  
 Ser Gly Tyr Ser Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Lys  
 35 40 45  
 65 Leu Glu Trp Met Gly Ser Ile Thr Tyr Asp Gly Ser Ser Asn Tyr  
 50 55 60

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Asn Pro Ser Leu Lys Asn Arg Ile Ser Val Thr Arg Asp Thr Ser  
65 70 75

Gln Asn Gln Phe Phe Leu Lys Leu Asn Ser Ala Thr Ala Glu Asp  
80 85 90

Thr Ala Thr Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His  
95 100 105

Trp His Phe Ala Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser  
110 115 120

Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Arg  
125 130 134

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val  
1 5 10 15

Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Ser  
20 25 30

Ser Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys  
35 40 45

Ala Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp  
50 55 60

Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile  
65 70 75

Ser Asn Val Gln Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln  
80 85 90

Tyr Tyr Thr Tyr Pro Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu  
95 100 105

Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro  
110 115 120

Pro Ser Thr Arg  
124

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 130 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser  
1 5 10 15

Gln Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Thr Ile Thr  
20 25 30

Ser Asp Asn Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Lys

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35 40 45  
 Leu Glu Trp Met Gly Tyr Ile Asn His Ser Gly Thr Thr Ser Tyr  
 50 55 60  
 5  
 Asn Pro Ser Leu Lys Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser  
 65 70 75  
 10  
 Lys Asn Gln Phe Phe Leu Gln Leu Asn Ser Val Thr Thr Glu Asp  
 80 85 90  
 Thr Ala Thr Tyr Tyr Cys Ala Trp Val Val Ala Tyr Ala Met Asp  
 95 100 105  
 15  
 Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr  
 110 115 120  
 Thr Pro Pro Ser Val Tyr Pro Leu Ala Arg  
 125 130

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

30 Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu  
 1 5 10 15  
 Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp  
 20 25 30  
 35 Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly  
 35 40 45  
 40 Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 50 55 60  
 Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 65 70 75  
 45 Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr  
 80 85 90  
 Tyr Cys Gln Gln Ser Asn Glu Asp Pro Phe Thr Phe Gly Ala Gly  
 95 100 105  
 50 Thr  
 106

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 137 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

65 Asp Val Gln His Gln Glu Ser Glu Pro Asp Leu Val Lys Pro Ser  
 1 5 10 15  
 Gln Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr  
 20 25 30

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Ser Gly Tyr Asn Arg His Trp Ile Arg Gln Phe Pro Gly Asn Lys  
 35 40 45  
 5 Leu Glu Trp Met Gly Tyr Ile His Tyr Ser Gly Ser Thr Asn Tyr  
 50 55 60  
 Asn Pro Ser Leu Lys Arg Arg Ile Ser Ile Thr Arg Asp Thr Ser  
 65 70 75  
 10 Lys Asn Gln Phe Phe Leu Gln Leu Asn Ser Val Thr Thr Glu Asp  
 80 85 90  
 Thr Ala Thr Tyr Tyr Cys Ala Arg Gly Ser Ile Tyr Tyr Tyr Gly  
 95 100 105  
 15 Ser Arg Tyr Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val  
 110 115 120  
 20 Thr Val Ser Ser Ala Lys Arg His Pro His Leu Ser Ile His Trp  
 125 130 135  
 Pro Gly  
 137  
 25 (2) INFORMATION FOR SEQ ID NO:8:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 453 amino acids  
 (B) TYPE: amino acid  
 30 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
 35 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly  
 1 5 10 15  
 Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr  
 20 25 30  
 40 Ser Gly Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly  
 35 40 45  
 Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr  
 50 55 60  
 45 Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser  
 65 70 75  
 50 Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
 80 85 90  
 Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His  
 95 100 105  
 55 Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser  
 110 115 120  
 Ser Ala Ser Thr Lys Gly Lys Gly Pro Ser Val Phe Pro Leu Ala  
 125 130 135  
 60 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys  
 140 145 150  
 65 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
 155 160 165  
 Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu

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180

5 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
185 190 195

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
200 205 210

10 Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser  
215 220 225

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
230 235 240

15 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
260 265 270

20 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
275 280 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
305 310 315

30 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser  
320 325 330

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala  
335 340 345

35 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
350 355 360

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
365 370 375

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
380 385 390

45 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
395 400 405

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
410 415 420

50 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
425 430 435

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
440 445 450

55 Pro Gly Lys  
453

60 (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 218 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val  
 1 5 10 15  
 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp  
 5 20 25 30  
 Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly  
 35 40 45  
 Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser  
 10 50 55 60  
 Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 65 70 75  
 Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr  
 15 80 85 90  
 Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly  
 20 95 100 105  
 Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe  
 110 115 120  
 Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser  
 25 125 130 135  
 Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val  
 140 145 150  
 Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 30 155 160 165  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
 35 170 175 180  
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val  
 185 190 195  
 Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr  
 40 200 205 210  
 Lys Ser Phe Asn Arg Gly Glu Cys  
 215 218

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Phe Asp Leu Phe Ile Arg Lys Ser  
 1 5 8

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Asp Thr Leu Met Ile Ser Arg Thr

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1

5

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## (2) INFORMATION FOR SEQ ID NO:12:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Pro Ser Lys Gly Thr  
1 5 6

## 15 (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
20 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

25 Ser His Glu Asp Pro Gln  
1 5 6

## (2) INFORMATION FOR SEQ ID NO:14:

- 30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

35 Ser Arg Ala Ser Gly Lys Pro Val Asn His Ser  
1 5 10 11

## (2) INFORMATION FOR SEQ ID NO:15:

- 40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Tyr Val Asp Gly Val Gln Val His Asn Gln Lys  
1 5 10 11

50

## (2) INFORMATION FOR SEQ ID NO:16:

- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

60 Gly Thr Arg Asp Trp Ile Glu Gly Glu Thr  
1 5 10

## (2) INFORMATION FOR SEQ ID NO:17:

- 65 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

5    Leu His Gln Asp Trp Leu Asp Gly Lys Glu  
     1                    5                    10

(2) INFORMATION FOR SEQ ID NO:18:

10    (i) SEQUENCE CHARACTERISTICS:  
      (A) LENGTH: 4 amino acids  
      (B) TYPE: amino acid  
      (D) TOPOLOGY: linear

15    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

     Arg Ala Leu Met  
      1                    4

20    (2) INFORMATION FOR SEQ ID NO:19:

     (i) SEQUENCE CHARACTERISTICS:  
      (A) LENGTH: 4 amino acids  
      (B) TYPE: amino acid  
25    (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

30    Ala Pro Ile Glu  
      1                    4

(2) INFORMATION FOR SEQ ID NO:20:

35    (i) SEQUENCE CHARACTERISTICS:  
      (A) LENGTH: 6 amino acids  
      (B) TYPE: amino acid  
      (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

40    Lys Glu Glu Lys Gln Arg  
      1                    5                    6

(2) INFORMATION FOR SEQ ID NO:21:

45    (i) SEQUENCE CHARACTERISTICS:  
      (A) LENGTH: 6 amino acids  
      (B) TYPE: amino acid  
      (D) TOPOLOGY: linear

50    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

55    Pro Arg Glu Gln Gln Tyr  
      1                    5                    6

(2) INFORMATION FOR SEQ ID NO:22:

60    (i) SEQUENCE CHARACTERISTICS:  
      (A) LENGTH: 5 amino acids  
      (B) TYPE: amino acid  
      (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

65    Gln Cys Arg Val Thr  
      1                    5

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## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ala Cys Ala Val Ala  
1 5

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Lys His Trp Leu Ser Asp Arg  
1 5 8

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Ala Ala Trp Leu Ala Ala Ala  
1 5 8

## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Tyr Asp Gly Asp  
1 4

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Phe Asp Leu Phe Ile Arg Lys Ser  
1 5 8

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Lys Asp Thr Leu Met Ile Ser Arg Thr  
1 5 9

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Phe Asp Leu Phe  
1 4

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Asp Leu His  
1 4

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Pro Ser Lys Gly Thr  
1 5 6

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ser His Glu Asp Pro Gln  
1 5 6

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Arg Ala Ser Gly Lys Pro Val Asn His Ser  
1 5 10 11

(2) INFORMATION FOR SEQ ID NO:34:

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- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Tyr Val Asp Gly Val Gln Val His Asn Ala Lys  
1 5 10 11

10

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

20

Ser Arg Ala Ser Gly Lys  
1 5 6

(2) INFORMATION FOR SEQ ID NO:36:

25

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ala Ala Ala Ala Gly Ala  
1 5 6

35

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

45

Lys Glu Glu Lys Gln Arg  
1 5 6

(2) INFORMATION FOR SEQ ID NO:38:

50

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Pro Arg Glu Gln Gln Tyr  
1 5 6

60

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

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Lys Glu Glu Lys Gln Arg  
1 5 6

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ala Glu Ala Lys Ala Arg  
1 5 6

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Glu Glu Lys Gln Arg  
1 5 6

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Lys Ala Glu Ala Gln Ala  
1 5 6

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Lys Glu Glu Lys Gln Arg  
1 5 6

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ala Ala Glu Ala Gln Ala  
1 5 6

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids

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(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Thr Arg Asp Trp Ile Glu Gly Glu Thr  
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Leu His Gln Asp Trp Leu Asp Gly Lys Glu  
1 5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Glu Gly Glu Thr  
1 4

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ala Gly Ala Ala  
1 4

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Thr Arg Asp Trp Ile Glu Gly Glu Thr  
1 5 9

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

His Gln Asp Trp Leu Asp Gly Lys Glu  
1 5 9

## (2) INFORMATION FOR SEQ ID NO:51:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Glu Gly Glu Thr

1 4

## (2) INFORMATION FOR SEQ ID NO:52:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Asp Gly Lys Glu

1 4

## (2) INFORMATION FOR SEQ ID NO:53:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gln Cys Arg Val Thr

1 5

## (2) INFORMATION FOR SEQ ID NO:54:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ala Cys Ala Val Ala

1 5

## (2) INFORMATION FOR SEQ ID NO:55:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Arg Ala Leu Met

1 4

## (2) INFORMATION FOR SEQ ID NO:56:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Ala Pro Ile Glu  
1 4

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gln Lys His Trp Leu Ser Asp Arg  
1 5 8

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Ala Ala Ala Trp Leu Ala Ala Ala  
1 5 8

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Pro Arg Ala Ala  
1 4

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 4 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gln Pro Arg Glu  
1 4

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Ala Ser Pro Ser Gln Thr  
1 5 6

(2) INFORMATION FOR SEQ ID NO:62:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu His Asn His Tyr  
1 5

10

## (2) INFORMATION FOR SEQ ID NO:63:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

20

Ser Pro Ser Gln Thr  
1 5

## (2) INFORMATION FOR SEQ ID NO:64:

25

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Ala Pro Ala Ala Ala  
1 5

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An antibody which is capable of binding to FcεRII-bound IgE but is substantially incapable of binding to FcεRI-bound IgE, comprising at least an Fab region of a human recipient antibody into which have been substituted at one or more positions 30, 30b, 30d, 33, 53, 91, 92, 93 and 94 in the light chain and positions 27, 28, 29, 29a, 31, 33, 34, 50, 52, 53, 54, 55, 58, 95, 97, 98, 99, 100 and 101 in the heavy chain, residues from analogous positions in donor antibody MAE11, MAE13 or MAE15 having the light and heavy chain amino acid sequences as set out in SEQ ID NO:2 to 7, or a donor antibody having the characteristics possessed by the MAE11 antibody, in particular in binding soluble IgE, binding IgE-bearing B cells, blocking IgE binding to FcεRI and FcεRII, inhibiting *in vitro* IgE production and failing to bind to IgE coated basophils.

2. An antibody according to Claim 1, wherein said donor antibody is MAE11.

3. An antibody according to Claim 1 or Claim 2, wherein the recipient human antibody has a Kabat human sub-group I kappa (light) chain and human sub-group III heavy chain.

4. An antibody according to any one of the preceding claims which is an IgG1, IgG2, IgG3 or IgG4 antibody.

5. An antibody according to Claim 4 which is a complement-fixing IgG antibody, or an IgG antibody capable of participating in ADCC.

6. An antibody comprising the Fab heavy and light chain sequences of humaellver.1, 2, 3, 4, 5, 6, 7, 7a, 8,

8a, 8b or 9, wherein humaellver.1 has the heavy and light chain amino acid sequences as set out in SEQ ID NO:8 and 9, and humaellver.2-9 have the heavy and light chain amino acid sequences of humaellver.1, further incorporating and modifications shown in Table 5.

7. An antibody comprising Fab heavy and light chain amino acid sequences of humaellver.1 as set out in SEQ ID NO:8 and 9, said heavy chain sequence being substituted at position 60 with asparagine, at position 61 with proline and position 67 with isoleucine.

8. An antibody according to any one of Claims 1 to 5, having additional substitution of one or more donor antibody framework residues at positions selected from 4, 13, 19, 58, 78 and 104 in the light chain, and 78, 48, 49, 60, 63, 67, 69, 82 and 82c in the heavy chain.

9. An antibody according to Claim 8, having donor framework hydrophobic residues at positions 13, 19, 58, 78 and 104 in the light chain and 48, 49, 63, 67, 69, 78, 82 and 82c in the heavy chain.

10. An antibody according to Claim 8, having additional substitutions of donor residues at positions 33 and 53 in the light chain and 24, 37, 50, 52, 58, 60, 61 and 95 in the heavy chain.

11. The antibody of any one of Claims 1 to 5 and 8 to 10, having substitution, deletion or insertion of a donor antibody residue at or adjacent to one or more sites selected from site 30, 30b, 30d, 33, 55, 57, 58, 78, 93, 94 or 104 in the light chain and sites 24, 37, 48, 49, 54, 57, 60, 61, 63, 65, 67, 69, 78, 82, 82c, 97, 100a or 100c in the heavy chain.

12. An antibody according to Claim 11, wherein V<sub>H</sub> 78 is substituted with phenylalanine.

13. An antibody according to Claim 11, wherein V<sub>H</sub> 78 is substituted with leucine, valine, isoleucine, methionine or alanine.
14. An antibody according to Claim 11, wherein V<sub>H</sub> 60 is substituted with asparagine.
15. An antibody according to Claim 11, wherein V<sub>H</sub> 60 is substituted with glutamine, histidine, lysine or arginine.
16. An antibody according to Claim 11, wherein V<sub>H</sub> 61 is substituted with proline.
17. An antibody according to Claim 11, wherein V<sub>H</sub> 61 is substituted with glycine, alanine, valine, leucine or isoleucine.
18. An antibody according to Claim 8, wherein residues are imported from the donor MAE11, including four inserts in V<sub>L1</sub> 30a-30d as well as V<sub>L3</sub> 91-94, V<sub>H1</sub> 27-29, 29a, 31, 33 and 34, V<sub>H2</sub> 53-55 and V<sub>H3</sub> 97-101.
19. An antibody according to Claim 11, wherein any of V<sub>H</sub> positions 97, 100a and 100c has a basic residue other than histidine.
20. An antibody according to claim 19 wherein the basic residue is lysine or arginine.
21. An antibody according to Claim 11, wherein any of V<sub>H</sub> positions 97, 100a and 100c has a residue selected from the group consisting of alanine, glycine, valine, isoleucine, serine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan and proline.

22. An antibody according to Claim 11, wherein any of  $V_L$  positions 30, 30b and 30d are glutamic acid.

23. An antibody according to Claim 11, wherein any of  $V_L$  positions 30, 30b and 30d has a residue selected from the group consisting of alanine, glycine, valine, isoleucine, serine, threonine, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan and proline.

24. An antibody according to Claim 11, wherein any of  $V_L$  positions 30, 30b and 30d are histidine and any of  $V_H$  positions 97, 100 a and 100c are aspartic acid.

25. An antibody according to Claim 11, wherein residues are inserted adjacent to any of  $V_H$  residues at positions 97, 100a, 100c, 60 or 61 or  $V_L$  residues at positions 30, 30b, 30d or 78.

26. An antibody according to Claim 24, wherein the inserted residues are of like kind, that is acid residues inserted adjacent to  $V_L$  30, 30b or 30d or basic residues adjacent to  $V_H$  97, 100a or 100c.

27. An antibody according to Claim 11, wherein residues at any of  $V_L$  30, 30b, 30d or 78 or of  $V_H$  97, 100a, 100c, 60 or 61 are deleted.

28. An antibody according to any one of claims 1 to 27, which is a bispecific antibody.

29. An antibody according to any one of claims 1 to 28, which is monovalent for Fc $\epsilon$ R11-bound IgE, and is capable of an immunoglobulin effector function and comprises an Fc domain containing at least two heavy chains.

30. An antibody according to any one of claims 1 to 29, comprising a human consensus heavy chain and light chain sequence.

31. An antibody according to any one of claims 1 to 30, which has an IgE affinity which is substantially the same as or greater than that of MAE11 for IgE.

32. A method of making an antibody which is capable of binding to FcεRII-bound IgE but is substantially incapable of binding to FcεRI-bound IgE, comprising substituting into at least an Fab region of a human recipient antibody into which has been substituted at one or more positions 30, 30b, 30d, 33, 53, 91, 92, 93 and 94 in the light chain and positions 27, 28, 29, 29a, 31, 33, 34, 50, 52, 53, 54, 55, 58, 95, 97, 98, 99, 100 and 101 in the heavy chain, residues from analogous positions in donor antibody MAE11, MAE13 or MAE15 having the light and heavy chain amino acid sequences as set out in SEQ ID NO:2 to 7, or a donor antibody having the characteristics possessed by the MAE11 antibody, in particular in binding soluble IgE, binding IgE-bearing B cells, blocking IgE binding to FcεRI and FcεRII, inhibiting *in vitro* IgE production and failing to bind to IgE coated basophils.

33. A method according to Claim 32, wherein said donor antibody is MAE11.

34. A method antibody according to Claim 32 or Claim 33, wherein the recipient human antibody has a Kabat human sub-group I kappa (light) chain and human sub-group III heavy chain.

35. A method according to any one of Claims 32 to 34 wherein the antibody produced is an IgG1, IgG2, IgG3 or IgG4 antibody.

36. A method according to Claim 35 wherein the antibody thus produced is a complement-fixing IgG antibody, or an IgG antibody capable of participating in ADCC.

37. A method according to any one of Claims 32 to 36, including additional substitution of one or more donor antibody framework residues at positions selected from 4, 13, 19, 58, 78 and 104 in the light chain, and 78, 48, 49, 60, 63, 67, 69, 82 and 82c in the heavy chain.

38. A method according to Claim 37, wherein the antibody thus produced has donor framework hydrophilic residues at positions 13, 19, 58, 78 and 104 in the light chain and 48, 49, 60, 63, 67, 69, 78, 82 and 82c in the heavy chain.

39. A method according to Claim 37, wherein the antibody thus produced has donor residues at positions 33 and 53 in the light chain and 24, 37, 50, 52, 58, 60, 61 and 95 in the heavy chain.

40. A method according to any one of Claims 32 to 39, including substitution, deletion or insertion of a donor antibody residue at or adjacent to one or more sites selected from sites 30, 30b, 30d, 33, 55, 57, 58, 78, 93, 94 or 104 in the light chain and sites 24, 37, 48, 49, 54, 57, 60, 61, 63, 65, 67, 69, 78, 82, 82c, 97, 100a or 100c in the heavy chain.

41. A method according to Claim 40, wherein V<sub>H</sub> 78 is substituted with phenylalanine.

42. A method according to Claim 40, wherein V<sub>H</sub> 78 is substituted with leucine, valine, isoleucine, methionine or alanine.

43. A method according to Claim 40, wherein V<sub>H</sub> 60 is substituted with asparagine.

44. A method according to Claim 40, wherein V<sub>H</sub> 60 is substituted with glutamine, histidine, lysine or arginine.

45. A method according to Claim 40, wherein V<sub>H</sub> 61 is substituted with proline.

46. A method according to Claim 40, wherein V<sub>H</sub> 61 is substituted with glycine, alanine, valine, leucine or isoleucine.

47. A method according to Claim 37, wherein residues are imported from the donor MAE11, including four inserts in V<sub>L1</sub> 30a-30d as well as 91-94, V<sub>H1</sub> 27-29, 29a, 31, 33 and 34, V<sub>H2</sub> 53-55 and V<sub>H3</sub> 97-101.

48. A method according to Claim 40, wherein in the antibody thus produced, any of V<sub>H</sub> positions 97, 100a and 100c has a basic residue other than histidine.

49. A method according to claim 48 wherein the basic residue is lysine or arginine.

50. A method according to Claim 40, wherein in the antibody thus produced, any of V<sub>H</sub> positions 97, 100a and 100c has a residue selected from the group consisting of alanine, glycine, valine, isoleucine, serine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan and proline.

51. A method according to Claim 40, wherein in the antibody thus produced, any of V<sub>L</sub> positions 30, 30b and 30d are glutamic acid.

52. A method according to Claim 40, wherein any of  $V_L$  positions 30, 30b and 30d has a residue selected from the group consisting of alanine, glycine, valine, isoleucine, serine, threonine, asparagine, glutamine, methionine, phenylalanine, tyrosine, tryptophan and proline.

53. A method according to Claim 40, wherein any of  $V_L$  positions 30, 30b and 30d are histidine and any of  $V_H$  positions 97, 100a and 100c are aspartic acid.

54. A method according to Claim 40, wherein residues are inserted adjacent to any of  $V_H$  residues at positions 97, 100a, 100c, 60 or 61 or  $V_L$  residues at positions 30, 30b, 30d or 78.

55. A method according to Claim 53, wherein the inserted residues are of like kind, that is acid residues inserted adjacent to  $V_L$  30, 30b or 30d or basic residues adjacent to  $V_H$  97, 100a or 100c.

56. A method according to Claim 40, wherein residues at any of  $V_L$  30, 30b or 30d or 78 or of  $V_H$  97, 100a, 100c, 60 or 61 are deleted.

57. A method according to any one of Claims 32 to 55, wherein the antibody thus produced is a bispecific antibody.

58. A method according to any one of Claims 32 to 57, wherein the antibody thus produced is monovalent for FcεRII-bound IgE, and is capable of an immunoglobulin effector function and comprises an Fc domain containing at least two heavy chains.

59. A method according to any one of Claims 32 to 58 wherein the antibody thus produced comprises a human consensus heavy chain and light chain sequence.

60. A method according to any one of Claims 32 to 59, wherein the antibody thus produced has an IgE affinity which is substantially the same as or greater than that of MAE11 for IgE.

61. A humanized antibody comprising:

(a) a VH domain and VL domain, wherein:

(i) the V H domain comprises the V H domain of SEQ ID NO:8 in which the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the aspartic acid residue at position 62 of SEQ ID NO:8 is replaced by a proline residue, the valine residue at position 64 of SEQ ID NO:8 is replaced by a leucine residue, and the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an isoleucine residue, in which said positions correspond to Kabat numbering 60, 61, 63 and 67, respectively, and

(ii) the VL domain comprises the V L domain of SEQ ID NO:9; or

(b) a VH domain and a VL domain, wherein:

(i) the VH domain comprises the V H domain of SEQ ID NO:8 in which the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the aspartic acid residue at position 62 of SEQ ID NO:8 is replaced by a proline residue, and the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an isoleucine residue, in which said positions

correspond to Kabat numbering 60, 61 and 67, respectively, and

- (ii) the V L domain comprises the V L domain of SEQ ID NO:9; or
- (c) a VH domain and a V L domain, wherein:
  - (i) the VH domain comprises the VH domain of SEQ ID NO:8 in which the valine residue at position 49 of SEQ ID NO:8 is replaced by a methionine residue, the alanine residue at position 50 of SEQ ID NO:8 is replaced by a isoleucine residue, the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the valine residue at position 64 of SEQ ID NO:8 is replaced by a leucine residue, the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an isoleucine residue, the isoleucine residue at position 70 of SEQ ID NO:8 is replaced by a valine residue, the methionine residue at position 83 of SEQ ID NO:8 is replaced by a leucine residue, and the leucine residue at position 86 of SEQ ID NO:8 is replaced by an alanine residue, in which said positions correspond to Kabat numbering 48, 49, 60, 63, 67, 69, 82 and 82c, respectively, and
  - (ii) the VL domain comprises the VL domain of SEQ ID NO:9 in which the alanine residue at position 13 of SEQ ID NO:9 is replaced by a valine residue, the valine

residue at position 19 of SEQ ID NO:9 is replaced by an alanine residue, the valine residue at position 62 of SEQ ID NO:9 is replaced by an isoleucine residue, the leucine residue at position 82 of SEQ ID NO:9 is replaced by a valine residue, and the valine residue at position 108 of SEQ ID NO:9 is replaced by a leucine residue, in which said positions correspond to Kabat numbering 13, 19, 58, 78 and 104, respectively.

62. The humanized antibody of Claim 61, which is an IgG1 antibody.

63. The humanized antibody of Claim 61, which is an IgG2 antibody.

64. The humanized antibody of Claim 61, which is an IgG3 antibody.

65. The humanized antibody of Claim 61, which is an IgG4 antibody.

66. A humanized antibody comprising a VH domain and a VL domain, wherein:

- (a) the V H domain comprises the V H domain of SEQ ID NO:8 in which the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the aspartic acid residue at position 62 of SEQ ID NO:8 is replaced by a

proline residue, the valine residue at position 64 of SEQ ID NO:8 is replaced by a leucine residue, and the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an isoleucine residue, in which said positions correspond to Kabat numbering 60, 61, 63 and 67, respectively and

(b) the VL domain comprises the VL domain of SEQ ID NO:9.

67. The humanized antibody of Claim 66, which is an IgG1 antibody.

68. The humanized antibody of Claim 66, which is an IgG2 antibody.

69. The humanized antibody of Claim 66, which is an IgG3 antibody.

70. The humanized antibody of Claim 66, which is an IgG4 antibody.

71. A humanized antibody comprising a VH domain and a VL domain, wherein:

(a) the VH domain comprises the VH domain of SEQ ID NO:8 in which the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the aspartic acid residue at position 62 of SEQ ID NO:8 is replaced by a proline residue, and the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an

isoleucine residue, in which said positions correspond to Kabat numbering 60, 61 and 67, respectively, and

(b) the VL domain comprising the VL domain of SEQ ID NO:9.

72. The humanized antibody of Claim 71, which is an IgG1 antibody.

73. The humanized antibody of Claim 71, which is an IgG2 antibody.

74. The humanized antibody of Claim 71, which is an IgG3 antibody.

75. The humanized antibody of Claim 71, which is an IgG4 antibody.

76. A humanized antibody comprising a VH domain and a VL domain, wherein:

(a) the VH domain comprises the VH domain of SEQ ID NO:8 in which the valine residue at position 49 of SEQ ID NO:8 is replaced by a methionine residue, the alanine residue at position 50 of SEQ ID NO:8 is replaced by a glycine residue, the alanine residue at position 61 of SEQ ID NO:8 is replaced by an asparagine residue, the valine residue at position 64 of SEQ ID NO:8 is replaced by a leucine residue, the phenylalanine residue at position 68 of SEQ ID NO:8 is replaced by an isoleucine residue, the

isoleucine residue at position 70 of SEQ ID NO:8 is replaced by a valine residue, the methionine residue at position 83 of SEQ ID NO:8 is replaced by a leucine residue, and the leucine residue at position 86 of SEQ ID NO:8 is replaced by an alanine residue, in which said positions correspond to Kabat numbering 48, 49, 60, 63, 67, 69, 82 and 82c, respectively, and

- (b) the VL domain comprises the VL domain of SEQ ID NO:9 in which the alanine residue at position 13 of SEQ ID NO:9 is replaced by a valine residue, the valine residue at position 19 of SEQ ID NO:9 is replaced by an alanine residue, the valine residue at position 62 of SEQ ID NO:9 is replaced by an isoleucine residue, the leucine residue at position 82 of SEQ ID NO:9 is replaced by a valine residue, and the valine residue at position 108 of SEQ ID NO:9 is replaced by a leucine residue, in which said positions correspond to Kabat numbering 13, 19, 58, 78 and 104, respectively.

77. The humanized antibody of Claim 76, which is an IgG1 antibody.

78. The humanized antibody of Claim 76, which is an IgG2 antibody.

79. The humanized antibody of Claim 76, which is an IgG3 antibody.

80. The humanized antibody of Claim 76, which is an IgG4 antibody.

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|     |                                                             |                   |         |
|-----|-------------------------------------------------------------|-------------------|---------|
|     | loop BC                                                     | <u>β-strand C</u> | loop CD |
| 390 | C L V V D L A P S K G T V N L T W S R X A S X X G K P V N H |                   |         |
|     | 2                                                           | 9                 | 3       |

|     |                   |                                           |    |                   |         |
|-----|-------------------|-------------------------------------------|----|-------------------|---------|
|     | <u>β-strand D</u> | loop                                      | DE | <u>β-strand E</u> | loop EF |
| 420 | S T R K E E K Q R | X N X X G T L T V T S T L P V G T R D W I |    |                   |         |
|     |                   |                                           |    | 10                | 4       |
|     |                   |                                           |    | 6                 |         |

β-strand F      loop      FG      β-strand G  
 450    E G E T Y Q C R V T H P H L P R A L X M R S T T K T S G P  
 11      12      5      12  
 FIG. 1

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## MaE11 Light Chain

DIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPILLIYAASYLG  
SEIPARFSGSGSGTDFTLNIHPVEEEDAATFYCQQSHEDPYTFGAGTKLEIK

## MaE11 Heavy Chain

DVQLQESGPGGLVKPSQSLSLACSVTGYSITSGYSWNWIRQFPGNKLEWMGSITYDGSS  
NYNPSLKNRISVTRDTSQNQFFLKLNSATAEDTATYYCARGSHYFGHWHFAVWGAGTTVT  
VSSAKTTPPSVYPLAR

## Mae13 Light Chain

DIVMTQSQKFMSTSVGDRVSVTCKASQNVSSNVAWYQQKPGQSPKALIYSASYRYSKV  
PDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYYTYPLYTFGGGTKLEIKRADAAPTVSI  
FPPSTR

## Mae13 Heavy Chain

DVQLQESGPGGLVKPSQSLSLTCTVTGYTITSDNAWNWIRQFPGNKLEWMGYINHSGTT  
SYNPSLKSRIISITRDTSKNQFFLQLNSVTTEDTATYYCAWVVAYAMDYWGQGTSVTVSSA  
KTPPSVYPLAR

## Mae15 Light Chain

DIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYAASNLES  
GIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPFTFGAGT

## Mae15 Heavy Chain

DVQHQESEPDLVKPSQSLSLTCTVTGYTITSGYNRHWIRQFPGNKLEWMGYIHYSGST  
NYNPSLKRRISITRDTSKNQFFLQLNSVTTEDTATYYCARGSIYYYGSRYRYFDVWGAGT  
TVTVSSAKRHPHLSIHWPG

FIG.2

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Humanized MaE11 Version 1 (intact IgG)

Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAVSGYSITSGYSWNWIRQAPGKGLEWVASITYDGSTNY  
ADSVKGRFTISRDDSKNTFYLMNSLRAEDTAVYYCARGSHYFGHWHFAVWGQGTLVTVS  
SASTKGKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL  
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK  
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light Chain

DIQLTQSPSSLSASVGDRVTITCRASQSVDYDGDSYMNWYQQKPGKAPKLLIYAASYLES  
GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQQSHEDPYTFGQGTKVEIKRTVAAPSVF  
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS  
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 3

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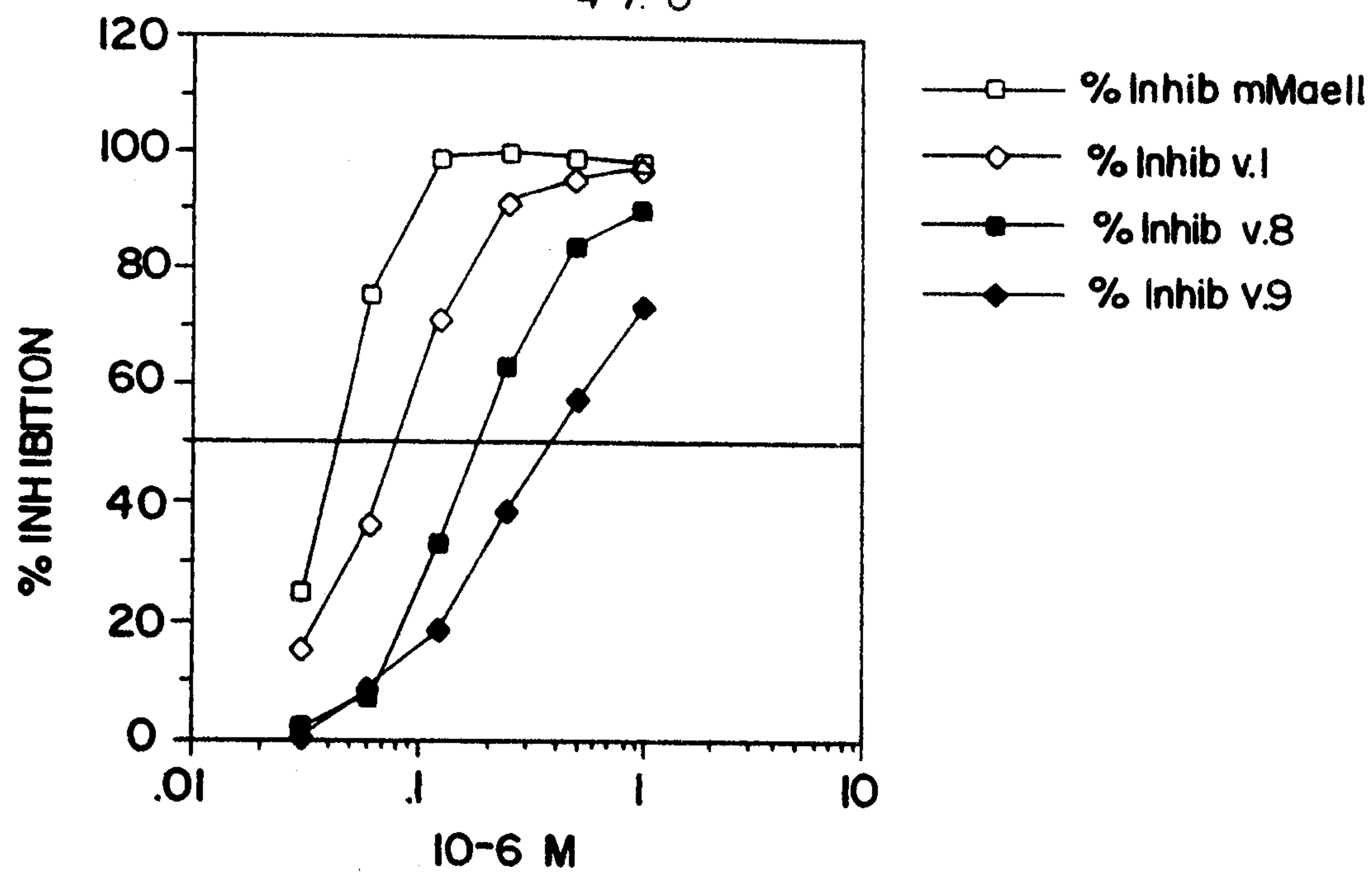


FIG. 4a

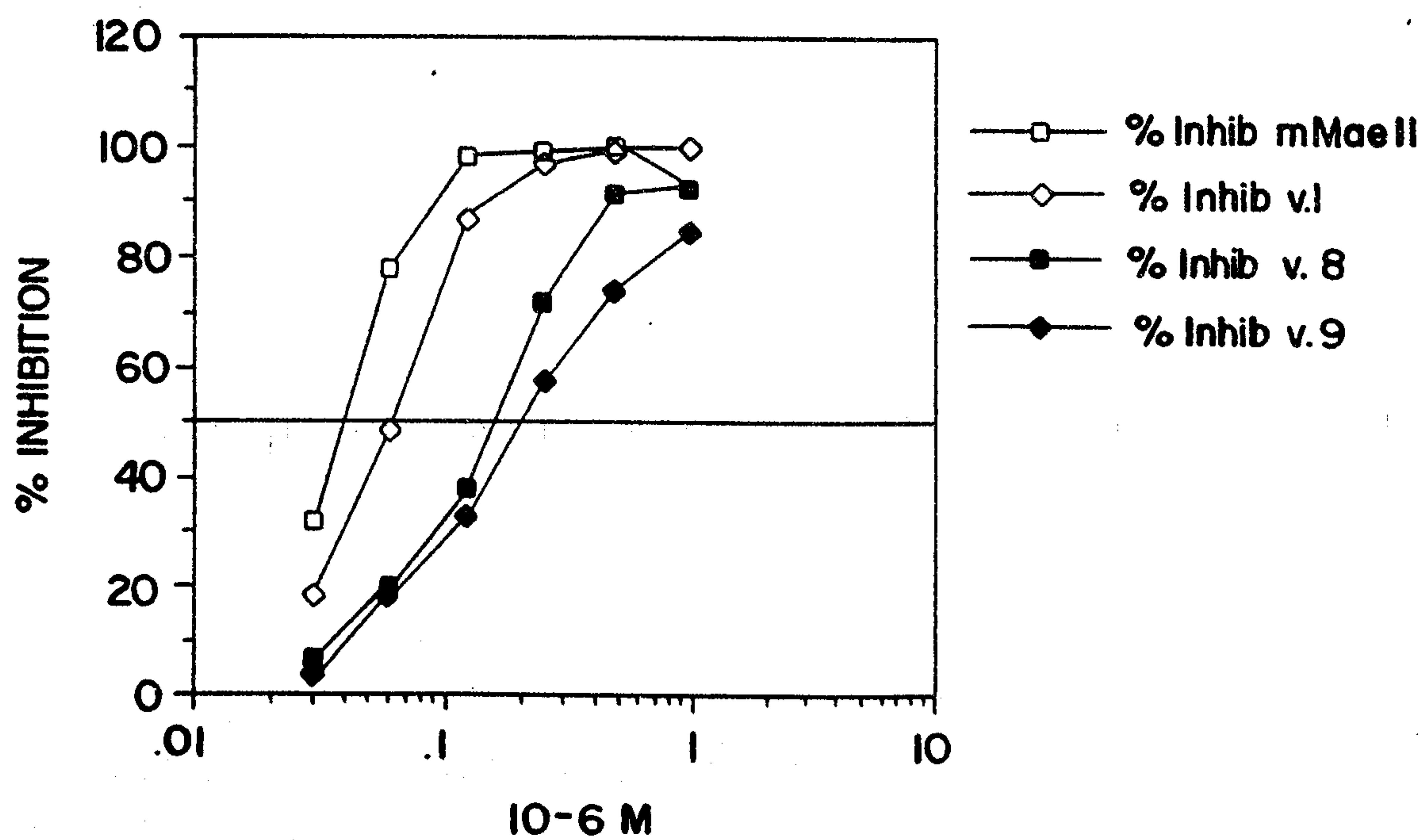


FIG. 4b

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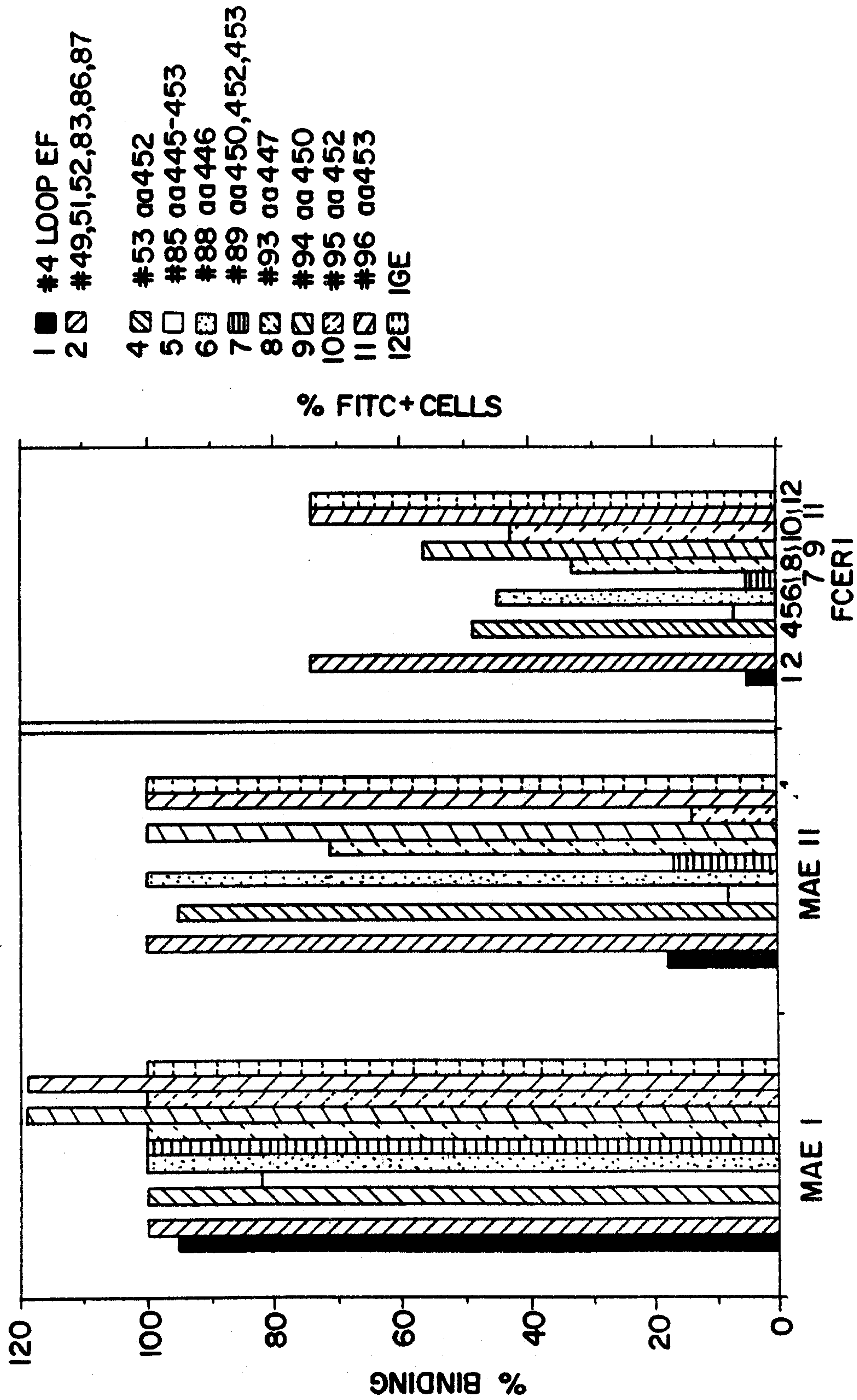


FIG.5a

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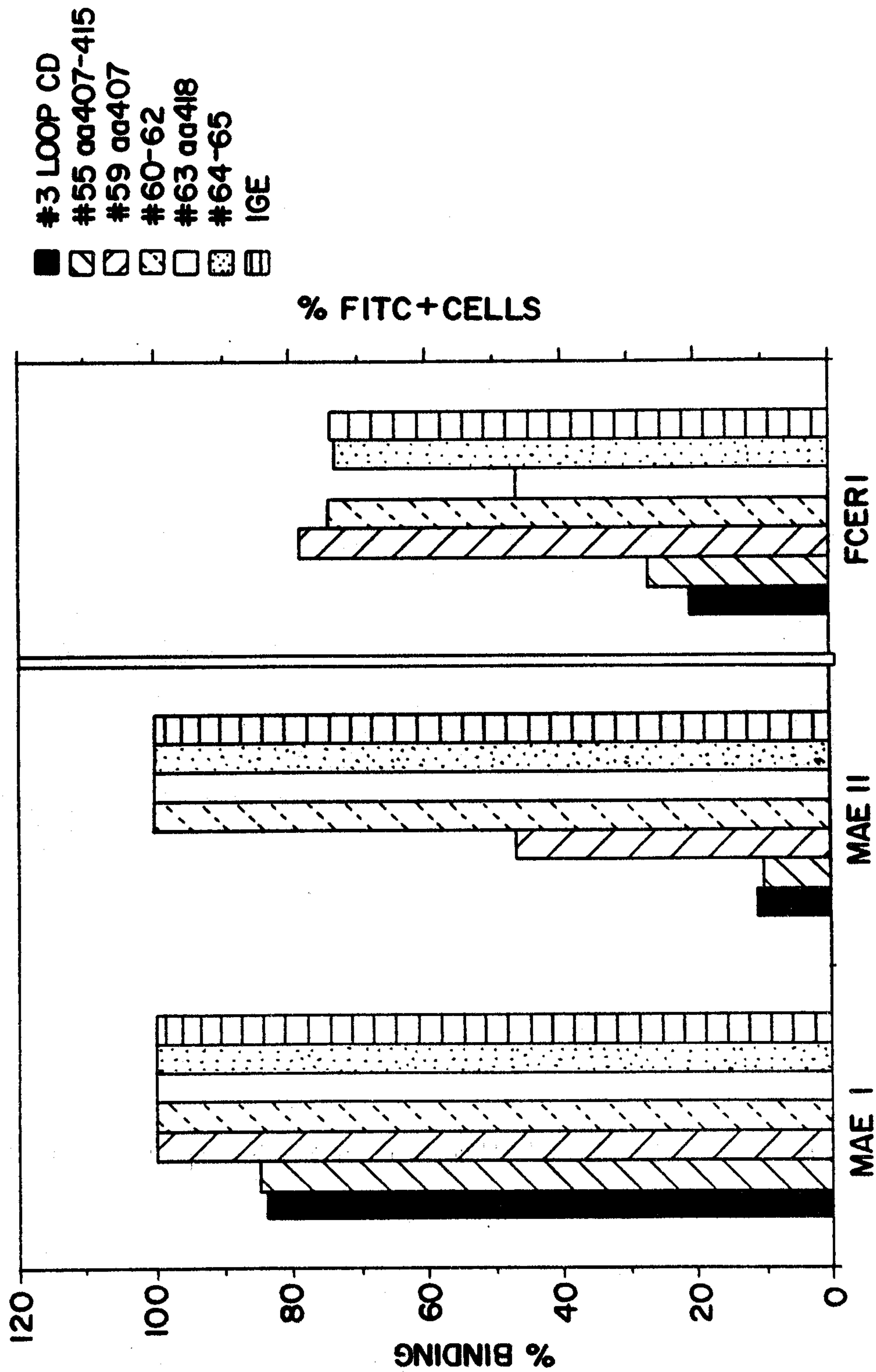


FIG.5b

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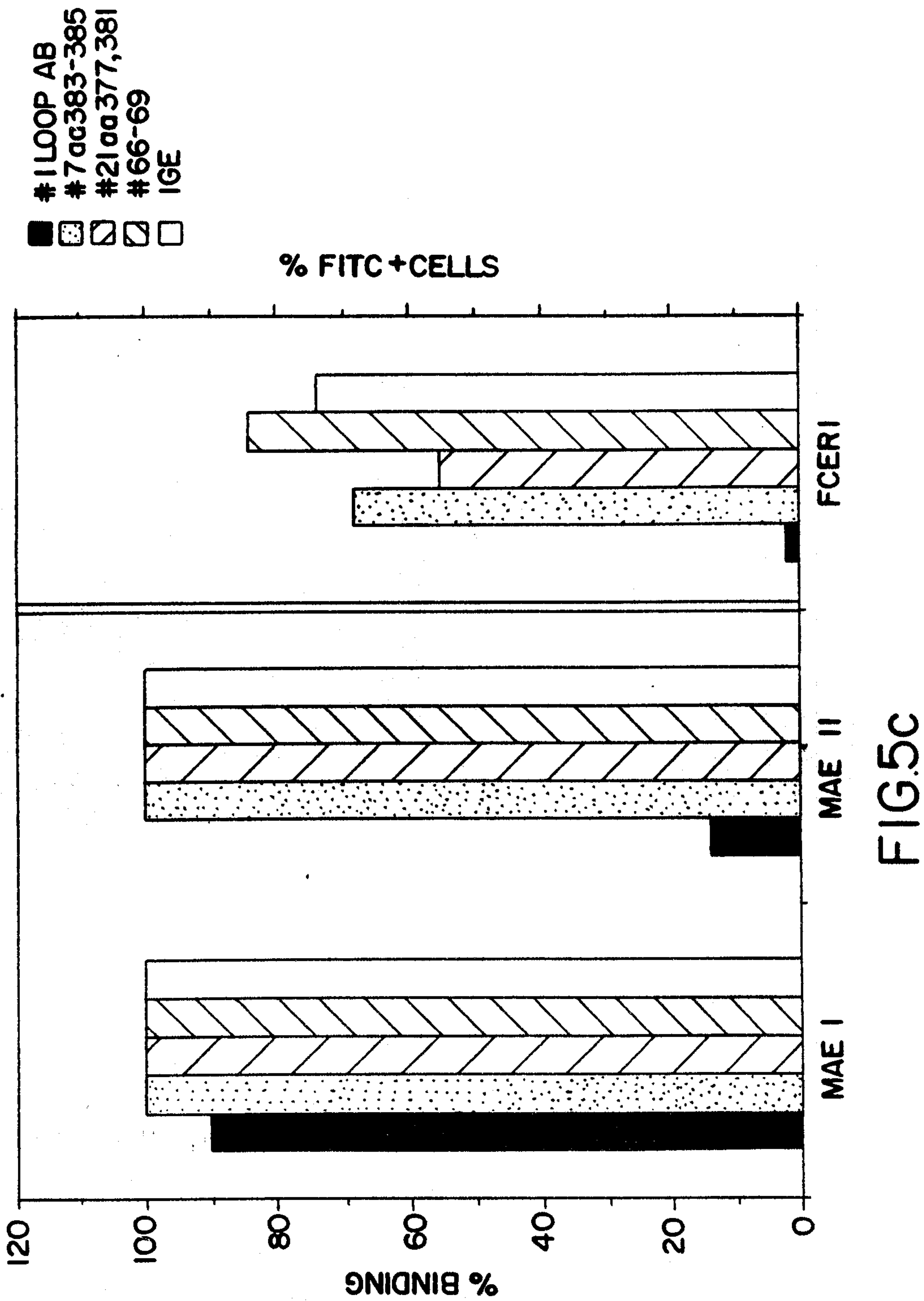


FIG.5C

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#1 LOOP AB  
#2 LOOP BC  
#3 LOOP CD  
#4 LOOP EF  
#5 LOOP FG  
#6 B STRAND D  
IGE

■ ▨ ▩ □ ▤ ▥ ▦

% FITC+ CELLS

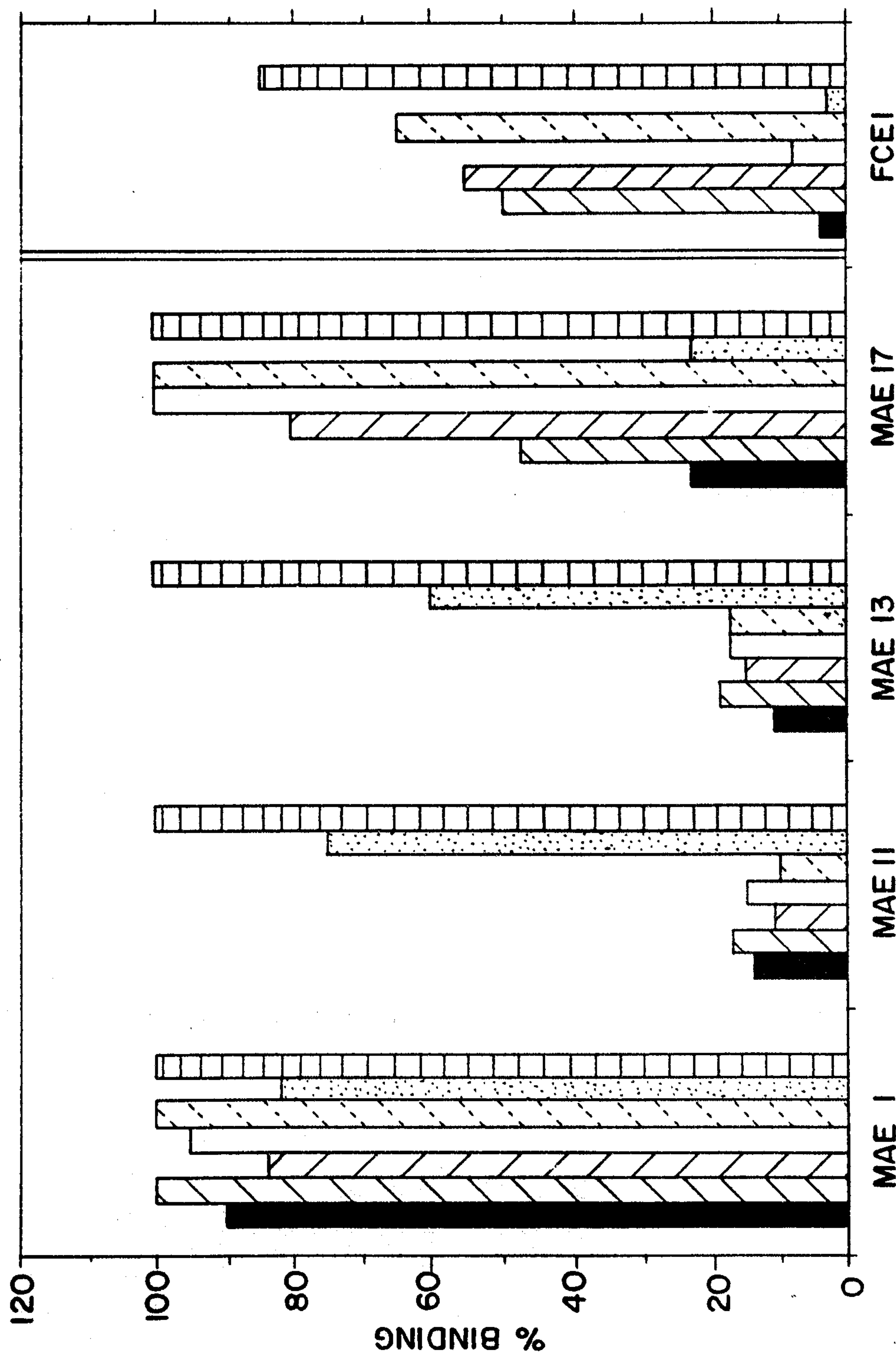


FIG. 5d