Anti-CεmX antibodies capable of binding to human mIgE on B lymphocytes

BACKGROUND OF THE INVENTION

IgE plays a central role in mediating type I hypersensitivity reactions that are responsible for causing allergic diseases, including allergic asthma, allergic rhinitis, atopic dermatitis, and others. Allergic reactions are the responses of the immune system toward harmless environmental substances, such as dust mites, tree and grass pollens, certain food and drugs, and bee and fire ant bites. In such reactions, the binding of an allergen to IgE on the surface of basophils and mast cells causes the cross-linking of IgE and the aggregation of the underlying receptors of IgE.Fc, the type I IgE.Fc receptors, or FcεRI. This receptor aggregation subsequently activates the signaling pathway leading to the exocytosis of granules and the release of pharmacologic mediators, such as histamine, leukotrienes, tryptase, cytokines and chemokines. The release of those mediators from mast cells and basophils causes the various pathological manifestations of allergy.

Anti-IgE antibodies that bind to free IgE in the blood and in interstitial fluid and to mIgE on B cells, but not to IgE bound by FcεRI on basophils and mast cells, have been developed for treating IgE-mediated allergic diseases. The treatment with a humanized anti-IgE antibody, omalizumab (trade name Xolair), has shown multiple pharmacologic effects in attenuating type I hypersensitivity in various allergic indications. The antibody binds to IgE with high affinity at a site in the CH3 domain of Fc that overlaps with the binding site of FcεRI. Hence, the therapy is based on the binding of the antibody to free IgE and to mIgE on B lymphoblasts and on memory B cells, which leads to the reduction of overall free IgE level in blood and interstitial fluid.

The binding of anti-IgE to free IgE further prevents IgE binding to FcεRI on the surface of basophils and mast cells. As the FcεRI unoccupied by IgE is unstable and subsequently internalized and degraded, the depletion of free IgE with anti-IgE binding also gradually down-regulates FcεRI on basophils and mast cells. Evidence for other effects of the antibody therapy has been found, including the neutralization of cytokinergic activities, the attenuation of overall inflammatory activity, and possibly the sweeping of allergens through the accumulation of IgE-anti-IgE immune complexes.

One of the inventors (T. W. Chang) of this invention discovered that in addition to the antigenic site on CH3 of IgE that omalizumab binds to, another antigenic site, referred to as CεmX, exists on human mIgE for the targeting of mIgE-expressing B lymphocytes. CεmX is a 52-amino acid segment located between the CH4 domain and the C-terminal membrane-anchoring segment of human membrane-bound ε chain (mε). It has been shown that in most human subjects studied, the mε without CεmX ($m\varepsilon_S$) accounts for minute proportions, whereas mε chain with CεmX ($m\varepsilon_L$) is dominantly expressed. The mRNAs for ε chain of free, secreted IgE and for $m\varepsilon_S$ and $m\varepsilon_L$ of mIgE are all derived from alternative splicing of the ε RNA transcript. The amino acid and nucleotide sequences of CεmX are unique in the entire protein and DNA databases. Therefore, CεmX provides a unique antigenic site for targeting mIgE and the mIgE-expressing B cells.

The research group of Chang previously reported the development of several CεmX-specific mouse monoclonal antibodies, including a20, which can bind to recombinant proteins containing CεmX segment and to cells of SKO-007 cell line, which was a human myeloma-derived cell line expressing human mIgE, and to cells of a CHO cell line, which was transfected with the gene corresponding to the segment from CH2 domain through the

cytoplasmic end of mε$_L$ (mε$_{L(CH2\text{-}CM)}$; CM: cytoplasm). The monoclonal antibody a20 and all antibodies developed earlier were found to bind to an 8-a. a. peptidic region, RADWPGPP, residues #45-52, at the C-terminal end of the 52 a. a. CεmX domain.

SUMMARY OF THE INVENTION

This invention pertains to the development and identification of antibodies that are specific for CεmX domain of human mIgE and that can bind to mIgE on human B lymphocytes. It also pertains to the utility of these antibodies in treating allergic and other diseases that are mediated by IgE.

In studying the anti-CεmX monoclonal antibody a20, which was developed by the research group of Chang, it was found that a20 has good binding to mε$_{L(CH2\text{-}CM)}$ gene- transfected cell lines, such as CHO cell line or NS0 cell line, that do not express Igα (CD79a), Igβ (CD79b), CD21, CD19, CD81, and other proteins associated with B cell receptor (BCR). However, a 20 was found to bind poorly to mε$_{L(CH2\text{-}CM)}$ gene- transfected cell lines that express Igα, Igβ, and other BCR-associated proteins, such as Ramos cell line. We hypothesized that the antigenic epitope on CεmX recognized by a20 may be blocked by certain BCR-associated protein(s). Therefore, a20 monoclonal antibody and its chimeric or humanized versions would not be suitable for use in human patients *in vivo* for the purpose of targeting mIgE expressing B lymphoblasts and memory cells.

If the peptidic epitope, RADWPGPP, is the only epitope for inducing antibody response, monoclonal antibodies generated from hybridoma methodology using mice that are immunized with human CεmX-containing proteins would all be specific for this peptide region. However, if this epitope is a dominant epitope, but not the only immunogenic epitope, monoclonal antibodies specific for other antigenic epitopes on CεmX could still be developed. It is possible that there exists an epitope(s) on CεmX that is not blocked by BCR-associated proteins for antibody binding. If so, an antibody that binds to IgE on B cells and that can be used for targeting those B cells may still be developed.

In the following examples, we have successfully shown that although RADWPGPP is a dominant epitope, it is not the only immunogenic and antigenic epitope on CεmX. Furthermore, we have discovered monoclonal antibodies, 4B12 and 26H2, that bind to CεmX on antigenic epitopes not located in the region of RADWPGPP. Those monoclonal antibodies do not compete with a20 antibody in binding to CεmX. They bind to mIgE on B cells much more strongly than a 20 and are much more effective than a20 in causing antibody-dependent cytolysis and apoptosis of mIgE-expressing cells.

The examples indicate that monoclonal antibodies, such as 4B12 and 26H2, can bind to mIgE on human B lymphocytes and are suitable for use to target mIgE-expressing B lymphoblasts and memory B cells for the down-regulation of IgE synthesis. The antibodies in chimeric or humanized forms will be useful for use in patients affected with IgE-mediated allergic diseases, such as allergic asthma, allergic rhinitis, and atopic dermatitis. Since neutralization of IgE by anti-IgE has been shown to effectively treat cold-induced urticaria, chronic urticaria, cholinergic urticaria, chronic rhinosinusitis, systemic mastocytosis, cutaneous mastocytosis, allergic bronchopulmonary aspergillosis, recurrent idiopathic angioedema, and interstitial cystitis, or eosinophil-associated gastrointestinal disorders, antibodies, such as 4B12 and 26H2, may also be applied to treat those various diseases.

The examples further suggest the potential utility of the peptides recognized by 4B12 and 26H2 in inducing immune response against CεmX and hence mIgE-expressing B cells. The peptides and their analogues with similar antigenic properties, i.e., with binding activity to anti-CεmX antibodies, such as 4B12 and 26H2, may be used individually or in combination in molecular constructs that also contain moieties that can induce T-cell help. Such constructs can induce active immunization against mIgE-expressing B cells and thus achieving the effects of down-regulating total IgE synthesis.

Example 1: New anti-CεmX monoclonal antibodies binding to antigenic sites other than RADWPGPP

To induce anti-CεmX immune response, BALB/c mice were immunized twice subcutaneously with 50 μg of n-undecyl-β-d-maltopyranoside (UDM; Anatrace)-solublized mIgE.Fc_L recombinant proteins that were emulsified in TiterMax Gold adjuvant (Sigma-Aldrich) according manufacturer's suggestions at 2 week intervals. We avoided hyper-immunization protocol, so that the mice would not produce antibodies only toward the dominant RADWPGPP epitope. A final boost was given intraperitoneally with 0.1 mg of UDM-solublized mIgE.Fc_L recombinant proteins without adjuvant. One day before fusion, NS0 cells were reseeded in fresh DMEM medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), and 1% penicillin-streptomycin mixture (100× Pen-Strep solution; Invitrogen) at a cell density of 5×10^5 cells/ml. Three days after the final boost, the spleen cells from two immunized mice were harvested and washed with serum-free DMEM medium twice. 5×10^7 NS0 cells were harvested and washed with serum-free DMEM medium twice. After washing, spleen cells and NS0 cells were fused by adding 1 ml of pre-warmed 50% polyethyleneglycerol 1500 (PEG 1500, Roche Applied Science) while continually stirring cells gently with the pipette tip over 1 min, stirring cells for further 1 min, adding 2 ml pre-warmed serum-free DMEM over 2 min, and finally adding 8 ml serum-free DMEM over 2 min. After centrifugation at 200×g for 10 min, fused cells were resuspended with 600 ml of HAT medium [DMEM medium supplemented with 2% hypoxanthine-aminopterin-thymidine mixture (50× HAT solution; Invitrogen), 10 % BM-Condimed H1 (Roche Applied Science), 10% heat-inactivated FBS, and 1% penicillin-streptomycin mixture] and distributed into 30 96-well culture plates at 200 μl/well. On days 3, 100μl of HAT medium was added to each well. On days 7 and 10, medium was freshened by aspiring half the volume of each well and replacing with HAT medium. On days 14, hybridoma supernatants were used to screen anti-CεmX mAbs for binding to UDM-solublized mIgE.Fc_L or mIgE.Fc_S proteins by enzyme-linked immunosorbent assay (ELISA).

To screen hybridomas secreting anti-CεmX mAbs by ELISA, purified UDM-solublized mIgE.Fc_L or mIgE.Fc_S proteins were coated on 96-well MaxiSorp plates (Nunc) at 50 ng/well in 0.1M $NaCO_3$ (pH 9.6) at 4°C overnight. Coated wells were blocked by 200 μl/well of 1% BSA in PBS at room temperature for 1 hour. Plates were washed three times with 200μl/well of PBS with 0.05% Tween-20, followed by adding 100μl of hybridoma supernatants to wells. The incubation was carried out at room temperature for 2 hours. All wells were aspirated and washed six times with 200 μl/well of PBS with 0.05% Tween-20. The plates were incubated with a 1:10,000 dilution of HRP-conjugated goat anti-mouse IgG antibody (Chemicon) for 1 hour (100μl/well). Then all wells were aspirated and washed six times with 200μl/well of PBS with 0.05% Tween-20. Finally, wells were developed by 50 μl/well of tetramethyl benzidine (TMB) substrate solution (SureBlue™, KPL) and the reaction was stopped by addition of 50 μl/well of 1N HCl. The absorbance was measured at OD_{450} on an ELISA reader. Of >4000 hybridoma clones screened from two fusions, 17 clones showed specificity for UDM-solublized mIgE.Fc_L rather than mIgE.Fc_S as determined by ELISA.

To explore the specificity of anti-CεmX mAbs to CεmX, the various CεmX-specific clones were then tested for reactivity with 3 synthetic peptides, representing 3 consecutive segments of CεmX, divided by a C residue located at residue #18 and a CHC segment at residues #39-41. Specifically, P1 peptide contains the last 4 amino acid residues of CH4 of mε and first 17 amino acid residues (#1-17), namely, GLAGGSAQSQRAPDRVL, of CεmX; P2 peptide contains 20 amino acid residues #19-38, namely, HSGQQQGLPRAAGGSVPHPR, of CεmX; P3 peptide contains the terminal 11 amino acid residues (#42-52), namely, GAGRADWPGPP, of CεmX and first 4 amino acid residues of the consecutive *migis* region, namely, the N-terminal extracellular region of the membrane anchor peptide of mε chain. All peptides were synthesized at Genomics Research Center, Academia Sinica (Taipei, Taiwan). The peptides were reconstituted with PBS at a concentration of 10 mg/ml. All peptides were coated on 96-well MaxiSorp plates at 500 ng/well in 0.1M $NaCO_3$ (pH 9.6) at 4°C overnight. Coated wells were blocked by 200 μl/well of 1% BSA in PBS at room temperature for 1 hour. Plates were washed three times with 200μl/well of PBS with 0.05% Tween-20, followed by adding 100μl of 1 μg/ml anti-CεmX mAbs to wells. The incubation was carried out at room temperature for 2 hours. All wells were aspirated and washed six times with 200 μl/well of PBS with 0.05% Tween-20. The plates were incubated with a 1:10,000 dilution of HRP-conjugated goat anti-mouse IgG antibody for 1 hour. After six times with 200 μl/well of PBS with 0.05% Tween-20, 50 μl/well of TMB substrate solution was added to the wells. The reaction was stopped by addition of 50 μl/well of 1N HCl. The absorbance was measured at OD_{450} on an ELISA reader. Of the many CεmX-specific monoclonal antibodies prepared in our experiments, only 4B12 and 26H2 do not react with RADWPGPP-containing P3 peptide. 4B12 reacted with P1 peptide, and 26H2 with P2 peptide. All of the other CεmX-specific monoclonal antibodies reacted with P3 (Figure 1). Thus, RADWPGPP is indeed a dominant immunogenic epitope. However, it is not the only immunogenic epitope.

Example 2: 4B12 and 26H2 bind to mIgE on mIgE-expressing B cells

We further tested the ability of various CεmX-specific monoclonal antibodies to bind to CHO and Ramos cell lines that were transfected with either recombinant DNA encoding $mε_{L(CH2\text{-}CM)}$ or $mε_{S(CH2\text{-}CM)}$. The two transfected CHO cell lines respectively produced mIgE.Fc_L or mIgE.Fc_S, both of which did not form complete B cell receptor with coreceptors such as Igα and Igβ, because the CHO cells did not express those proteins. The transfected two Ramos cell lines respectively produced mIgE.Fc_L or mIgE.Fc_S, both of which form complexes with their native coreceptors. To investigate the binding of anti-CεmX mAbs to native CεmX, CHO or Ramos cells expressing mIgE.Fc_L or mIgE.Fc_S were resuspended in FACS buffer [PBS, 1% FBS, 0.1% sodium azide, and 2 mM EDTA (pH 8.0)] at a cell density of 10^7 cells/ml. 10^6 cells were then incubated for 30 min on ice with 100 μl of hybridoma supernatants, followed by washing with FACS buffer. Bound antibodies were detected by incubation for 30 minutes on ice with FITC-labeled rabbit F(ab')$_2$ fragment specific for mouse IgG (AbD Secrotec), followed by washing twice with FACS buffer prior to analysis. Flow cytometry experiments were performed using a FACSCanto II flow cytometer (BD Bioscience) and analyzed using FCSExpress software (De Novo Software). All CεmX-specific monoclonal antibodies were found not to bind to CHO and Ramos cells expressing mIgE.Fc_S. All CεmX-specific monoclonal antibodies were found to bind to CHO cells expressing $mIgE_L$. However, only 4B12 and 26H2 could bind to Ramos cells expressing mIgE.Fc_L, while all other CεmX-specific monoclonal antibodies could not bind to Ramos cells expressing mIgE.Fc_L (Figure 2).

Example 3: 4B12 and 26H2 induce antibody-dependent cellular cytotoxicity against mIgE-expressing B cells

To investigate the ADCC activity of chimeric anti-CεmX mAbs, we used peripheral blood mononuclear cells (PBMCs) as effector cells to target mIgE.Fc_L-expressing Ramos cells. PBMCs were purified from buffy coats of healthy donors (Taiwan Blood Service Foundation) by centrifugation over a Ficoll-Paque Plus (GE Healthcare) density gradient and cryopreserved in 90% FBS/10% DMSO (Hybri-Max™; Sigma-Aldrich). Prior to use, PBMCs were thawed and cultured at 2×10^6 cells/ml overnight in IMDM medium (Invitrogen) supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin mixture. To identify target cells in coculture with PBMCs, mIgE.Fc_L-expressing Ramos cells were labeled with 2.5 μM 5-(and -6)-carboxyfluorescein diacetate, succinimidyl ester (CFDA, SE; Invitrogen) in 0.1% BSA/PBS for 10 min at 37°C. After three washes with cold RPMI medium (Invitrogen) containing 10% FBS, cells were adjusted to 10^5 cells/ml. For effector-target (E/T) ratio titrations, 20,000 labeled cells in 200μl of complete RPMI medium were coated with antibodies at 1 μg/ml for 30 min at 37°C, and then combined with an equal volume of PBMCs at multiple E/T ratios from 50 to 3.125. For antibody titrations, 20,000 labeled cells in 200μl of complete RPMI medium were opsonized with antibody at various concentrations (1000~0.01 ng/ml) for 30 minutes at 37°C, and then combined with PBMCs at an E/T ratio of 25:1. To measure antibody-independent killing, labeled target cells were also incubated with PBMCs in the absence of antibodies at given E/T ratios. At the end of 24-hour incubation, dead cells were stained with 2.5 μg/ml 7-amino actinomycin (7-AAD; Invitrogen) for 15 min on ice. Cells were analyzed on a Becton Dickinson FACSCanto II flow cytometer. Living target cells were defined as the percentage of CFSE-positive/7-AAD-negative cells on dot-plot analyses. The percentage of cells killed at a given E/T ratio was calculated according the following formula: 100 × [(% of living target cells in the antibody-independent control - % of living target cells in the sample) / % of living target cells in the antibody-independent control]. The ADCC activity of c4B12, c26H2 and omalizumab was observed at multiple E/T ratios. At an E/T ratio of 50, c4B12, c26H2 and omalizumab gave up to 60% specific lysis; in contrast, ca20 was less active and gave only 10~20% specific lysis (Figure 3A). Besides, significant ADCC was observed when the concentration of c4B12 and c26H2 was higher than 0.01μg/ml. At the maximum dose of 10 μg/ml, specific lysis of target cells by c4B12 and c26H2 ranged from 80% to 90%, while ca20 gave up to 50% specific lysis (Figure 3B). The positive control rituximab, which directed to CD20, and omalizumab effectively induced ADCC at multiple E/T ratios and in dose-responsive manner. Thus, we concluded that c4B12 and c26H2 are more potent anti-CεmX mAbs than ca20 in mediating ADCC and could efficiently recruit effector cells to target mIgE-expressing B cells *in vivo*.

Example 4: Chimeric anti-CεmX mAbs induce apoptosis of membrane-bound IgE.Fc_L-expressing Ramos cells

To detect phosphatidylserine (PS) exposure, mIgE.Fc_L-expressing Ramos cells (5×10^5 cell/ml) were incubated with chimeric anti-CεmX mAbs, omalizumab or control antibodies at indicated concentrations in complete culture medium for 1 hour at 37°C. Cells were then treated with goat $F(ab')_2$ fragment specific for the Fc fragment of human IgG (Jackson ImmunoResearch Laboratories Inc.) at a concentration of 10 μg/ml and further incubated for 24 hours at 37°C. The detection of phosphatidylserine (PS) exposure was assessed by staining cells in 200μl of Annexin buffer [10 mM HEPES/NaOH (pH 7.4), 140 mM NaCl, 5 mM $CaCl_2$]

containing fluorescein isothiocyanate (FITC)-labeled Annexin V (BioVision), diluted 1/200, and 2.5 μg/ml propidium iodide (PI, Sigma-Aldrich) for 15 min in dark at room temperature. Cells were analyzed on a FACSCanto II flow cytometer. Apoptotic cells were defined as the percentage of Annexin V-positive/PI-negative cells on dot-plot analyses. Approximately 80% of mIgE.Fc_L-expressing Ramos cells were dead through apoptosis by increasing concentration of c4B12, c26H2, or omalizumab, but not ca20, with maximal induction at 1 μg/ml (Figure 4A).

For detection of apoptotic nuclei, mIgE.Fc_L-expressing Ramos cells (5×10^5 cell/ml) were incubated with chimeric anti-CεmX mAbs, omalizumab or control antibodies at a concentration of 1 μg/ml in complete culture medium for 1 hour at 37°C. Cells were then treated with goat $F(ab')_2$ fragment specific for the Fc fragment of human IgG at a final concentration of 10 μg/ml and further incubated for 48 hours at 37°C. 5×10^5 cells were incubated in 0.5 ml of propidium iodide (PI)/Triton solution (0.1% sodium citrate, 0.1% Triton X-100, 15 μg/ml PI, and 100 μg/ml RNase A in PBS; all from Sigma-Aldrich) for one hour in dark on ice. PI fluorescence was determined on a FACSCanto II flow cytometer. The DNA content of intact of nuclei was recorded on a linear scale. Apoptotic nuclei containing hypodiploid DNA emitting fluorescence in channels below the G_0/G_1 peak were enumerated as a percentage of the total population. A significant increase in cell population with hypodiploid DNA was observed in c4B12, c26H2, or omalizumab-treated mIgE.Fc_L-expressing Ramos cells (Figure 4B).

For detection of caspase 3 and poly(ADP-ribose) polymerase (PARP) cleavage, mIgE.Fc_L-expressing Ramos cells cells (5×10^5 cell/ml) were incubated with chimeric anti-CεmX mAbs, omalizumab or control antibodies at a concentration of 1 μg/ml in complete culture medium for 1 hour at 37°C. Cells were then treated with goat $F(ab')_2$ fragment specific for the Fc fragment of human IgG at a final concentration of 10 μg/ml and further incubated for 24 hours at 37°C. 5×10^6 cells were washed in ice-cold PBS and resuspended in 100μl of ice-cold modified RIPA lysis buffer [20 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton-X 100, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5 mM EDTA, and protease inhibitor (Sigma-Aldrich)]. The lysates were incubated for 20 min on ice. Samples were centrifuged for 20 min at 16000×g and 4°C. The supernatants were transferred to a fresh 1.5 ml tube and stored at -80°C. The amount of protein in each clarified lysate was quantified using the Protein DC assay (Bio-Rad Laboratories) according manufacturer's suggestions. Each sample was normalized for total protein content and was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to PVDF membranes (GE Healthcare). Rabbit polyclonal antibodies to caspase-3 and PARP were obtained from Cell Signaling Techonology and were used at 1:500 dilutions. HRP-conjugated goat anti-rabbit IgG secondary antibody (Sigma-Aldrich) was used at 1:10,000 dilutions. Membranes were developed with an ECL reagent (Immobilon™ Western; Millipore). Equivalent protein loading was verified by probing the blot with an antibody to β-actin (Sigma-Aldrich). 24 hours after mIgE.Fc_L-expressing Ramos cells were treated by c4B12, c26H2 and omalizumab, rather than ca20, cleavage of caspase-3 into M_r 19- and 17-kDa fragments was evident. Besides, the cleavage of PARP was detectable in c4B12-, c26H2-, and omalizumab-treated mIgE.Fc_L-expressing Ramos cells using an antibody recognized the M_r 116 kDa intact PARP and the M_r 89 kDa cleavage product (Figure 4C).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the three synthetic peptides representing the consecutive segments of CεmX and the reactivities of various anti-CεmX mAbs with those peptides. The amino acid residues of the CεmX domain are shown in bold face.

Figure 2 shows the binding of various anti-CεmX mAbs to CHO or Ramos cell lines that express mIgE.Fc_L or mIgE.Fc_S.

Figure 3A shows that chimeric c4B12 and c26H2 induce ADCC against mIgE.Fc_L-expressing Ramos cells at multiple E/T ratios. Figure 3B shows that chimeric c4B12 and c26H2 induce ADCC against mIgE.Fc_L-expressing Ramos cells in a dose-responsive manner.

Figure 4A shows that PS exposure induced by chimeric c4B12 and c26H2 in mIgE.Fc_L-expressing Ramos cells is does-dependent. Figure 4B shows that apoptotic nuclei were observed in chimeric c4B12- and c26H2-treated mIgE.Fc_L-expressing Ramos cells. Figure 4C shows that the cleavage of caspase 3 and PARP were observed in chimeric c4B12- and c26H2-treated mIgE.Fc_L-expressing Ramos cells.

Figure 5 shows the amino acid sequence alignment of the V_L and V_H of parental mouse 4B12, the chosen human germ-line templates KV2 and HV4 for V_L and V_H, respectively, and the humanized 4B12 (hu4B12), labeled as "Replace" in the alignments. This hu4B12 has the same binding affinity to CεmX recombinant proteins and to mIgE.Fc_L-expressing Ramos cells as chimeric 4B12 (c4B12).

REFERENCES CITED

Related Patent Documents

US5,091,313 2/1992 Chang
US5,254,671 10/1993 Chang
US5,260,416 11/1993 Chang
US5,274,075 12/1993 Chang
US5,292,867 3/1994 Chang
US5,342,924 8/1994 Chang
US2009/0010924A1 Wu

Other References

Davis FM, Gossett LA, Chang TW (1991) An epitope on membrane-bound but not secreted IgE: implications in isotype-specific regulation. Bio/Technology 9: 53-56.

Peng C, Davis FM, Sun LK, Liou RS, Kim YW, Chang TW (1992) A new isoform of human membrane-bound IgE. J Immunol 148: 129-136.

Chen, H.Y., Liu, F.T., Hou, C.M.H., Huang, J.S.W., Sharma, B.B., and Chang, T.W. (2002) Monoclonal antibodies against CεmX domain in human membrane-bound IgE and their potential on targeting IgE-expressing B cells. Int. Archives Allergy & Immunol. 128, 315-324.

CLAIMS

WHAT IS CLAIMED:

1. A CεmX-specific antibody capable of binding to membrane-bound IgE on human B lymphocytes and incapable of binding to RADWPGPP peptide.

2. An antibody of claim 1, wherein the antibody is a mouse monoclonal antibody.

3. An antibody of claim 1, wherein the antibody is a chimeric antibody comprising the variable regions of a mouse monoclonal antibody and constant regions of human antibodies.

4. An antibody of claim 1, wherein the antibody is a humanized monoclonal antibody comprising essentially the hypervariable regions of a mouse monoclonal antibody and the framework regions and constant regions of human antibodies.

5. An antibody of claim 1, wherein the antibody is a human antibody.

6. A fragment of a CεmX-specific antibody of claim 1, capable of binding to membrane-bound IgE on human B lymphocytes and incapable of binding to RADWPGPP peptide.

7. A fragment of an antibody of claim 6, wherein the fragment is Fab, $F(ab)'_2$, or single-chain Fv.

8. A therapeutic method of using an antibody of claim 1 to treat IgE-mediated diseases.

9. A therapeutic method of claim 8, wherein the IgE-mediated disease is allergic asthma, allergic rhinitis, or atopic dermatitis.

10. A therapeutic method of claim 8, wherein the IgE-mediated disease is cold-induced urticaria, chronic urticaria, cholinergic urticaria, chronic rhinosinusitis, systemic mastocytosis, cutaneous mastocytosis, allergic bronchopulmonary aspergillosis, recurrent idiopathic angioedema, and interstitial cystitis, or eosinophil-associated gastrointestinal disorders.

11. An antibody of claim 1, wherein the antibody binds to GLAGGSAQSQRAPDRVL or an analogue with similar antigenic property.

12. An antibody of claim 1, wherein the antibody binds to HSGQQQGLPRAAGGSVPHPR or an analogue with similar antigenic property.

13. A therapeutic method of inducing immune response in patients *in vivo* by employing an immunogen containing GLAGGSAQSQRAPDRVL or an analogue with similar antigenic property.

14. A therapeutic method of inducing immune response in patients *in vivo* by employing an immunogen containing HSGQQQGLPRAAGGSVPHPR or an analogue with similar antigenic property.

15. A therapeutic method of inducing immune response in patients *in vivo* by employing an immunogen containing GLAGGSAQSQRAPDRVL or an analogue with similar antigenic property and HSGQQQGLPRAAGGSVPHPR or an analogue with similar antigenic property.