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 a first aspect, there is provided an isolated CεmX-specific antibody or an antigen binding fragment thereof, wherein the antibody is capable of binding to GLAGGSAQSQRAPDRVL (SEQ ID NO:2).

In a second aspect, there is provided an isolated antibody or antigen binding fragment thereof, wherein the antibody comprises the same hypervariable regions as antibody 4B12, and wherein the hypervariable regions are set forth as 24-39 of SEQ ID NO:5, 55-61 of SEQ ID NO:5, 94-102 of SEQ ID NO:5, 26-36 of SEQ ID NO:8, 51-66 of SEQ ID NO:8, and 98-106 of SEQ ID NO:8.

In a third aspect, there is provided the use of the antibody or antigen binding fragment as defined herein in the manufacture of a medicament for treating an IgE-mediated disease.

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.

A098870036US01-SEQ-YJC
SEQUENCE LISTING

<110> CHANG, Tsewen
CHEN, Jiun-bo
WU, Pheidias C
HUNG, Alfur F

<120> ANTI-C(epsilon)mX ANTIBODIES CAPABLE OF BINDING TO HUMAN mIgE

<130> A0988.70036US01

<140> US 13/203,483
<141> 2011-08-25

<150> 61/155,224
<151> 2009-02-25

<150> PCT/CN2010/000232
<151> 2010-02-25

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35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Lys Ile
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35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Ser Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Met Gly Ser Ile Ser Tyr Ser Gly Ile Thr Gly Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Ile Ser Val Thr Arg Asp Thr Ser Lys Asn Gln Phe Phe
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35 40 45

Met Ile Ser Ile Tyr His Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
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35 40 45

Met Ile Ser Ile Ser Tyr Ser Gly Ile Thr Gly Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
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Pro Asp Arg Val Leu Cys His Ser Gly Gln Gln Gln Gly Leu Pro Arg
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Pro Asp Arg Val Leu
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