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(54) **CDH26 BASED THERAPEUTIC AGENTS
AND THEIR USE**

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A61P 29/00 (2006.01)

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(US)

(52) **U.S. Cl.**
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A61K 38/177 (2013.01)

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§ 371 (c)(1),
(2) Date: **Nov. 28, 2017**

(57) **ABSTRACT**

Related U.S. Application Data

(60) Provisional application No. 62/168,087, filed on May
29, 2015.

The invention provides (CDH26)-based therapeutic agent,
compositions comprising same, and methods of treating
inflammatory conditions using same.

Fig. 1A

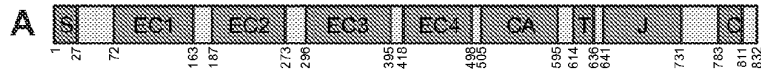


Fig. 1B

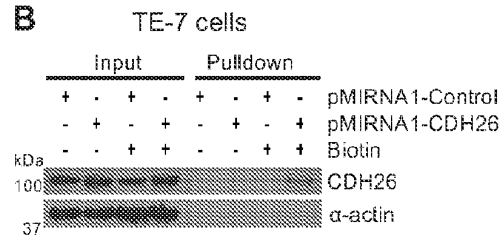


Fig. 1C

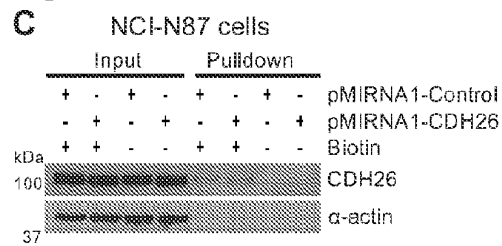


Fig. 1D

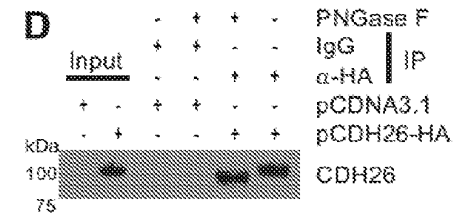


Fig. 2A

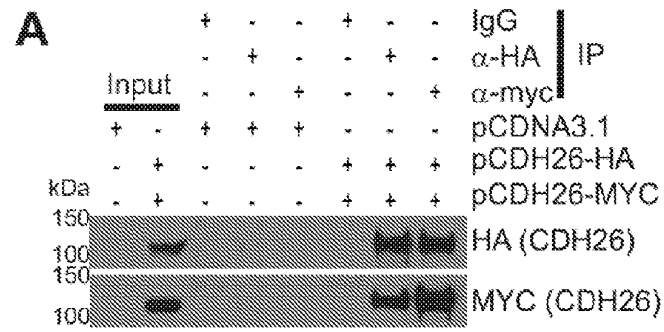


Fig. 2B

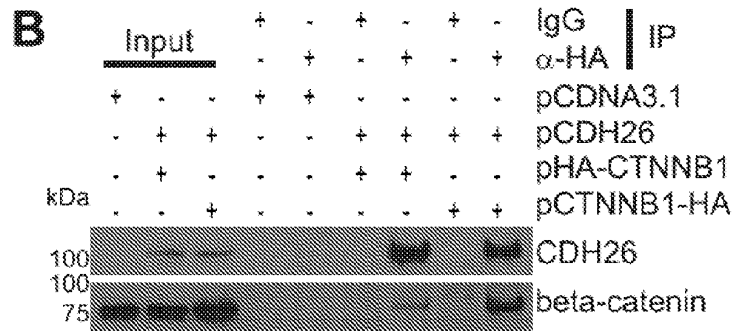


Fig. 2C

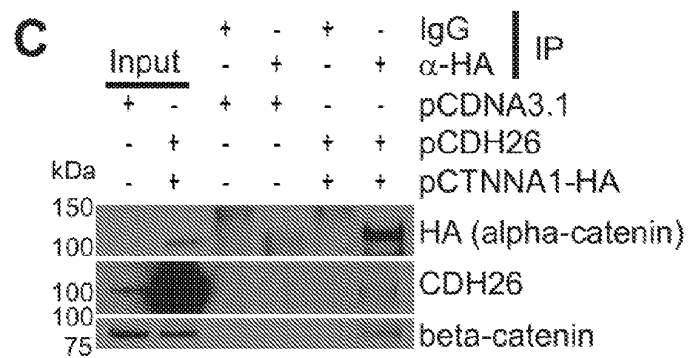


Fig. 2D

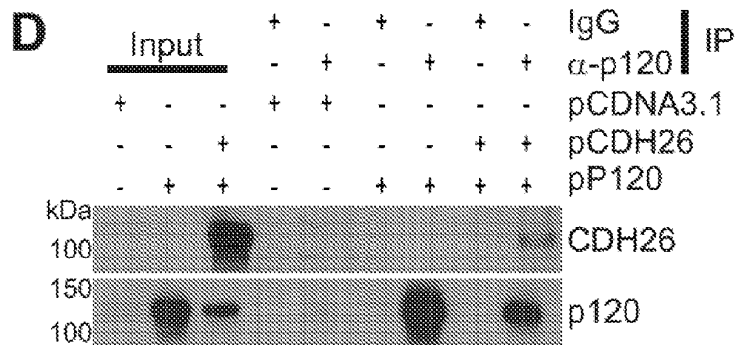


Fig. 2E

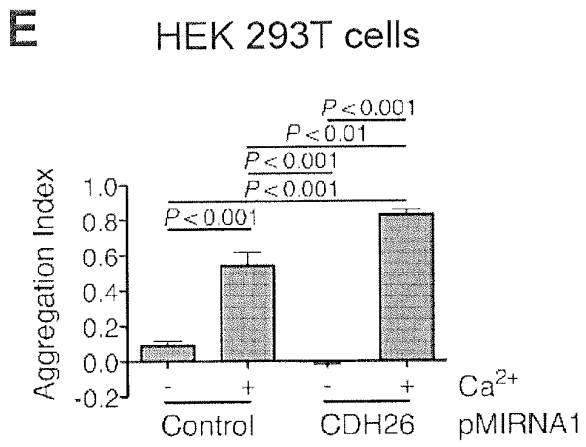


Fig. 2F

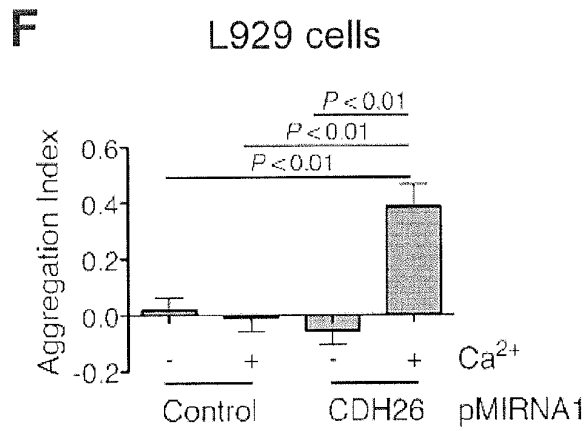


Fig. 3A

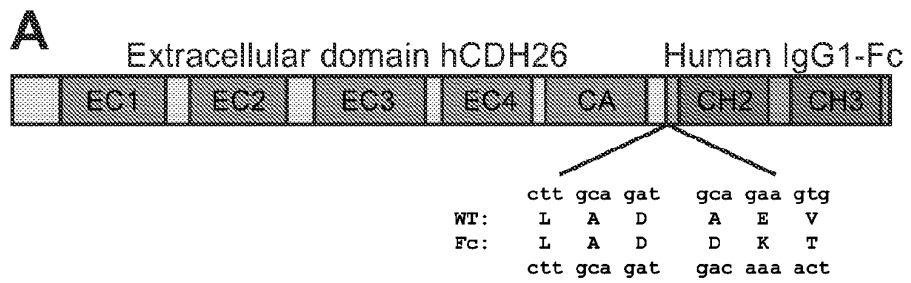


Fig. 3B

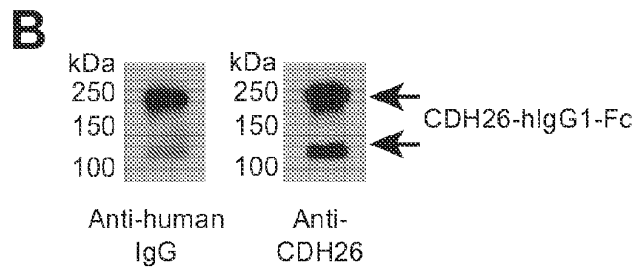


Fig. 3C

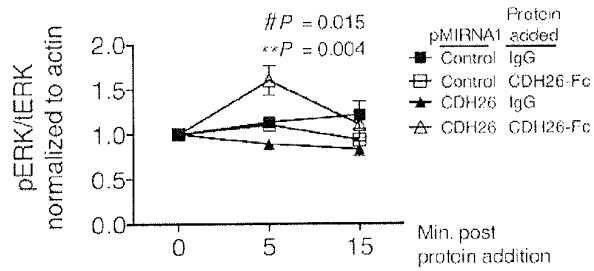
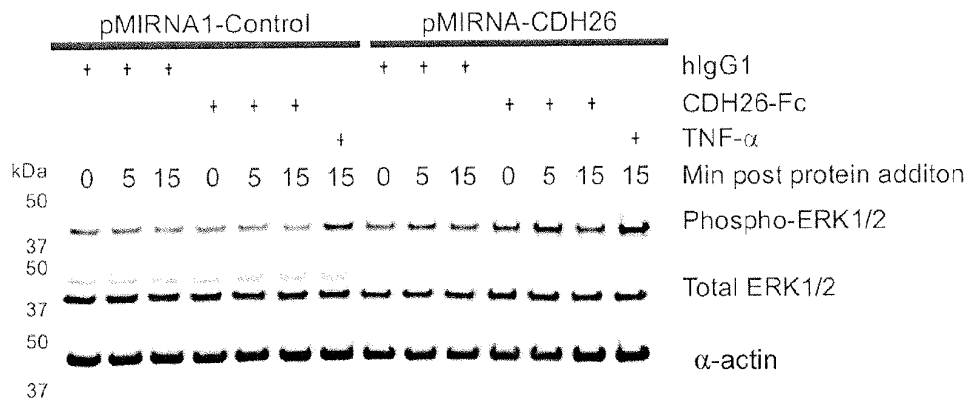


Fig. 3D

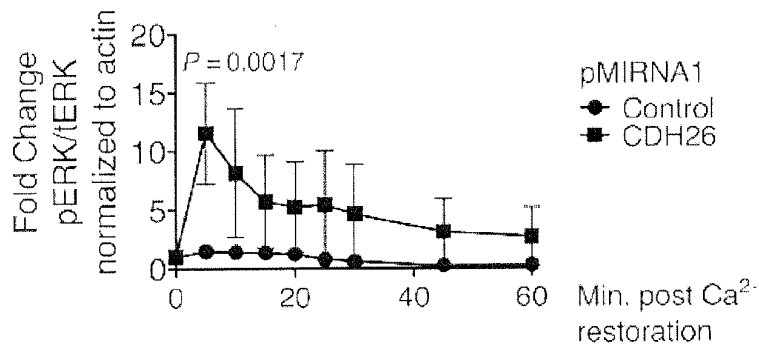
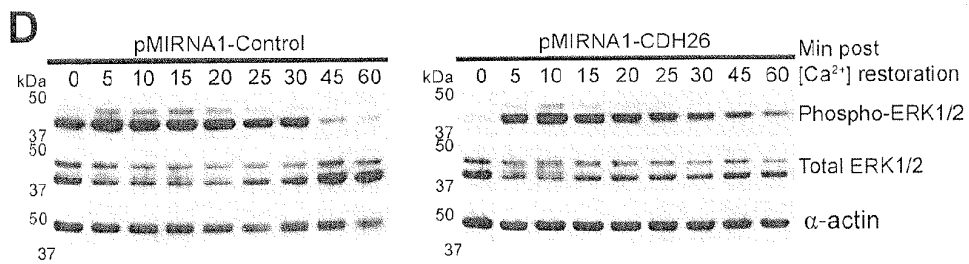


Fig. 4

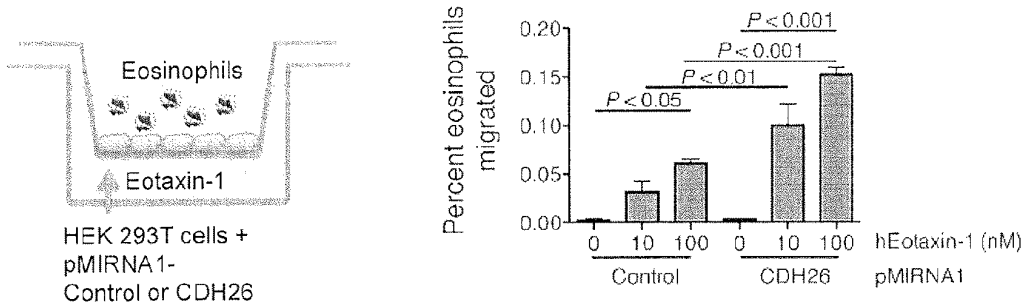


Fig. 5A

A

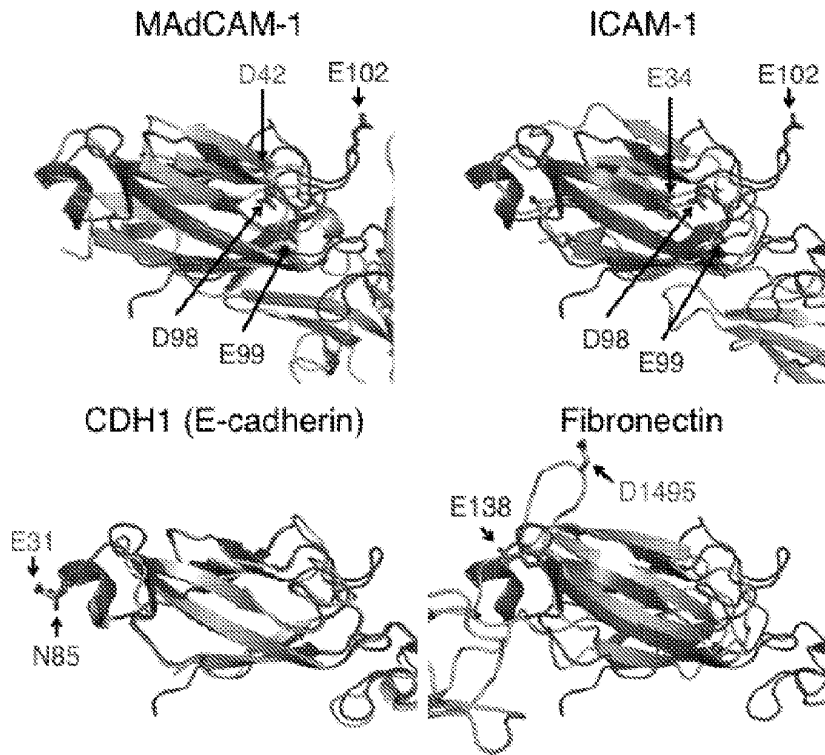


Fig. 5B

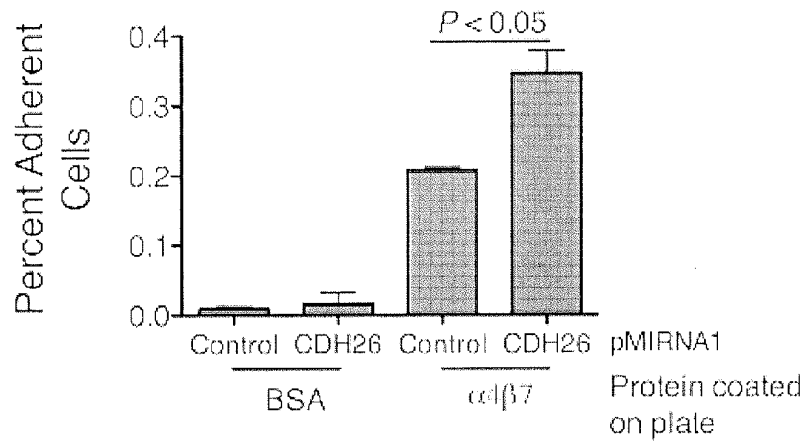


Fig. 5C

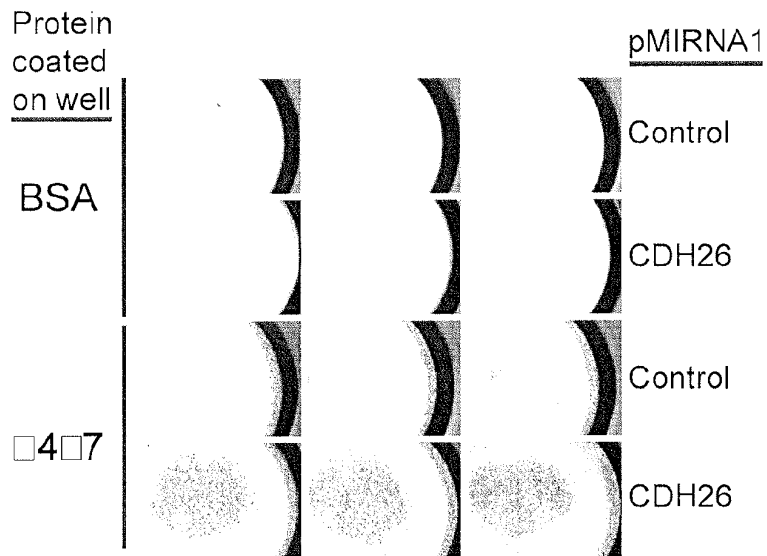


Fig. 5D

D

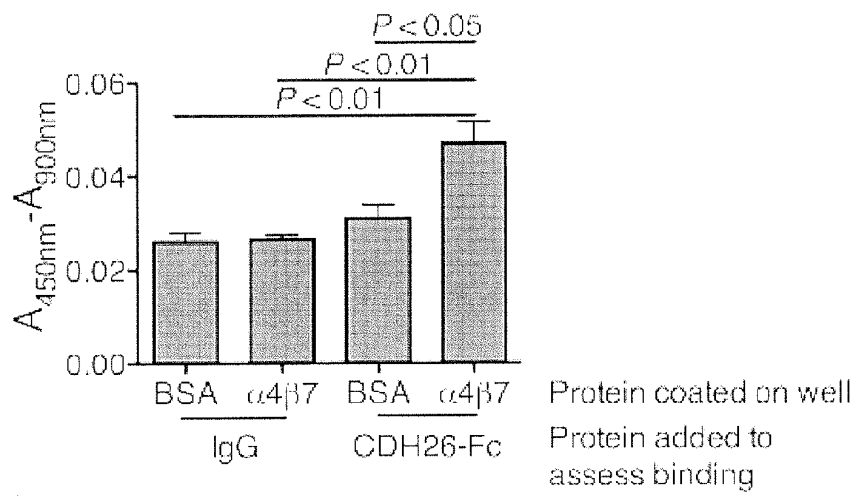


Fig. 6A

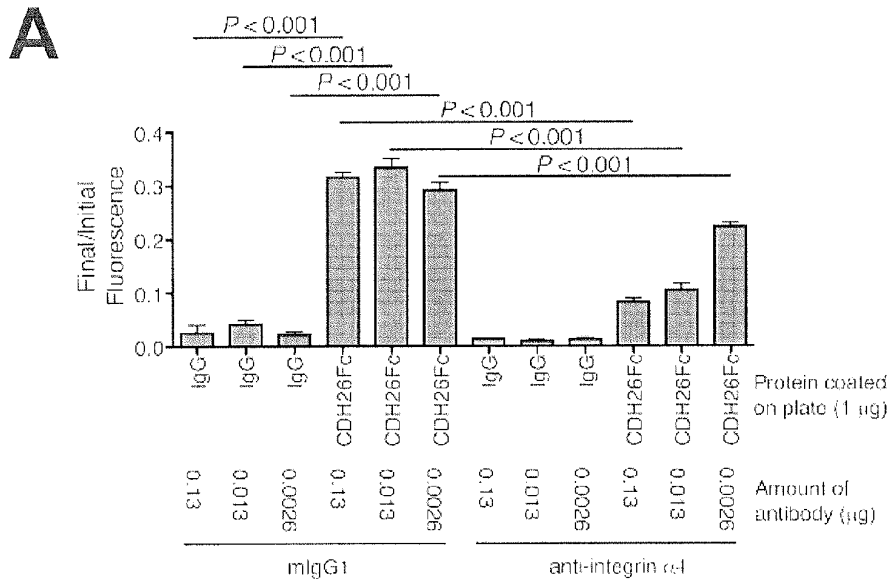


Fig. 6B

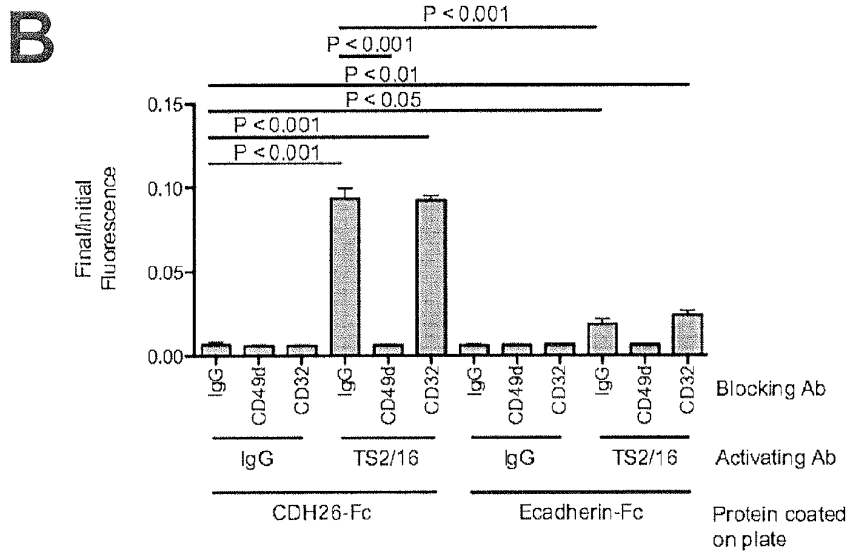


Fig. 6C

C

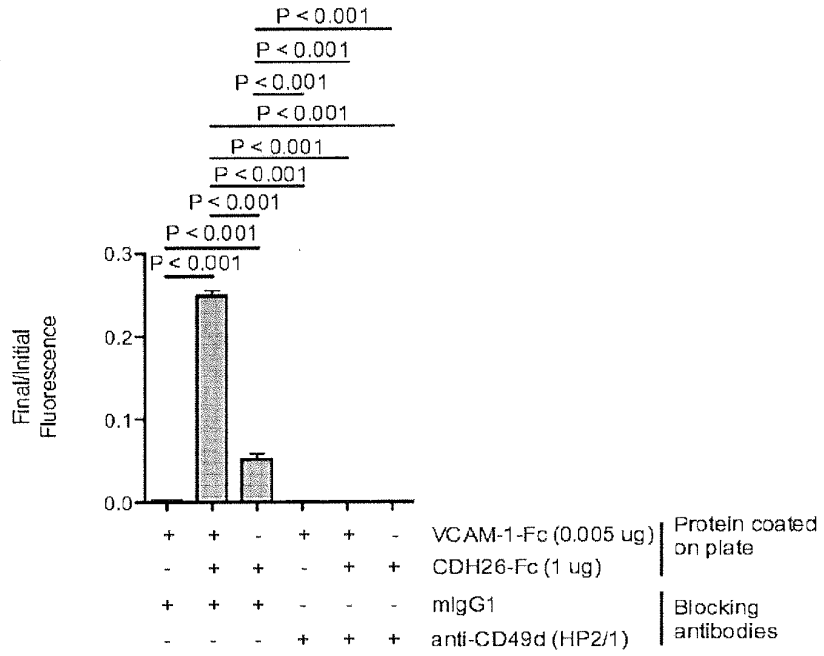


Fig. 6D

D

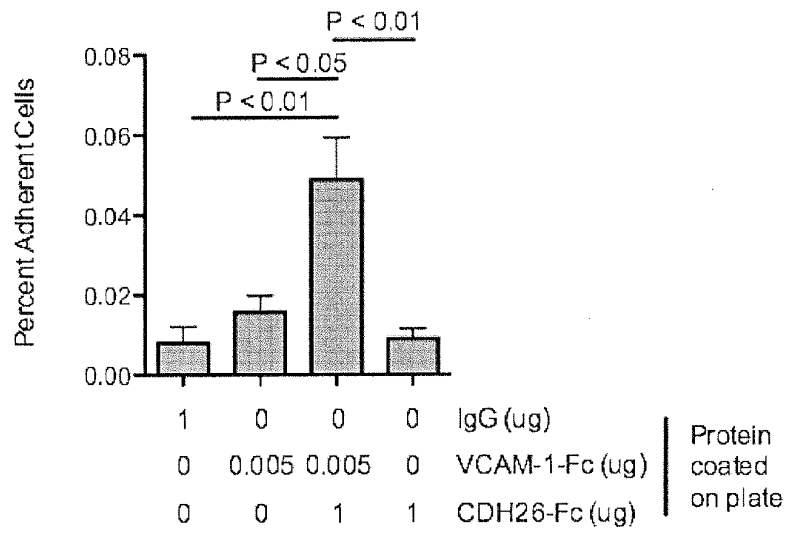


Fig. 6E

E

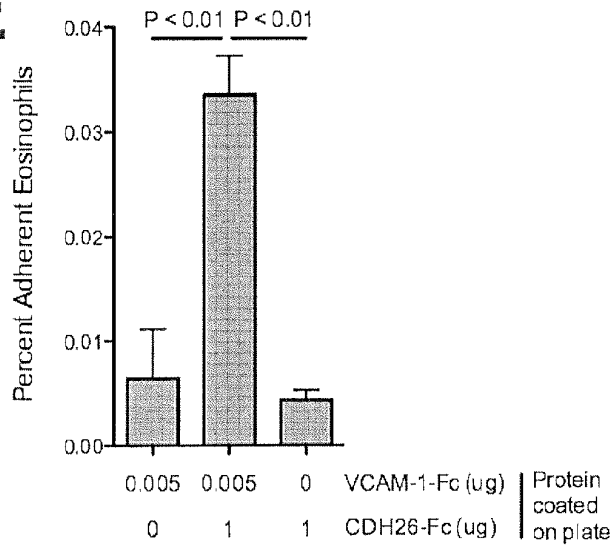


Fig. 7A

A

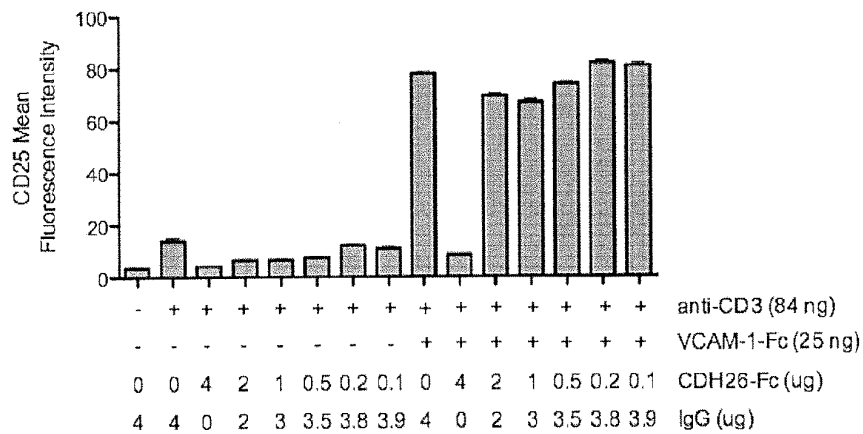
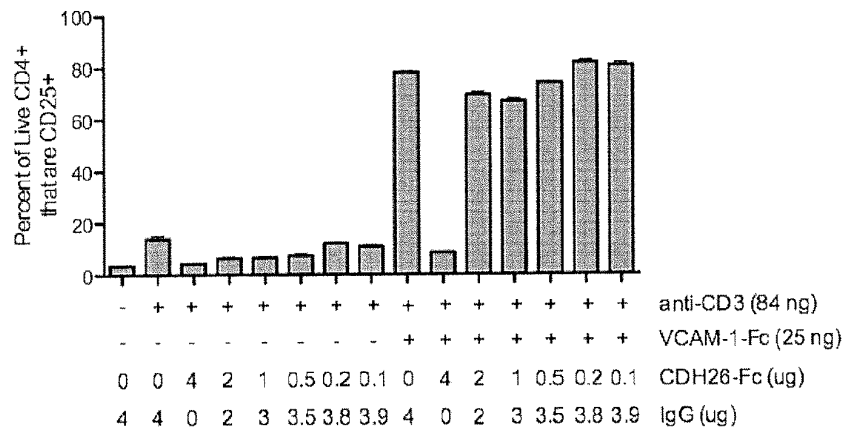


Fig. 7B

B

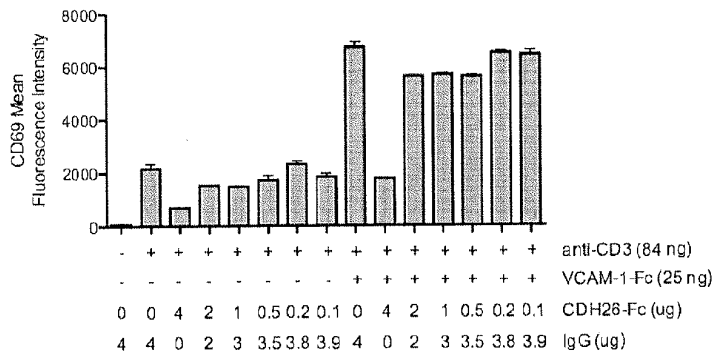
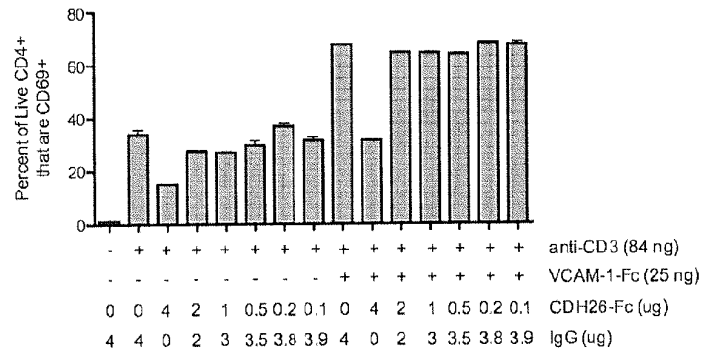


Fig. 7C

C

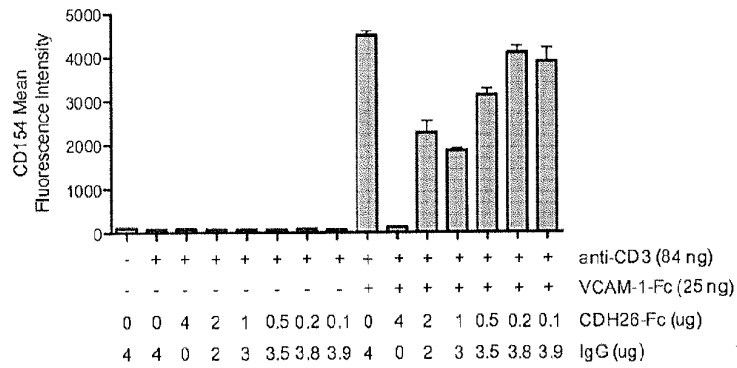
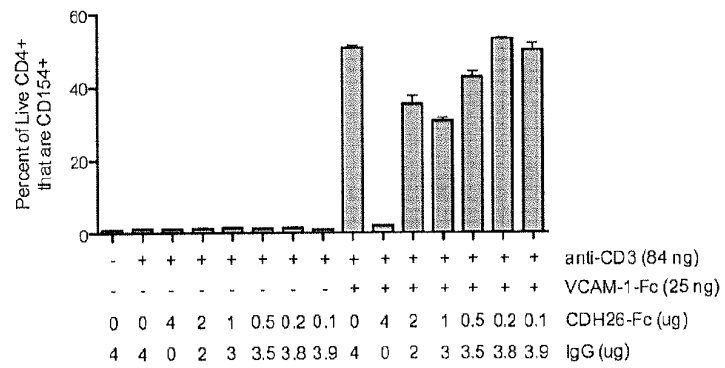


Fig. 7D

D

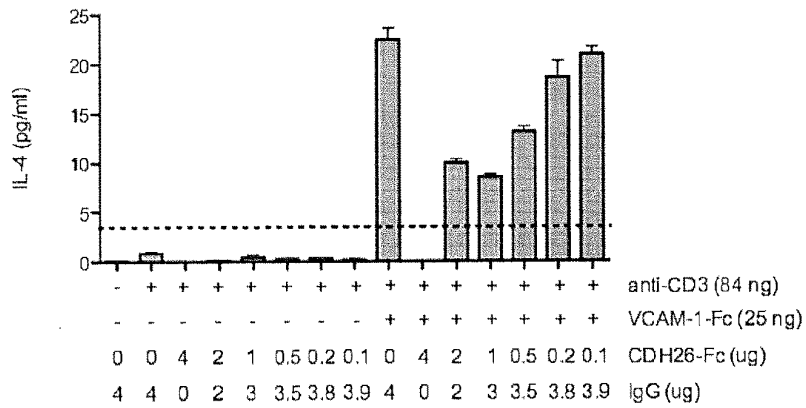
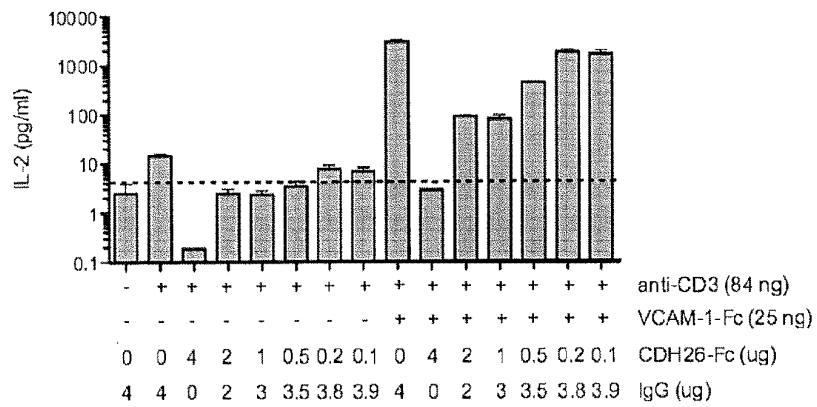


Fig. 8

**RWVITLLELEEEDPGPFKLI GELFNNMSYNMSL MYLISGPGVDEYPEIGLFSLEDH
ENGRIVVHRPVDREMTSPSFTVYFDVVERSTGKIVDTSLIFNIRISDVNDHAPQFPEKE
FNITYQENQSAGQPIFQMLAVDLDEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEI
RLSGCLDYETAPQFTLLIRARDCGEP SLSSTTTVHVDVQEGNNHRPAFTQENYKVQ
IPEGRASQGVLRLLVQDRDSPFTSAWRAKFNILHGNEEGHFDISTDPETNEGILNVI
KPLDYETRPAQSLIIVVENEERLVFCERGLQPPR KAAASATVSVQVTDANDPPAFH
PQSFIVNKEEGARPGTLLGTFNAMDPDSQIRYELVHDPANWVSVDKNSGVVITVEPI
DRESPHVNNSFYVIIIHAVDDGFPPQTATGTLMLFLSDINDNVPTLRPRSR YMEVCES
AVHEPLHIEAEDPDLEPFSDPFTFELDNTWGN AEDTWKLGRNWGQSVELLTLRSLP
RGNYLVP LFIGDKQGLSQKQTVHVRICPCAS**

SEQ ID NO: 10

Fig. 9

SEQ ID NO: 9

ATGGCCATGAGATCCGGGAGGCACCCCTCGCTGCTGCTTCTAGTGTCTGCTGCTGTGG
CTGCTGCAGGTCAGTATCATT²GACAGTGTTCACAGGAAACAGATGATCTTACTAAGCAA
ACAAAGGAAAAGATCTACCAGCCTCTACGGCGATCCAAGAGAAGATGGGTATCACCACC
TTGGAGCTGGAGGAGGAAGACCCGGGACCCCTTCCCAACICATTGGTGAGCTGTTCAT
AATATGTCTTATAACATGTCACTAATGTATCTAATCAGTGGACCTGGTGTGGATGAATAT
CCAGAGATTGGTTGTTTTCTCTAGAAGATCATGAGAACGGAGGATATATGTTCAACCGC
CCTGTCGATCGAGAAATGACACCATCTTTCACGGTTTATTTTGATGTTGTGGAGCGCTCA
ACAGGAAAAATTGTGGATACATCCTTGATTTTCAACATTAGGATCAGTGTGTGAATGAT
CATGCACCC²CAGTTTCCAGAGAAGGAATTAACATCACTGTGCAAGAAAACCAATCTGCA
GGCAACCTATTTTTCAGATGTTAGCAGTCGATTTGGATGAAGAAAACACTCCAATTTCT
CAAGTCC²TTACTTCCTCATTTC²CAAAACCCATTACTGAAGAAAGTGGTTTCCGGGT
GATCGCCTTAGTGGAGAAATACGACTCTCTGGCTGCTTAGATTATGAGACCGCTCCTCAG
TTTACACTGCTAATCAGAGCCAGGGACTGTGGAGAACCCTCACTGTCTCCACGACCACC
GTTACCGTGGATGTGCAAGAAGGCAACAACCACAGGCCT³GCATTTACCCAGGAGAACTAT
AAGGTTCAGATTCCTGAAGGCCGAGCCAGCCAGGGCGTGTGCGTCTCCTGGTTCAGAT
CGAGATTCTCCATTACATCAGCTTGGAGAGCAAATTCACATATTGCATGGCAATGAA
GAGGGGCATTTTGACATTTGACTGACCCTGAGACCAACGAAGGGATATTAATGTTATC
AAGCCTTTGGATTATGAGACTCGCCAGCGCAAAGCCTCATCATTGTCGTGGAGAATGAG
GAGAGGCTCGTCTTCTGTGAGAGAGGAAAGCTTCAGCCGCCAAGGAAGGCAGCCAGC
GCCACTGTGAGTGTGCAGGTGACAGACGCCAAGCCAGCCACCAGCC⁴TTTCAACCCAGGAGC
TTCAATTGTCAATAAAGAGGAGGGCGCCAGGCCTGGGACCTGTTGGGAAC⁴TTTAATGCC
ATGGATCCAGACAGCCAGATAAGATATGAAGTGGTTCATGACCCAGCAAATTGGGTACGC
GTCGACAAAACTCCGGAGTGGTCATCACCGTGGAGCCAATTGACCGAGAATCCCCTCAT
GTAATAACAGT⁵TTTATGTAATCATCATTACGCTGTTGATGATGGCTTCCACCCGAG
ACTGTACAGGGACCC⁵TAATGCTCTTCCCTGTCTGACATCAATGACAACGTC⁵ACTCTC
CGGCCACGTTCCCGCTACATGGAGGTCTGTGAGTCTGCTGTGCATGAGCCCTCCACATC
GAGGCAGAGGATCCGGACCTGGAGCCGTTCTCTGACCCATTTACATTTGAATTGGACAAT
ACCTGGGGAAATGCGGAGGACACATGGAAGTGGGGAGAAATGGGGTCAATCAGTTGAA
CTTTAACCTTGAGAAGCCTGCCACGTGGTAATTACTTGGTGCCACTCTTCATTGGAGAC
AAACAGGGACTTTCCAGAAAGCAAAC⁶TGTCATGTAAGGATCTGC⁶CCCTGTGCCAGTGGG
CTCACATGTGTGGAGCTTGCAGATGACAAAAC⁶TACACATGCCACCCGTCGCCAGCACCT
GACTCCTGGGGGACCGTCAGTCTTCCCTCTTCCCCCAAACCCAAAGGACACCCTCATG
ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTTGAGCCACGAGACCCTGAG
GTCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGG
GAGGAGCAGTACAACAGCACGTACCGTGTGGTTCAGCGTCC⁷TACCGTCC⁷TGCACCAGGAC
TGGCTGAATGGCAAGGAGTACAAGTGC⁷AAGGTC⁷TCCAACAAGCCCTCCAGCCCCATC
GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC⁸TACAAG
ACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGAAAGCTCTTCTCATGCTCCGTGATGCACGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGA⁸

Fig. 10

SEQ ID NO: 8

MAMRSGRHPSSLLLLLWLLQVSI¹DSVQQETDDLTQTKKEIYQPLRRSKRRWVITTT
LELEEDPGFPKLI GELFNMSYNMSL MYLISGPGVDEYPEIGLFSLEDHENGRIYVHR
PVDREMT²SETVYFDVVERSTGKI VDTSLIFENIRISDVNDHAP²QFPEKEFENITVQENQSA
GQPIFQMLAVDLDEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEIRLSGCLDYETAPO
FTLLIRARDCGEPSSSTTVVHVVDVQEGNNHRP³AFTQENYKVQIPEGRASQGVLRLLVQD
RDSPFTSAWRAKENILHGNEEGHEDISTDPETNEGILNVIKPLDYETRPAQSLIIVVENE
ERLVEFERGKLP⁴PRKAAASATVSVQVTDANDPPAF⁴HPQSFIVNKEEGARPGTLLGTFNA
MDRDSQIRYELVHDPANWVSV⁵DKNSG⁵VI⁵TVEPI⁵DRESPHVNN⁵SFYV⁵IIH⁵AVDDGEP⁵PQ
TATGTLML⁶FLSDINDNVP⁶TLRPRSR⁶YMEVCE⁶SAVHEPLHIEAED⁶PDLEP⁶FSDF⁶TFELDN
TWGN⁷AEDTWK⁷LGRNW⁷QSV⁷EL⁷TL⁷RS⁷LPR⁷GN⁷YL⁷VL⁷FL⁷IGDK⁷QGL⁷SOK⁷Q⁷TVH⁷VR⁷IC⁷PCASG
LTCVELAD⁸DK⁸TH⁸TC⁸PP⁸CP⁸AP⁸ELLG⁸GP⁸SV⁸FL⁸FP⁸PK⁸DT⁸LM⁸ISRT⁸PEVTCV⁸VVDV⁸SHEDPE
VKENWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTI⁹SKAKGQPREPQVY⁹TLPP⁹SREEMTK⁹NOV⁹SLTCLV⁹KGFY⁹PSDIAVEWESNGCPENNYK
TT¹⁰PPV¹⁰LDS¹⁰DG¹⁰SFFLY¹⁰SKLT¹⁰VDKSRW¹⁰QGNV¹⁰FSCSVMHEALHNHY¹⁰TOK¹⁰SL¹⁰SPGK¹⁰*

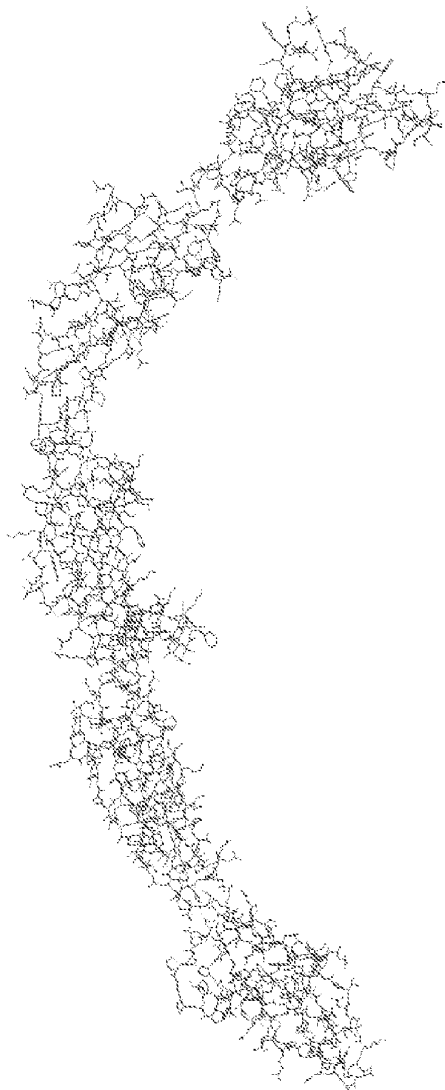


Fig. 11A

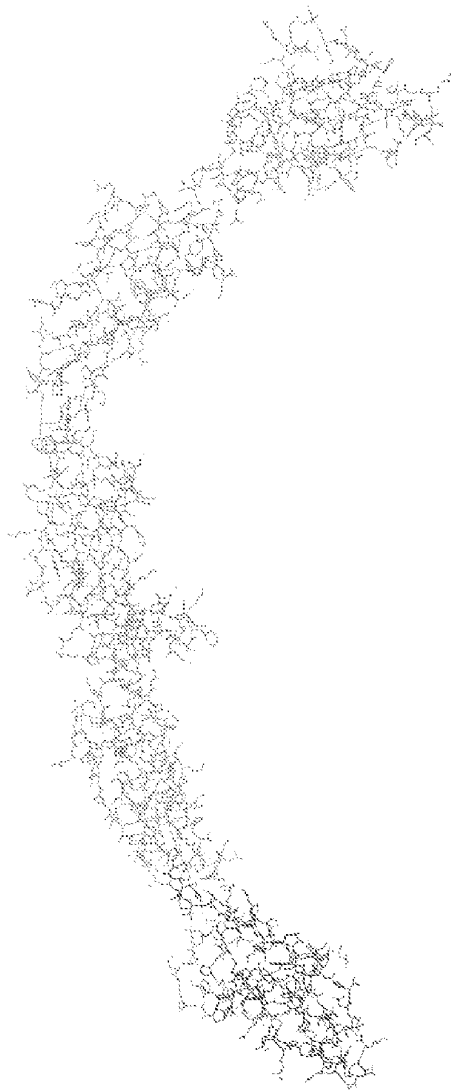


Fig. 11B
EC1

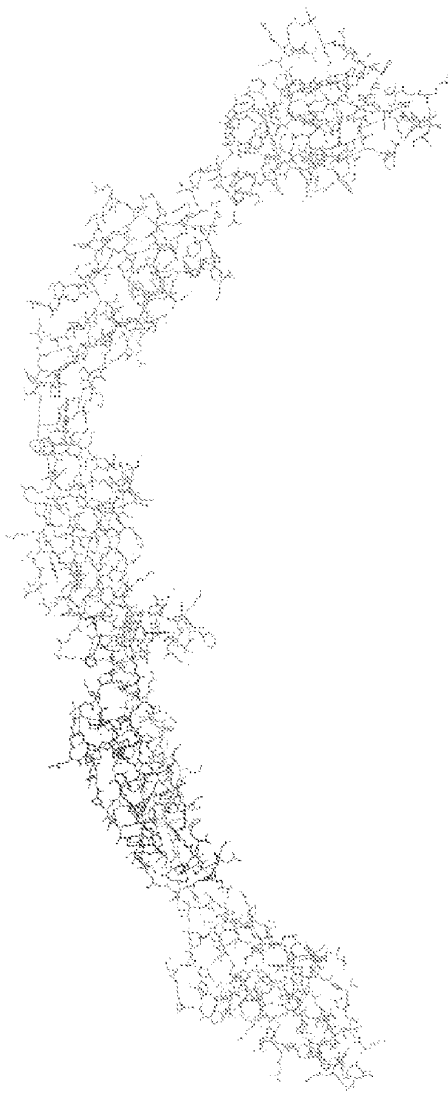


Fig. 11C
EC2

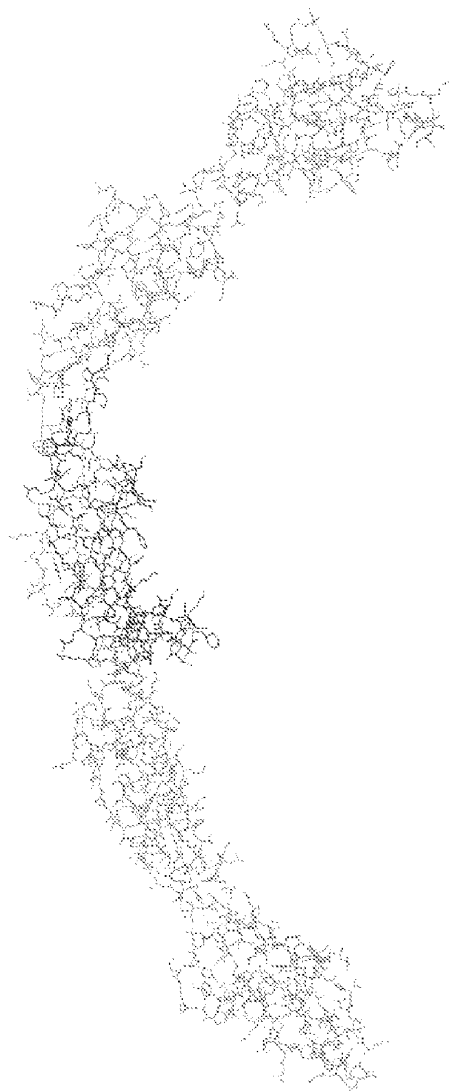


Fig. 11D
EC3

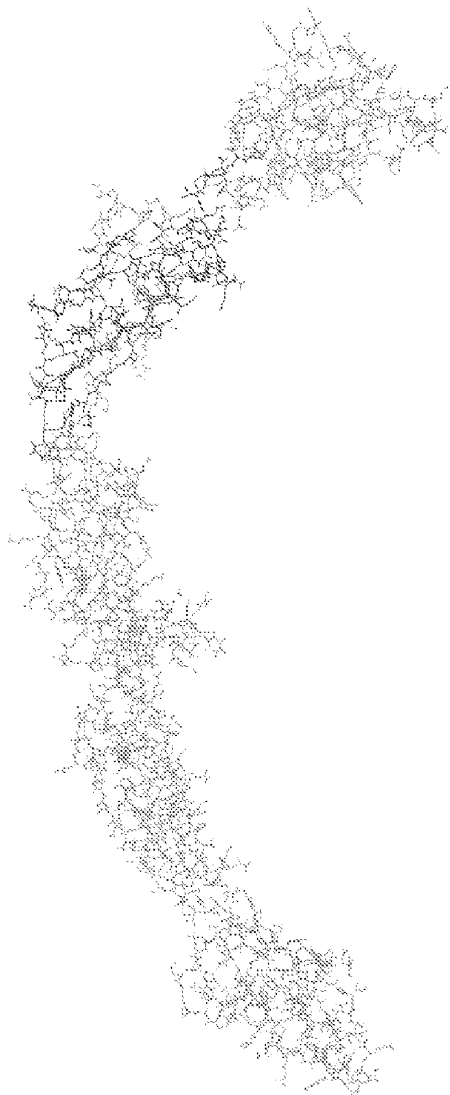


Fig. 11E
EC4

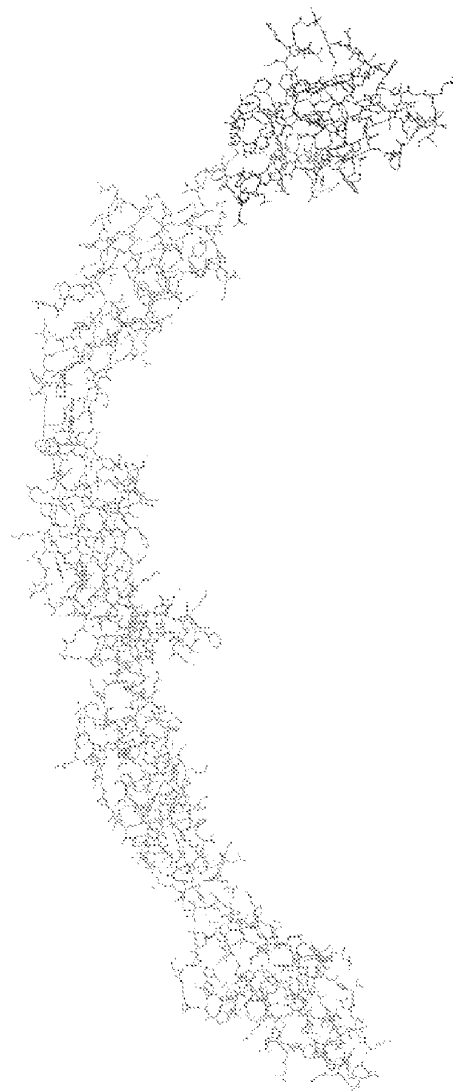


Fig. 11F
EC5

Fig. 12

Human CDH26 primary amino acid sequence (SEQ ID NO: 11)

MAMRSGRHPSSL LLLVLLWLLQVSIIDSVQQETDDLTKQTKEKIYQPLRRSKRRWVITT
LELEEDPGPPKLLIGELFNNMSYNMSLMYLISGPGVDEYPEIGLFSLEDHENGRIYVHR
PVDREMTPSFTVYFDVVERSTGKIVDTSLIFNIIRISDVNDHAPQFPEKEFNITVQENQSA
GQPIFQMLAVDLDEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEIRLSGCLDYETAPQ
FTLLIRARDCCGEPSSSTTTVHVVDVQEGNNHRPAFTQENYK VQIPEGRASQGVLRLLVQD
RDSFFTSAWRAKFNILHGNEEGHFIDISTDPETNEGILNVIKPLDYETRPAQSLIIVENE
ERLVFCERGKLLQPPRKAASAATVSVQVTDANDPPAFHPQSFIVNKEEGARPGTLLGTENA
MDPDSQIRYELVHDPANWVSDKNVSGVVITVEPIDRESPHVNNSFYVIIIHAVDDGFPPQ
TATGTLMLFLSDINDNVPTLRPRSRVMEVCESA VHEPLHIEAEDPDLEPFSDDPFTFELDN
TWGNAEDTWKLRNWWGQSVELLTLRSLPRGNYL VPLFIGDKQGLSQKQTVHVRI PCASG
LTCVELADAEVGLHVGFVCAAFVALAVALLFLRCYFVLEPKRHGCSVSNDEGHQTL
VMYNAESKGTSAQTWSDVEGQRPALLICTAAAGPTQGVKDLEEVPPSAASQSAQARCALG
SWGYGKPFEPRSVKNIHSTPAYPDATMHRQLLAPVEGRMAETLNQKLLHVANVLEDDPGYL
PHVYSEEGECGAPSLSSLASLEQELQPDLLDSLGSKATPFEIYSESGVPS

CDH26 BASED THERAPEUTIC AGENTS AND THEIR USE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/168,087, filed May 29, 2015, the content of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

[0002] This invention was made with government support under U19 AI070235 and R01 DK076893, awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention relates to cadherin based compounds and related compositions, and their use for treating inflammatory conditions.

BACKGROUND

[0004] Cadherin-mediated interactions between epithelial cells contribute to the maintenance of mucosal barriers that prevent access of environmental agents such as pathogens and allergens to underlying tissue and immunocytes. Several observations suggest a link between epithelial cadherin dysregulation, barrier disruption, and allergic disease. For example, reduced E-cadherin (CDH1) protein in asthmatic patients is associated with decreased airway epithelial barrier function (Trautmann et al., Apoptosis and loss of adhesion of bronchial epithelial cells in asthma. *Int Arch Allergy Immunol.* 2005; 138(2):142-50; de Boer W et al., Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol.* 2008; 86(3):105-12), and levels of membrane-localized CDH1 are decreased in the lesional skin of atopic dermatitis patients (Trautmann et al. The differential fate of cadherins during T-cell-induced keratinocyte apoptosis leads to spongiolysis in eczematous dermatitis. *J Invest Dermatol.* 2001; 117(4):927-34). In addition to affecting barrier function, inhibition of CDH1 expression in vitro results in increased expression of the chemoattractant cytokine CCL17 by human bronchial epithelial cells (Heijink I H, et al. Down-regulation of E-cadherin in human bronchial epithelial cells leads to epidermal growth factor receptor-dependent Th2 cell-promoting activity. *J Immunol.* 2007; 178(12):7678-85). Whether other cadherins influence allergic disease through impacting epithelial barrier maintenance or gene expression is not known.

[0005] In addition to homotypic interaction of cadherin molecules expressed by adjacent epithelial cells, cadherin-mediated interactions occur between epithelial cells and leukocytes. For example, CDH1 expressed by epithelial cells can bind CDH1 expressed by leukocytes or heterotypic molecules expressed by leukocytes such as integrins, in both cases influencing biological outcomes. However, reports of such epithelial cadherin/leukocyte integrin interactions are rare and to our knowledge limited to the observation that CDH1 binds lymphocyte integrin α F β 7 and regulates the activation and localization of epidermal and intestinal intra-epithelial lymphocytes (Cepek K L et al., Integrin alpha E

beta 7 mediates adhesion of T lymphocytes to epithelial cells. *J Immunol.* 1993; 150(8 Pt 1):3459-70; Cepek K L et al., Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature.* 1994; 372(6502):190-3; Schon et al. Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice. *J Immunol.* 1999; 162(11): 6641-9; Uchida et al. Role for E-cadherin as an inhibitory receptor on epidermal gammadelta T cells. *J Immunol.* 2011; 186(12):6945-54). Despite these observations regarding CDH1, the involvement of other cadherins in the regulation of immunologic processes mediated by the mucosal epithelium such as their binding to integrins has not been described.

[0006] A central feature of chronic allergic inflammation is T helper cell type 2 (Th2)-driven eosinophil accumulation typically mediated by an IL-13-driven cascade that elicits eosinophil trafficking from the blood to the tissue. This process involves the coordinate expression and activation of eosinophil-expressed selectins and integrins and their counter-receptors on activated endothelium, as well as the induction of eosinophil-activating chemokines such as the eotaxins (Broide D, and Sriramarao P. Eosinophil trafficking to sites of allergic inflammation. *Immunological reviews.* 2001; 179 163-72; Tachimoto et al. Cross-talk between integrins and chemokines that influences eosinophil adhesion and migration. *Int Arch Allergy Immunol.* 2002; 128 Suppl 1 18-20; Rosenberg et al. Eosinophil trafficking in allergy and asthma. *J Allergy Clin Immunol.* 2007; 119(6): 1303-10; quiz 11-2; Bochner B S. Road signs guiding leukocytes along the inflammation superhighway. *J Allergy Clin Immunol.* 2000; 106(5):817-28). While the mechanism of eosinophil transendothelial migration is well described, the processes involved in eosinophil transepithelial migration are not understood as completely. Moreover, much remains to be learned regarding the integrin/counter-receptor interactions occurring between epithelial cells and leukocytes and their consequences in the context of allergic inflammation.

[0007] The present inventors identified a previously uncharacterized cadherin, CDH26, that was markedly over-expressed in human gastrointestinal tissue with active eosinophilic inflammation. No studies of this molecule have been reported, although its transcript appears to be upregulated in epithelial cells under Th2-associated conditions (Woodruff et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci USA.* 2007; 104(40):15858-63; Shum et al. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J Clin Invest.* 2006; 116(8):2183-92; Zhen et al. IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. *Am J Respir Cell Mol Biol.* 2007; 36(2):244-53; Li R W, and Gasbarre L C. A temporal shift in regulatory networks and pathways in the bovine small intestine during *Cooperia oncophora* infection. *Int J Parasitol.* 2009; 39(7):813-24).

SUMMARY OF THE INVENTION

[0008] The present inventors sought to elucidate the function of CDH26 in allergic inflammation. As described in detail in the examples section, eosinophilic gastroenteropathy was used as a model system for allergic inflammation. The data presented in the examples confirm that CDH26 is

a functional cadherin upregulated specifically during allergic responses and demonstrate that CDH26 affects epithelial gene expression and modulates leukocyte responses. The immunomodulatory activity of CDH26 is shown to be mediated through its regulation of leukocyte migration, adhesion, and activation via binding $\alpha 4$ and αE integrins. Moreover, T cell activation is inhibited by a CDH26-Fc fusion protein, which is shown here to have potent immunosuppressive activity. Since the fusion protein inhibits T cell activation, it may be useful in treating various diseases and disorders characterized by an underlying aberrant T cell activation.

[0009] In embodiments, the disclosure provides a fusion protein comprising two polypeptide segments, 1 and 2, segment 1 comprising or consisting of an extracellular region of the human cadherin 26 (CDH26) protein defined by EC1 (SEQ ID NO: 2), or fragment thereof, said fragment consisting of at least 3 contiguous amino acids of SEQ ID NO: 2, and segment 2 comprising or consisting of a heavy chain constant region (Fc) of a human immunoglobulin (Ig) molecule.

[0010] In embodiments, segment 1 comprises or consists of SEQ ID NO: 10.

[0011] In embodiments, segment 1 comprises one or more additional extracellular cadherin repeat domains (EC) of human CDH26 selected from the group consisting of EC2, EC3, EC4, and EC5. In embodiments, the fragment of EC1 consists of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 2. In embodiments, segment 1 further comprises EC2 (SEQ ID NO: 3), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 3. In embodiments, segment 1 further comprises EC3 (SEQ ID NO: 4), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 4. In embodiments, segment 1 comprises or consists of EC4 (SEQ ID NO: 5), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 5. In embodiments, segment 1 comprises or consists of EC5 (SEQ ID NO: 6), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 6.

[0012] In accordance with any of the embodiments above, the Ig molecule may be selected from the group consisting of IgG, IgE, IgM, IgD, IgA and IgY.

[0013] In embodiments, segment 1 comprises or consists of SEQ ID NO: 10 and the Ig molecule is selected from the group consisting of IgG, IgE, IgM, IgD, IgA and IgY.

[0014] In accordance with any of the embodiments above, the Ig molecule may be an IgG or IgA molecule. In embodiments, the IgG or IgA molecule is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. In embodiments, the Ig molecule is selected from the group consisting of IgG1, IgG4, IgA1 and IgA2. In embodiments, the Ig molecule is IgG1. In embodiments, segment 2 comprises or consists of SEQ ID NO: 7.

[0015] In accordance with any of the embodiments above, the fusion protein may further comprise a signal sequence or a linker sequence, or both. In embodiments, the signal sequence is selected from a signal peptide of any one of interleukin-2, CD5, immunoglobulin kappa light chain, trypsinogen, serum albumin, and prolactin, and functional

fragments thereof. In embodiments, the linker sequence is located between segments 1 and 2 of the fusion protein. In embodiments, the linker sequence comprises or consists of from 2 to 10, or from 10 to 20, or from 20 to 50 amino acids. In embodiments, the fusion protein comprises or consists of SEQ ID NO: 8.

[0016] The disclosure also provides a composition or pharmaceutical composition comprising a fusion protein as described above or *infra*. In embodiments, the composition or pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient. In embodiments, the composition or pharmaceutical composition is formulated for topical administration. In embodiments, the composition or pharmaceutical composition further comprises at least one additional active agent selected from an IL-13 inhibitor, a non-steroidal anti-inflammatory drug (NSAID), a cytokine inhibitor, an asthma medicine such as a bronchodilator, omalizumab, mepolizumab, or reslizumab, an immunosuppressive agent such as 6-mercaptopurine, or a steroid. In embodiments, the composition or pharmaceutical composition is for treating an inflammatory disease, disorder, or condition in a subject in need thereof.

[0017] The disclosure also provides modified fusion proteins which are modified relative to any of the fusion proteins described above or *infra*. In embodiments, the amino acid sequence of a modified fusion protein is from 90 to 99% identical to the unmodified sequence. In embodiments, the sequence of segment 1 is modified to alter one or more of integrin binding, glycosylation, or homodimer formation. In embodiments, the sequence of segment 2 is modified to improve the aqueous solubility, stability, avidity, and/or pharmacokinetics of the fusion protein. In embodiments, the sequence of segment 2 is modified to reduce complement binding or antibody dependent cytotoxicity. In accordance with any of the foregoing embodiments, the sequence is modified by one or more amino acid insertions, substitutions or deletions.

[0018] In embodiments, the present disclosure provides a CDH26-Fc fusion protein for treating an inflammatory disease, disorder, or condition in a subject in need thereof.

[0019] In embodiments, the disclosure provides methods of treating an inflammatory disease, disorder, or condition in a subject in need thereof, the methods comprising administering to the subject an amount of a cadherin 26 (CDH26)-based therapeutic agent. In embodiments, the CDH26-based therapeutic agent is administered in an amount effective to suppress T cell activation in target cells of the subject, thereby treating the inflammatory condition in the subject. In embodiments, the target cells are epithelial cells. In embodiments, the target cells are cells of a target tissue in the subject. In embodiments, the target tissue is skin, nasal, tracheal, lung, or esophageal tissue.

[0020] In embodiments, the CDH26-based therapeutic agent is effective to suppress a CDH26 activity in the cells of the subject, relative to untreated cells. In embodiments, the CDH26-based therapeutic agent is effective to suppress CDH26 gene expression or protein expression, or both. In embodiments, the amount of the CDH26-based therapeutic agent is effective to ameliorate one or more symptoms of the inflammatory disease, disorder, or condition.

[0021] In embodiments, the CDH26-based therapeutic agent is a CDH26-Fc fusion protein, a CDH26 anti-sense polynucleotide, a CDH26-directed miRNA, a CDH26-directed shRNA, or a CDH26-directed antibody.

[0022] In embodiments, the inflammatory condition is characterized by an interleukin 13 (IL-13) mediated inflammation. In embodiments, the inflammatory condition is an allergic inflammatory condition. In embodiments, the allergic inflammatory condition is selected from asthma, atopic dermatitis, allergic rhinitis, eosinophilic duodenitis, eosinophilic colitis, eosinophilic gastritis (EG), and eosinophilic esophagitis (EoE). In embodiments, the inflammatory condition is associated with an autoimmune disease or disorder. In embodiments, the inflammatory condition is associated with an infection, for example a viral or bacterial infection. In embodiments, the inflammatory condition is associated with a cancer.

[0023] In embodiments, the methods of treating an inflammatory disease, disorder, or condition described herein further comprise administering to the subject at least one additional active agent. In embodiments, the at least one additional active agent is an anti-inflammatory agent. In embodiments, the at least one additional active agent is an IL-13 inhibitor, a non-steroidal anti-inflammatory drug (NSAID), an asthma medicine such as a bronchodilator, omalizumab, mepolizumab, or reslizumab, an immunosuppressive agent such as 6-mercaptopurine, a cytokine inhibitor, or a steroid.

[0024] The disclosure also provides methods of treating eosinophilic esophagitis (EoE) or eosinophilic gastritis (EG) in a subject in need thereof, comprising administering to the subject an amount of a (CDH26)-based therapeutic agent. In embodiments, the CDH26-based therapeutic agent is a CDH26-Fc fusion protein, a CDH26 anti-sense polynucleotide, a CDH26-directed miRNA, a CDH26-directed shRNA, or a CDH26-directed antibody.

[0025] The disclosure also provides methods of suppressing CDH26 activity in a cell, the method comprising contacting the cell with an anti-CDH26 agent in an amount effective to suppress CDH26 expression in the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1. Biochemical analysis of CDH26. (A) Schematic representing human CDH26 domain structure. S: signal peptide, EC1-EC4: extracellular cadherin repeat 1-4, CA: cadherin domain, T: transmembrane domain, J: juxtamembrane domain, C: catenin binding domain. (B and C) Surface biotinylation of TE-7 (B) and NCI-N87 (C) cells. Cell surface proteins were labeled with biotin and pulled down with streptavidin beads. Total cell lysates (input) and proteins bound to the streptavidin beads were subjected to SDS-PAGE and western blot analysis. Predicted CDH26 molecular weight: 92.4 kDa. (D) Immunoprecipitates from transiently transfected HEK 293T cells were treated with either peptide: N-glycosidase F (PNGase F) (+) or heat-inactivated PNGase F (-). Inputs (1/10 of amount used for IP) and treated immunoprecipitates were subjected to SDS-PAGE and western blot analysis.

[0027] FIG. 2. Biochemical and functional properties of CDH26. (A-D) Immunoprecipitates from transiently transfected HEK 293T cells and inputs (1/10 of amount used for IP) were subjected to SDS-PAGE and western blot analysis. Each blot shown is representative of 3 independent experiments. (E) Transduced HEK 293T cells were dispersed, incubated in buffer either containing or lacking 1 mM CaCl₂, and assessed for the degree of aggregation. (F) Aggregation assays were carried out as in E, except that transduced L929

cells were used. For E and F, data show 1 experiment representative of 3 and were analyzed by one-way ANOVA followed by Tukey post-test.

[0028] FIG. 3. CDH26 engagement activates ERK. (A) Schematic representation of CDH26-hIgG1-Fc fusion protein, including the nucleotide sequence encoding the junction between the CDH26 extracellular domain and the Fc portion of human IgG1 (Fc) compared to the corresponding sequence encoding the wild-type protein (WT). (B) CDH26-hIgG1-Fc fusion protein isolated by protein G affinity chromatography was subjected to western blot analysis. Lower arrow, CDH26-hIgG1-Fc monomer; Upper arrow, CDH26-hIgG1-Fc dimer. (C) CDH26-hIgG1-Fc (CDH26-Fc) fusion protein (20 ug/ml), hIgG1 control antibody (20 ug/ml), or TNF- α (10 ng/ml) was added to the supernatant of transduced L929 cell clones. Protein lysates were collected and subjected to SDS-PAGE and western blot analysis. The ratio of (phospho-ERK1/2/total ERK1/2) to actin signal was graphed as fold change relative to untreated for each cell type. The graph represents the average of 3 experiments that each included 3 separate control and 3 separate CDH26-overexpressing clones. A representative blot of one control and one CDH26-overexpressing clone from one experiment is shown. Data were analyzed by t test. **pMIRNA1-CDH26/CDH26-Fc 0 min vs. 5 min; #pMIRNA1-control/CDH26-Fc 5 min vs. pMIRNA1-CDH26/CDH26-Fc 5 min. (D) Transduced L929 cell clones were incubated in media lacking calcium for 3 h, followed by restoration of calcium concentration to 1.8 mM. Protein lysates were collected and subjected to SDS-PAGE and western blot analysis. The ratio of (phospho-ERK1/2/total ERK1/2) to actin signal was graphed as fold change relative to pre-calcium restoration for each cell type. The graph represents the average of 3 experiments that each included 3 separate control and 3 separate CDH26-overexpressing clones. Data were analyzed by 2-way ANOVA; a significant interaction was observed when comparing pMIRNA1-control to pMIRNA1-CDH26.

[0029] FIG. 4. Effect of CDH26 on eotaxin-1-mediated eosinophil cell transmigration. Transduced HEK 293T cells were seeded on top of transwells. Left: Eosinophils were added to the top chamber, and buffer containing the indicated concentration of eotaxin-1 was added to the bottom chamber (n=3 samples per group). Right: The fraction of eosinophils present in the bottom chamber after 1.5 h is shown. One representative experiment is shown (n=3). Data were analyzed by one-way ANOVA followed by Tukey post-test.

[0030] FIG. 5. Binding of CDH26 to integrin $\alpha 4\beta 7$. (A) Pairwise structure alignment of CDH26 with known integrin ligands. CDH26 structure was modeled (dark gray) and aligned to the resolved structures of MAdCAM-1 (PDB ID 1BQS), ICAM-1 (PDB ID 1IC1), CDH1 (PDB ID 1EDH), and fibronectin (PDB ID 1FNF) (light gray). For each pair, integrin binding amino acids and the corresponding CDH26 residues are labeled (arrows) and rendered using a stick representation. (B) Transduced L929 cell clones were dispersed and added to wells coated with either BSA or recombinant $\alpha 4\beta 7$. The percentage of adherent cells remaining after wells were washed is shown. The graph represents seven experiments combined that each involved separate control and CDH26-overexpressing clones. (C) Pictures of Giemsa-stained wells from B were taken (magnification=4 \times), with 1 control and 1 CDH26-overexpressing clone shown. (D) Wells were coated with or without recombinant

$\alpha 4\beta 7$ and then blocked with BSA, followed by addition of either IgG1 κ or CDH26-hIgG1-Fc (CDH26-Fc). Bound antibody or fusion protein was then detected and expressed as A450 nm-A900 nm. Each condition was performed in triplicate. This graph shows 1 experiment representative of 3. For B and D, data were analyzed by one-way ANOVA followed by Tukey post-test.

[0031] FIG. 6. Cell adhesion to CDH26-Fc and requirement for integrin $\alpha 4$. (A-C) Fluorescently labeled Jurkat cells (untreated [A] or incubated with TS2/16 integrin $\beta 1$ -activating antibodies [B and C]) pre-incubated with the indicated amount of either control mIgG1 or anti-integrin $\alpha 4$ (HP2/1) antibodies were added to wells coated with control hIgG1, CDH26-hIgG1-Fc (CDH26-Fc), E-cadherin-hIgG1-Fc (Ecadherin-Fc), and/or VCAM-1-hIgG1-Fc (VCAM-1-Fc), as indicated. The graph indicates the percentage of fluorescence remaining after wells were washed. (D) Fluorescently labeled human peripheral blood CD4⁺ T cells treated with TS2/16 integrin $\beta 1$ -activating antibodies were added to wells coated with control hIgG1, CDH26-hIgG1-Fc (CDH26-Fc) and/or VCAM-1-hIgG1-Fc (VCAM-1-Fc), as indicated. The graph indicates the percentage of fluorescence remaining after wells were washed. (E) Human peripheral blood eosinophils were added to wells coated with control hIgG1, CDH26-hIgG1-Fc (CDH26-Fc), and/or VCAM-1-hIgG1-Fc (VCAM-1-Fc), as indicated. The graph indicates the percentage of eosinophils remaining after wells were washed as determined by residual eosinophil peroxidase activity. Data were analyzed by one-way ANOVA followed by Tukey post-test.

[0032] FIG. 7. Effect of CDH26-Fc on CD4⁺ T cell activation. Human peripheral blood CD4⁺ T cells were isolated and cultured for 48 h in wells coated with the indicated amounts of proteins (IgG, anti-CD3, VCAM-1-Fc, and/or CDH26-Fc). Cells were stained for flow cytometry analysis to detect CD4, CD25, CD69, and CD154, and supernatants were analyzed to detect IL-2 and IL-4 levels by ELISA. For (A-C, top), the percent of live CD4⁺ cells that are (A) CD25⁺, (B) CD69⁺, or (C) CD154⁺ are shown, and for (A-C, bottom), the mean fluorescence intensity of (A) CD25, (B) CD69, or (C) CD154 signal for CD4⁺ cells is shown. For (D), the amount of (top) hIL-2 and (bottom) hIL-4 detected in the supernatant is shown. The dotted lines represent the detection limit for each ELISA.

[0033] FIG. 8. Primary amino acid sequence used for modelling of the 3-dimensional structure of human CDH26 putative extracellular domain. Includes Asparagine 55 to Serine 599 of human CDH26 (SEQ ID NO: 10).

[0034] FIG. 9. Nucleotide sequence of human CDH26-hIgG1-Fc (SEQ ID NO: 9). Specific regions of the sequence are underlined and denoted by superscript numbers at the end of the denoted underlined sequence. The indicated nucleotides encode the amino acids that compose the following domains of the protein: (1) signal peptide, (2) extracellular cadherin repeat domain 1 (EC1), (3) extracellular cadherin repeat domain 2 (EC2), (4) extracellular cadherin repeat domain 3 (EC3), (5) extracellular cadherin repeat domain 4 (EC4), (6) extracellular cadherin repeat domain 5 (EC5), (7) human IgG1-Fc.

[0035] FIG. 10. Primary amino acid sequence of a human CDH26-hIgG1-Fc fusion protein (SEQ ID NO: 8). Specific regions of the sequence are underlined and denoted by superscript numbers at the end of the denoted underlined sequence. The indicated amino acids form the following

domains of the protein: (1) signal peptide, (2) extracellular cadherin repeat domain 1 (EC1), (3) extracellular cadherin repeat domain 2 (EC2), (4) extracellular cadherin repeat domain 3 (EC3), (5) extracellular cadherin repeat domain 4 (EC4), (6) extracellular cadherin repeat domain 5 (EC5), (7) human IgG1-Fc.

[0036] FIG. 11. Modeling the 3-dimensional structure of the extracellular domain of human CDH26. The model includes amino acids Asparagine 55 to Serine 599 (A). The N-terminus is located on the left side of the figure, and the C-terminal portion is located on the right side of the figure. The model was obtained using the Phyre2 server and was visualized using Swiss-PdbViewer. In panels B-F, each of the extracellular cadherin repeat domains is individually denoted in black, with the remainder of the putative extracellular domain shown in gray as follows: (B) EC1, (C) EC2, (D) EC3, (E) EC4, (F) EC5.

[0037] FIG. 12: Human CDH26 amino acid reference sequence (SEQ ID NO: 11).

DETAILED DESCRIPTION OF THE INVENTION

[0038] Previous work by the inventors established a biological connection between high levels of CDH26 expression and the behavior of the inflammatory cells that underlie the inflammatory condition eosinophilic gastritis (EG) (WO 2012/178188). As discussed in more detail infra, the inventors have extended this work to show that CDH26 affects epithelial gene expression, binds $\alpha 4$ and αE integrins, and regulates leukocyte migration and activation. Importantly, the present disclosure further demonstrates that a CDH26-Fc fusion protein acts as an immunosuppressive agent in a model system of T cell activation which is relevant to allergic inflammation.

[0039] The present disclosure provides CDH26-based therapeutic agents for the treatment of an inflammatory condition. In embodiments, the CDH26-based therapeutic agent is effective to suppress T cell activation, especially CD4⁺ T cell activation. In embodiments, the agent suppresses T cell activation in a target cell or tissue. In embodiments, the agent suppresses T cell activation in an in vitro assay, such as a T cell activation assay.

[0040] In embodiments, the CDH26-based therapeutic agent is a CDH26-Fc fusion protein, a CDH26 anti-sense polynucleotide, a CDH26-directed miRNA, a CDH26-directed shRNA, or a CDH26-directed antibody, including a humanized antibody. In embodiments, the CDH26-based therapeutic agent is a fusion protein, as discussed in more detail below.

[0041] The present disclosure also provides methods for treating an inflammatory condition in a subject by modulating CDH26 activity, and related methods. The methods comprise administering a CDH26-based therapeutic agent to a subject in need of treatment for an inflammatory condition.

CDH26 Fusion Proteins

[0042] The present disclosure provides CDH26 fusion proteins comprising two polypeptide segments, denoted 1 and 2. Segment 1 consists of a polypeptide comprising a CDH26 extracellular region. Segment 2 consists of a polypeptide comprising a heavy chain constant region (Fc) of a human immunoglobulin (Ig) molecule. The term "fusion protein" in this context refers to a polypeptide comprising

the polypeptide segments 1 and 2, and one or more additional, optional polypeptides. For example, the fusion protein may contain an optional polypeptide that functions as a signal sequence, also referred to as a signal peptide (these terms are used interchangeably herein). Exemplary signal sequences include the signal peptides of interleukin-2, CD5, immunoglobulin kappa light chain, trypsinogen, serum albumin, and prolactin, which are known in the art. The fusion protein may also contain an optional polypeptide that functions as a linker sequence joining segments 1 and 2 together. In embodiments, the linker sequence comprises or consists of from 2 to 10, or from 5 to 10, or from 10 to 25 amino acids. In embodiments the linker is selected from a flexible linker and a rigid linker. In embodiments, the linker is a cleavable linker, e.g., one that can be cleaved *in vivo* by an endogenous enzyme. Exemplary linkers are known in the art and are reviewed, for example, in Chen et al., 2013, *Adv Drug Deliv Rev*.

[0043] In embodiments, segment 1 comprises or consists of the CDH26 extracellular region defined by amino acids 1-608 of the human CDH26 protein (SEQ ID NO: 11). In embodiments, segment 1 comprises or consists of the CDH26 extracellular region defined by amino acids asparagine 55 (Asp55) to serine 599 (Ser599) (SEQ ID NO: 10) of the human CDH26 protein. In embodiments, segment 1 comprises one or more of amino acids 609-613 of the human CDH26 protein.

[0044] In embodiments, segment 1 comprises or consists of one or more CDH26 extracellular domains selected from the group consisting of extracellular cadherin repeat domain (EC) 1, EC2, EC3, EC4, and EC5 of the human CDH26 protein (SEQ ID NO: 11). In embodiments, segment 1 comprises at least EC1 (SEQ ID NO: 2). In embodiments, segment 1 comprises or consists of (SEQ ID NO: 2). In embodiments, segment 1 comprises or consists of EC1 (SEQ ID NO: 2) and at least one of EC2 (SEQ ID NO: 3), EC3 (SEQ ID NO: 4), and EC4 (SEQ ID NO: 5). In embodiments, segment 1 comprises or consists of EC1 (SEQ ID NO: 2) and at least two of EC2 (SEQ ID NO: 3), EC3 (SEQ ID NO: 4), and EC4 (SEQ ID NO: 5). In embodiments, segment 1 comprises or consists of EC1 (SEQ ID NO: 2) and all three of EC2 (SEQ ID NO: 3), EC3 (SEQ ID NO: 4), and EC4 (SEQ ID NO: 5). In embodiments, segment 1 comprises or consists of each of the five EC domains of CDH26 (SEQ ID Nos 2-6).

[0045] In embodiments, segment 2 comprises or consists of the Fc region of an Ig molecule. The term "Fc" refers to "fragment crystallizable", the part of an antibody molecule that interacts with Fc receptors which are cell surface and intracellular molecules that bind to the Fc region of antibodies. The Fc region contains the carboxy-terminal heavy chains of the antibody molecule disulfide bonded to each other through the hinge region.

[0046] In embodiments, the Ig molecule is a human Ig molecule selected from the group consisting of IgG, IgE, IgM, IgD, IgA and IgY.

[0047] In embodiments, the human Ig molecule is an IgG or IgA molecule. In embodiments, the IgG or IgA molecule is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. In embodiments, the Ig molecule is selected from the group consisting of IgG1, IgG4, IgA1 and IgA2.

[0048] In embodiments, the Fc polypeptide of segment 2 is comprises or consists of SEQ ID NO: 7.

Modifications

[0049] In embodiments, the polypeptide of a fusion protein described herein may be modified by one or more amino acid insertions, substitutions or deletions, for example in order to alter the stability or biological activity of the fusion protein.

[0050] In embodiments, the polypeptide of segment 1 is modified by one or more amino acid insertions, substitutions or deletions for example, to alter integrin binding, glycosylation, or homodimer formation, as described in more detail below.

[0051] In embodiments, the Fc polypeptide of segment 2 is modified by one or more amino acid insertions, substitutions or deletions, for example, to improve the aqueous solubility, stability, avidity, and/or pharmacokinetics of the CDH26-Fc fusion protein. In embodiments, the Fc polypeptide of segment 2 is modified to reduce or eliminate complement binding or antibody dependent cellular cytotoxicity, or both. In some embodiments, the Fc polypeptide is modified to improve the aqueous solubility and stability of the fusion protein, or to promote its purification during manufacturing. In embodiments, the Fc polypeptide is modified to polymerize, e.g., through engineered disulfide bridges and carboxy-terminal extensions, thereby increasing the avidity of the fusion protein. In embodiments, the Fc polypeptide is modified to improve the pharmacokinetics of the fusion protein, e.g., to improve its *in vivo* half-life. Examples of such Fc modifications are known in the art and described, for example in Czajkowsky et al. (2012) Fc-fusion proteins: new developments and future perspectives in *EMBO Molecular Medicine*.

[0052] In embodiments, the amino acids inserted or substituted in the polypeptide of segment 1 and/or segment 2 of a fusion protein described herein are naturally occurring amino acids selected from the group consisting of: alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamine (Gln, Q); glutamic acid (Glu, E); glycine (Gly, G); histidine (His, H); Isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V). In embodiments, the amino acids inserted or substituted are naturally or non-naturally occurring amino acids. A non-naturally occurring amino acid refers to an amino acid other than those listed above, which is able to covalently bind adjacent amino acids to form a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, omithine, norvaline, and homoserine.

[0053] In embodiments, the polypeptide of segment 1 or segment 2, or both, is modified by a conservative or non-conservative amino acid substitution. A conservative amino acid substitution refers to substituting an amino acid with another amino acid in the same chemical class. A non-conservative amino acid substitution refers to substituting an amino acid with another amino acid having different chemical and/or physical (e.g., in terms of size or bulkiness) properties. Amino acids are generally classified according to the chemical and physical properties of their side chains, for example by hydrophobicity, acidity, basicity, polarity, large or small. Generally included among the hydrophobic amino acids are norleucine, methionine, alanine, valine, leucine, and isoleucine. Generally classified as neutral and large nonpolar are isoleucine, leucine, valine, methionine, and

phenylalanine; generally classified as neutral and small nonpolar are alanine and threonine; generally classified as neutral and small polar are glycine, serine, and asparagine; generally classified as neutral and intermediate or large polar are proline, tyrosine, glutamine, and tryptophan; generally classified as acidic are aspartic acid and glutamic acid; generally classified as basic are arginine and lysine, and generally classified as weakly basic are histidine and cysteine.

[0054] In embodiments, the one or more amino acid insertions, substitutions or deletions in segment 1 is relative to the amino acid sequence of one or more of SEQ ID NOs: 2-6. In embodiments, the one or more amino acid insertions, substitutions or deletions in segment 2 is relative to the amino acid sequence of SEQ ID NO: 7.

[0055] In embodiments, one or more amino acid residues in segment 1 is substituted with a non-conservative amino acid in order to change the activity of the fusion protein, for example, to alter the binding of the fusion protein to an integrin, reduce the formation of CDH26-Fc homodimers with other CDH26 molecules, or change the glycosylation of the fusion protein. In this context, a non-conservative amino acid substitution refers to one which would be expected to abolish the functionality of the amino acid residue it replaces. For example, an acidic residue is needed for integrin binding. Accordingly, a non-conservative amino acid substitution at a residue which functions in integrin binding would be the substitution of an acidic residue with a residue that is not acidic.

[0056] In embodiments, segment 1 comprises or consists of EC1 (SEQ ID NO: 2) and the one or more amino acid residues in segment 1 that is modified as discussed above is selected from the group consisting of the amino acids corresponding to W56, N81, N85, D98, E99, E102, E138, N171, N177 and N462 of CDH26 (SEQ ID NO:11). In this context, "corresponding to" means the amino acid residues in segment 1 of the fusion protein that map to the indicated residues in SEQ ID NO:11 when the amino acid sequences of segment 1 and the EC1 of CDH26 (SEQ ID NO:11) are aligned.

[0057] In embodiments, the one or more amino acid residues is selected from the group consisting of the amino acids corresponding to N85, D98, E99, E102 and E138 of

CDH26 (SEQ ID NO:11) and the amino acid substitution is effective to enhance or reduce the binding of the fusion protein to an integrin molecule. For example, mutation of any one of these amino acid residues to alanine would be expected to reduce integrin binding. Mutation of N85 to aspartic acid or glutamic acid might be expected to enhance integrin binding. Mutation of any one of these residues to another negatively charged amino acid (i.e. aspartic acid or glutamic acid) might be expected to maintain similar integrin binding or to enhance integrin binding.

[0058] In embodiments, the amino acid residue is that corresponding to W56 of CDH26 (SEQ ID NO:11) and the amino acid substitution is effective to reduce the formation of CDH26-Fc homodimers in trans with other CDH26 molecules. For example, mutation of W56 to alanine would be expected to reduce homodimer formation.

[0059] In embodiments, the one or more of the amino acid residues is selected from the group consisting of the amino acids corresponding to N81, N85, N171, N177 and N462 of CDH26 (SEQ ID NO:11) and the amino acid substitution is effective to prevent glycosylation. For example, mutation of any one of these residues to alanine would be expected to prevent glycosylation.

[0060] In embodiments, the signal peptide of a fusion protein described herein comprises or consists of the endogenous signal peptide of CDH26, which is amino acids 1-27 of SEQ ID NO: 11. In embodiments, the signal peptide comprises or consists of the signal peptide of a molecule selected from the group consisting of interleukin-2, CD5, immunoglobulin kappa light chain, trypsinogen, serum albumin, and prolactin. These peptides are known in the art to enhance protein secretion and are described, for example, in Dalton and Barton 2014 Protein Science.

[0061] Table 1 below shows the key sequences of an exemplary CDH26-Fc fusion protein. Shown are (1) signal peptide, (2) extracellular cadherin repeat domain 1 (EC1), (3) EC2, (4) EC3, (5) EC4, (6) EC5, and (7) human IgG1-Fc. The complete annotated amino acid sequence of the exemplary fusion protein (SEQ ID NO: 8) is shown in FIG. 10 and the complete annotated nucleotide sequence (SEQ ID NO: 9) is shown in FIG. 9.

TABLE 1

Key sequences of an exemplary CDH26-Fc fusion protein		
SEQ ID NO	Designation	Amino acid sequence
1	signal	MAMRSGRHPSLLLLLVLLLWLLQVSII
2	EC1	PKLIGELFNNMSYNMSLMYLI SGPGVDEYPEI GLFSL EDHENG RIYVHRPVDREMTPSFTVYFDVVERSTGKI VDTSLIFNIRISDV NDHAP
3	EC2	QMLAVDLDEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEIR LSGCLDYETAPQFTLLIRARDCEPSSLSTTTVHVVDVQEGNN HRP
4	EC3	LLVQDRDSPPTSAWRAKFNILHGNEEGHFDISTDPETNEGILN VIKPLDYETRPAQSLII VVENEERLVFCERGKLPQRKAASAT VSVQVTDANDPPAF
5	EC4	FNAMDPDSQIRYELVHDPANWVSDKNSGVVITVEPIDRESP HVNNSFYVII IHAVDGFPPTATGTLMLFLSDINDNV

TABLE 1-continued

Key sequences of an exemplary CDH26-Fc fusion protein		
SEQ ID NO	Designation	Amino acid sequence
6	EC5	RYMEVCESAVHEPLHIEAEDPDLEPFSDPFTFELDNTWGNAE DTWKLGRNWGQSVELLTLRSLPRGNLVLPLFIGDKQGLSQK QVHVRIC
7	Fc	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVSM HEALHNHYTQKSLSLSPGK

Methods of Use

[0062] The present disclosure provides methods for the treatment of an inflammatory condition in a subject in need thereof by administering to the subject an effective amount of a CDH26-based therapeutic agent. In embodiments, the effective amount is a therapeutically effective amount. In embodiments, the effective amount is the amount effective to ameliorate one or more symptoms of the inflammatory condition. In embodiments, the effective amount is the amount effective to reduce the inflammatory response underlying the allergic reaction of the inflammatory condition, thereby treating the inflammatory condition. In embodiments, the inflammatory response is reduced in cells of a target tissue affected by an inflammatory condition. In embodiments, the tissue is skin tissue, esophageal tissue, nasal tissue, tracheal tissue, or lung tissue, such as tissue of the bronchi or bronchioles.

[0063] In embodiments, the inflammatory condition is characterized by an interleukin 13 (IL-13) mediated allergic inflammation. In embodiments, the inflammatory condition is asthma, atopic dermatitis, allergic rhinitis, eosinophilic gastritis (EG), or eosinophilic esophagitis (EoE).

[0064] The present disclosure also provides methods comprising combination therapy for the treatment of inflammatory condition. As used herein, “combination therapy” or “co-therapy” includes the administration of an effective amount of a CDH26-based therapeutic agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of the CDH26-based therapeutic agent and an additional active agent, e.g., an additional active pharmaceutical ingredient (API). The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic compounds. The beneficial effect of the combination may also relate to the mitigation of a toxicity, side effect, or adverse event associated with another agent in the combination. “Combination therapy” is not intended to encompass the administration of two or more of these therapeutic compounds as part of separate monotherapy regimens that incidentally and arbitrarily result in a beneficial effect that was not intended or predicted.

[0065] In embodiments, at least one additional active agent is administered in combination therapy with a CDH26-based therapeutic agent described herein. In embodiments, the additional active agent may be a therapeutic agent, for example an anti-inflammatory agent, or a non-therapeutic agent, and combinations thereof. With

respect to therapeutic agents, the beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutically active compounds. With respect to nontherapeutic agents, the beneficial effect of the combination may relate to the mitigation of a toxicity, side effect, or adverse event associated with a therapeutically active agent in the combination.

[0066] In embodiments, the at least one additional active agent is an anti-inflammatory agent selected from an IL-13 inhibitor, a non-steroidal anti-inflammatory drug (NSAID), a steroid, and a cytokine inhibitor.

[0067] In one embodiment, the at least one additional agent is a non-therapeutic agent which mitigates one or more side effects of the CDH26-based therapeutic agent in the composition, or which mitigates one or more side effects of the at least one additional active agent in the composition.

[0068] In the context of combination therapy, the administration of the CDH26-based therapeutic agent may be simultaneous with or sequential to the administration of the one or more additional active agents. In another embodiment, administration of the different components of a combination therapy may be at different frequencies. The one or more additional agents may be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a compound of the present disclosure.

[0069] The one or more additional active agents can be formulated for co-administration with the CDH26-based therapeutic agent in a single dosage form. The one or more additional active agents can be administered separately from the dosage form that comprises the CDH26-based therapeutic agent. When the additional active agent is administered separately from the CDH26-based therapeutic agent, it can be by the same or a different route of administration as the CDH26-based therapeutic agent.

[0070] Preferably, the administration of a composition comprising the CDH26-based therapeutic agent in combination with one or more additional active agents provides a synergistic response in the subject being treated. In this context, the term “synergistic” refers to the efficacy of the

combination being more effective than the additive effects of either single therapy alone. The synergistic effect of a combination therapy according to the disclosure can permit the use of lower dosages and/or less frequent administration of at least one agent in the combination compared to its dose and/or frequency outside of the combination. Additional beneficial effects of the combination can be manifested in the avoidance or reduction of adverse or unwanted side effects associated with the use of either therapy in the combination alone (also referred to as monotherapy).

[0071] “Combination therapy” also embraces the administration of the compounds of the present disclosure in further combination with non-drug therapies (e.g., surgery or radiation treatment). Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic compounds and non-drug treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic compounds, perhaps by days or even weeks.

[0072] In embodiments, the amount of the CDH26-based therapeutic agent administered to the subject is a therapeutically effective amount. The term “therapeutically effective amount” refers to an amount sufficient to treat, ameliorate a symptom of, reduce the severity of, or reduce the duration of the disease or disorder being treated or enhance or improve the therapeutic effect of another therapy, or sufficient to exhibit a detectable therapeutic effect in the subject.

[0073] An effective amount of the CDH26-based therapeutic agent can be administered once or twice daily, from two to five times daily, up to two times or up to three times daily, or up to eight times daily.

[0074] In accordance with the methods described herein, a “subject in need thereof” is a subject having an inflammatory disease, disorder, or condition, or a subject having an increased risk of developing an inflammatory disease, disorder, or condition relative to the population at large. The subject in need thereof can be one that is “non-responsive” or “refractory” to a currently available therapy. In this context, the terms “non-responsive” and “refractory” refer to the subject’s response to therapy as not clinically adequate to relieve one or more symptoms associated with the inflammatory disease, disorder, or condition. In one aspect of the methods described here, the subject in need thereof is a subject having an inflammatory disease, disorder, or condition that is refractory to standard therapy.

[0075] A “subject” includes a mammal. The mammal can be any mammal, for example, a human, primate, vertebrate, bird, mouse, rat, fowl, dog, cat, cow, horse, goat, camel, sheep or a pig. Preferably, the mammal is a human. The term “patient” refers to a human subject.

[0076] The present disclosure also provides a monotherapy for the treatment of inflammatory disease, disorder, or condition as described herein. As used herein, “monotherapy” refers to the administration of a single active or therapeutic compound, e.g., an CDH26-based therapeutic agent, to a subject in need thereof.

[0077] As used herein, “treatment”, “treating” or “treat” describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of an CDH26-based therapeutic

agent to alleviate the symptoms or complications of the inflammatory disease, disorder, or condition.

[0078] As used herein, “prevention”, “preventing” or “prevent” describes reducing or eliminating the onset of the symptoms or complications of the inflammatory disease, disorder, or condition and includes the administration of an CDH26-based therapeutic agent to reduce the onset, development or recurrence of symptoms of the disease, disorder, or condition.

[0079] In one embodiment, the administration of a CDH26-based therapeutic agent leads to the elimination of a symptom or complication of the inflammatory disease, disorder, or condition being treated, however elimination of the disease, disorder, or condition is not required. In one embodiment, the severity of the symptom is decreased.

Pharmaceutical Compositions and Formulations

[0080] The present disclosure provides pharmaceutical compositions comprising an amount of a CDH26-based therapeutic agent.

[0081] In one embodiment, the CDH26-based therapeutic agent is combined with at least one additional active agent in a single dosage form. In embodiments, the at least one additional active agent is selected from an anti-inflammatory agent selected from an IL-13 inhibitor, a non-steroidal anti-inflammatory drug (NSAID), a steroid, an asthma medicine such as a bronchodilator, omalizumab, mepolizumab, or reslizumab, an immunosuppressive agent such as 6-mercaptopurine, and a cytokine inhibitor, and combinations thereof.

[0082] A “pharmaceutical composition” is a formulation containing a CDH26-based therapeutic agent in a pharmaceutically acceptable form suitable for administration to a subject. The term subject is used in the same context as note above, namely to refer to a mammal, for example, a human, primate, vertebrate, bird, mouse, rat, fowl, dog, cat, cow, horse, goat, camel, sheep or a pig. Preferably, the subject is a human subject, and most preferably a human in need of treatment for an inflammatory condition. The term “patient” refers to a human subject who has been diagnosed or is otherwise under the care of a medical professional. The phrase “pharmaceutically acceptable” refers to those compounds, materials, compositions, carriers, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0083] “Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. Examples of pharmaceutically acceptable excipients include, without limitation, sterile liquids, water, buffered saline, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), oils, detergents, suspending agents, carbohydrates (e.g., glucose, lactose, sucrose or dextran), antioxidants (e.g., ascorbic acid or glutathione), chelating agents, low molecular weight proteins, or suitable mixtures thereof.

[0084] A pharmaceutical composition can be provided in bulk or in dosage unit form. It is especially advantageous to formulate pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. The

term “dosage unit form” as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved. A dosage unit form can be an ampoule, a vial, a suppository, a dragee, a tablet, a capsule, an IV bag, or a single pump on an aerosol inhaler.

[0085] In therapeutic applications, the dosages vary depending on the agent, the age, weight, and clinical condition of the recipient subject or patient, and the experience and judgment of the clinician or practitioner administering the therapy, among other factors affecting the selected dosage. Generally, the dose should be a therapeutically effective amount. Dosages can be provided in mg/kg/day units of measurement (which dose may be adjusted for the patient’s weight in kg, body surface area in m², and age in years). An effective amount of a pharmaceutical composition is that which provides an objectively identifiable improvement as noted by the clinician or other qualified observer. For example, alleviating a symptom of a disorder, disease or condition. As used herein, the term “dosage effective manner” refers to amount of a pharmaceutical composition to produce the desired biological effect in a subject or cell.

[0086] For example, the dosage unit form can comprise 1 nanogram to 2 milligrams, or 0.1 milligrams to 2 grams; or from 10 milligrams to 1 gram, or from 50 milligrams to 500 milligrams or from 1 microgram to 20 milligrams; or from 1 microgram to 10 milligrams; or from 0.1 milligrams to 2 milligrams.

[0087] The pharmaceutical compositions can take any suitable form (e.g. liquids, aerosols, solutions, inhalants, mists, sprays; or solids, powders, ointments, pastes, creams, lotions, gels, patches and the like) for administration by any desired route (e.g. pulmonary, inhalation, intranasal, oral, buccal, sublingual, parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, intrapleural, intrathecal, transdermal, transmucosal, rectal, and the like). For example, a pharmaceutical composition of the disclosure may be in the form of an aqueous solution or powder for aerosol administration by inhalation or insufflation (either through the mouth or the nose), in the form of a tablet or capsule for oral administration; in the form of a sterile aqueous solution or dispersion suitable for administration by either direct injection or by addition to sterile infusion fluids for intravenous infusion; or in the form of a lotion, cream, foam, patch, suspension, solution, or suppository for transdermal or transmucosal administration.

[0088] In embodiments, the pharmaceutical composition is in a form suitable for topical administration. For example, the composition may be formulated as a gel or cream using suitable excipients. In embodiments, the composition may be formulated as an aqueous, aqueous/alcoholic or oily solution; a dispersion of the lotion or serum type; an anhydrous or lipophilic gel; an emulsion of liquid or semi-liquid consistency, for example of the type that are obtained by dispersion of a fatty phase in an aqueous phase; or a suspension or emulsion of smooth, semi-solid or solid consistency of the cream or gel type. In embodiments, the composition may comprise one or more of a thickener, a

coloring agent, an emollient, a skin moisturizing agent, an emulsifier, a fragrance, an anti-microbial agent, a sunscreen, a pH modulator, and a preservative. A thickener may be present in amounts anywhere from about 0.1% or less to about 20% or more by weight, such as from about 0.5% to about 10% by weight of the composition. Exemplary thickeners may be cross-linked polyacrylate materials available under the trademark Carbopol. Gums may be employed such as xanthan, carrageenan, gelatin, karaya, pectin and locust beans gum. Under certain circumstances the thickening function may be accomplished by a material also serving as a silicone or emollient. For instance, silicone gums in excess of 10 centistokes and esters such as glycerol stearate have dual functionality.

[0089] A pharmaceutical composition can be in the form of an orally acceptable dosage form including, but not limited to, capsules, tablets, buccal forms, troches, lozenges, and oral liquids in the form of emulsions, aqueous suspensions, dispersions or solutions. Capsules may contain mixtures of a compound of the present disclosure with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g., corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, can also be added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the compound of the present disclosure may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

[0090] A pharmaceutical composition can be in the form of a tablet. The tablet can comprise a unit dosage of a compound of the present disclosure together with an inert diluent or carrier such as a sugar or sugar alcohol, for example lactose, sucrose, sorbitol or mannitol. The tablet can further comprise a non-sugar derived diluent such as sodium carbonate, calcium phosphate, calcium carbonate, or a cellulose or derivative thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose, and starches such as corn starch. The tablet can further comprise binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures.

[0091] The tablet can be a coated tablet. The coating can be a protective film coating (e.g. a wax or varnish) or a coating designed to control the release of the active agent, for example a delayed release (release of the active after a predetermined lag time following ingestion) or release at a particular location in the gastrointestinal tract. The latter can be achieved, for example, using enteric film coatings such as those sold under the brand name Eudragit®.

[0092] Tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents,

including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine.

[0093] A pharmaceutical composition can be in the form of a hard or soft gelatin capsule. In accordance with this formulation, the compound of the present disclosure may be in a solid, semi-solid, or liquid form.

[0094] A pharmaceutical composition can be in the form of a sterile aqueous solution or dispersion suitable for parenteral administration. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

[0095] A pharmaceutical composition can be in the form of a sterile aqueous solution or dispersion suitable for administration by either direct injection or by addition to sterile infusion fluids for intravenous infusion, and comprises a solvent or dispersion medium containing, water, ethanol, a polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, or one or more vegetable oils. Solutions or suspensions of the compound of the present disclosure as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant. Examples of suitable surfactants are given below. Dispersions can also be prepared, for example, in glycerol, liquid polyethylene glycols and mixtures of the same in oils.

[0096] The pharmaceutical compositions for use in the methods of the present disclosure can further comprise one or more additives in addition to any carrier or diluent (such as lactose or mannitol) that is present in the formulation. The one or more additives can comprise or consist of one or more surfactants. Surfactants typically have one or more long aliphatic chains such as fatty acids which enables them to insert directly into the lipid structures of cells to enhance drug penetration and absorption. An empirical parameter commonly used to characterize the relative hydrophilicity and hydrophobicity of surfactants is the hydrophilic-lipophilic balance ("HLB" value). Surfactants with lower HLB values are more hydrophobic, and have greater solubility in oils, while surfactants with higher HLB values are more hydrophilic, and have greater solubility in aqueous solutions. Thus, hydrophilic surfactants are generally considered to be those compounds having an HLB value greater than about 10, and hydrophobic surfactants are generally those having an HLB value less than about 10. However, these HLB values are merely a guide since for many surfactants, the HLB values can differ by as much as about 8 HLB units, depending upon the empirical method chosen to determine the HLB value.

[0097] Among the surfactants for use in the compositions of the disclosure are polyethylene glycol (PEG)-fatty acids and PEG-fatty acid mono and diesters, PEG glycerol esters, alcohol-oil transesterification products, polyglyceryl fatty acids, propylene glycol fatty acid esters, sterol and sterol derivatives, polyethylene glycol sorbitan fatty acid esters, polyethylene glycol alkyl ethers, sugar and its derivatives, polyethylene glycol alkyl phenols, polyoxyethylene-polyoxypropylene (POE-POP) block copolymers, sorbitan fatty acid esters, ionic surfactants, fat-soluble vitamins and their salts, water-soluble vitamins and their amphiphilic derivatives, amino acids and their salts, and organic acids and their esters and anhydrides.

[0098] The present disclosure also provides packaging and kits comprising pharmaceutical compositions for use in the methods of the present disclosure. The kit can comprise one or more containers selected from the group consisting of a bottle, a vial, an ampoule, a blister pack, and a syringe. The kit can further include one or more of instructions for use in treating and/or preventing a disease, condition or disorder of the present disclosure, one or more syringes, one or more applicators, or a sterile solution suitable for reconstituting a pharmaceutical composition of the present disclosure.

[0099] All percentages and ratios used herein, unless otherwise indicated, are by weight. Other features and advantages of the present disclosure are apparent from the different examples. The provided examples illustrate different components and methodology useful in practicing the present disclosure. The examples do not limit the claimed disclosure. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present disclosure.

Examples

[0100] The following non-limiting examples are provided to further illustrate the invention disclosed herein. It will be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches that have been found to function well in the practice of the invention and thus can be considered to constitute examples of modes for its practice. However, those of skill in the art will, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0101] Cadherins mediate diverse processes critical in inflammation including cell adhesion, migration, and differentiation. Here, we report that the previously uncharacterized cadherin 26 (CDH26) is highly expressed by epithelial cells in human allergic gastrointestinal tissue and is induced by the Th2 cytokine IL-13. We further demonstrate that CDH26 promotes calcium-dependent cellular adhesion by a mechanism involving homotypic binding and interaction with catenin family members (alpha, beta, and p120) and that CDH26 overexpression enriches epithelial transcripts involved in cell adhesion, differentiation, and the IL-13 response. CDH26 enhances cellular adhesion to recombinant integrin $\alpha 4 \beta 7$; conversely, recombinant CDH26 bound αE and $\alpha 4$ integrins in biochemical and cellular functional assays, respectively. CDH26-overexpressing cells displayed increased eotaxin-mediated eosinophil migration. In addition, CDH26-Fc inhibited activation of CD4+ T cells mediated by either suboptimal T cell receptor activation or by costimulation with suboptimal T cell receptor activation and

VCAM-1-Fc, as indicated by surface expression of CD25, CD69, and CD154 as well as secretion of IL-2 and IL-4. Taken together, we have identified a novel functional cadherin regulated during allergic responses with unique immunomodulatory properties, as it impacts epithelial gene expression, binds $\alpha 4$ and αE integrins, and regulates leukocyte migration and activation.

Modeling the 3-Dimensional Structure of the Extracellular Domain of Human CDH26

[0102] Heterotypic binding of cadherins and integrins has been reported and there are a number of proteins known to bind integrins, including CDH1 as well as ICAM-1, MAD-CAM-1, and fibronectin, for which 3D structures have been resolved and integrin binding sites have been localized. To map these sites to CDH26, we generated a 3D model of CDH26 structure using homology modeling with the CDH1 structure (PDB ID 3Q2V) as a primary template.

[0103] FIG. 11 shows the predicted 3-D structure of the extracellular domain of human CDH26. The model includes amino acids Asparagine 55 to Serine 599 (A). The N-terminus is located on the left side of the figure, and the C-terminal portion is located on the right side of the figure. The model was obtained using the Phyre2 server and was visualized using Swiss-PdbViewer. In panels B-F, each of the extracellular cadherin repeat domains (EC1 to EC 5) is individually denoted in black, with the remainder of the putative extracellular domain shown in gray.

[0104] The pairwise structure alignment of the model with other resolved structures indicates putative integrin binding sites in CDH26. We found the CDH26 putative integrin binding sites to be located in unstructured loops of EC1. For example, D42 of MADCAM-1 and E34 of ICAM-1 overlap with the acidic residues D98, E99, and E102 in the EC1 of CDH26. D1495 of fibronectin corresponds to E138 in the EC1 of CDH26. The presence of these negatively charged residues in unstructured loops on the surface suggests that they are involved in CDH26 binding to integrins. Also possibly involved is N85 in the EC1 of CDH26 which maps to E31 of CDH1. However, N85 is less likely an integrin binding site, since a negatively charged residue is required and CDH26 does not contain any Asp or Glu residues on the surface in close proximity to this location. However, this residue may be involved in the mechanism by which CDH26 binds integrins.

[0105] It is known that certain subfamilies of cadherins, including type I and type II cadherins, contain a tryptophan residue at position 2 of the most N-terminal EC domain that is critical for dimerization in trans of type I and type II cadherins. Based on comparison of the primary amino acid sequence of CDH26 to other cadherins, we identified that CDH26 contains a tryptophan residue (W56) corresponding to this position. W56 in the EC1 of CDH26 is predicted to function in the dimerization of the cadherin molecule, for example in promoting homotypic interaction of CDH26 molecules, but it is not predicted to be involved in CDH26 binding to integrins.

CDH26 is Overexpressed During Pathologic Allergic Inflammation

[0106] Following global transcript analysis of gastric tissue of control patients and patients with an allergic eosinophilic gastroenteropathy, eosinophilic gastritis (EG) (data

not shown), we identified that the most upregulated transcript (passing the criteria $P < 0.01$ and 2-fold filter) in patients with active EG was the uncharacterized cadherin family member cadherin 26 (CDH26) (12.3 fold, $P < 0.005$; data not shown). We verified by real time polymerase chain reaction (RT-PCR) analysis that the CDH26 mRNA level was highly increased in the gastric tissue of EG patients within the same cohort subjected to microarray (15.3-fold, $n = 5$ EG vs. $n = 5$ control, $P = 0.0317$; data not shown) as well as in additional EG patient gastric tissue (35.6 fold, $n = 10$ EG vs. $n = 10$ control, $P < 0.0001$; data not shown). Comparison of the genes differentially regulated in EG (data not shown) and in eosinophilic esophagitis (EoE) (Blanchard et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest.* 2006; 116(2):536-47) revealed that CDH26 was the only cadherin family member that exhibited a significant change in gene expression in both allergic disorders. Indeed, as previously observed (18), CDH26 mRNA expression was significantly increased (115 fold) in the esophageal tissue of patients with active EoE compared to control patients (data not shown). Only CDH1 (E-cadherin) and CDH26 exhibited raw signal indicative of the transcript being substantially expressed in the gastric tissue of patients with active EG (data not shown). Raw signals for cadherin transcripts in esophageal tissue from EoE patients only showed high values for CDH1, CDH3 (P-cadherin), and CDH26 (data not shown). As a control, no significant change in CDH26 or CCL26 (eotaxin-3) expression was observed in gastric tissue of patients with *H. pylori* gastritis compared to control patients, although as a control C3 transcript was elevated in the *H. pylori* cohort as previously reported. Notably, a microarray study of gastric antrum tissue of patients with *H. pylori* gastritis did not identify CDH26 as being upregulated compared to normal tissue (Ikuse T, et al. Microarray analysis of gastric mucosa among children with *Helicobacter pylori* infection. *Pediatr Int.* 2012; 54(3):319-24). As such, CDH26 is a unique cadherin in terms of its expression level and regulation in two distinct allergic states.

CDH26 Protein Expression is Increased in Inflamed Allergic Gastrointestinal Tissue and is Localized to Epithelial Cells

[0107] Immunohistochemical staining for CDH26 showed cytoplasmic localization with focal membrane accentuation in the surface epithelial cells in gastric tissue of patients with EG. The peak number of CDH26-positive cells was 241 cells/400 \times high-power field (HPF) (mean \pm SEM, 104.2 \pm 40.8) in EG patients compared with no expression above background in controls. Western blot revealed an increased level of gastric CDH26 (4.9 fold) in EG patients compared to control samples. Patients with active EoE had high levels of esophageal CDH26 protein expression compared to control patients. In control esophageal biopsies, the staining was confined to epithelial cells near the surface, but in active EoE the staining was both more intense and prevalent and included cells in the expanded basal layer. Consistent with the immunohistochemistry results, by western blot analysis, esophageal tissue of EoE patients showed 3.4-fold increased CDH26 protein levels compared to control tissue.

IL-13 is Sufficient to Promote Epithelial CDH26 Expression

[0108] IL-13 is a key Th2 cytokine that has been shown to be important in allergic and eosinophilic responses. Indeed,

IL13 transcript levels were elevated in the gastric tissue of patients with EG (mean 44 fold). Upon IL-13 stimulation, CDH26 mRNA increased in a dose-dependent manner in primary esophageal epithelial cells, the esophageal cell line TE-7, and the gastric cell line NCI-N87. As a control, TNF- α stimulation did not induce CDH26. Expression of dominant-negative STATE in TE-7 cells attenuated IL-13-mediated induction of CDH26 (2.6 fold vs. non-targeting siRNA).

CDH26 is a Functional Cadherin

[0109] CDH26 exhibits sequence homology to the cadherin family of proteins, with 4 extracellular cadherin repeats (EC) and a cadherin domain in the putative extracellular portion of the protein, a predicted transmembrane domain, and a C-terminal cytoplasmic region (FIG. 1A). To determine if CDH26 localized to the cell membrane of esophageal and gastric epithelial cells, affinity isolation of biotinylated surface proteins was performed. Western blot for CDH26 indicated that it was present at the cell surface in TE-7 and NCI-N87 cells that express high levels of CDH26 (FIGS. 1, B and C). CDH26 contains five asparagine residues in its extracellular domain located within the consensus sequence for N-glycosylation (N81, N85, N171, N177, N462). Immunoprecipitated CDH26 treated with peptide: N-glycosidase F (PNGase F), but not heat-inactivated PNGase F, exhibited increased mobility compared to CDH26 from total cell lysates (FIG. 1D), indicating that the protein is modified by N-glycosylation under baseline conditions.

[0110] CDH26 contains a tryptophan residue at position 2 of the most N-terminal EC domain known to be critical for dimerization in trans of type I and type II cadherins (Shapiro L, and Weis W I. Structure and biochemistry of cadherins and catenins. Cold Spring Harb Perspect Biol. 2009; 1(3): a003053). Therefore, we tested whether CDH26 molecules interact in a homotypic manner. HEK 293T cells were co-transfected with two separate expression constructs containing either CDH26-MYC or CDH26-HA. Myc-tagged CDH26 co-immunoprecipitated with HA-tagged CDH26, and the reciprocal immunoprecipitation confirmed that CDH26-HA co-immunoprecipitated with CDH26-MYC (FIG. 2A).

[0111] We next tested whether CDH26 interacted with beta-catenin, which binds other cadherin molecules to link them indirectly to the actin cytoskeleton. The region of CDH1 known to interact with beta-catenin exhibited 68% similarity to the same region of CDH26 (Jou T S, et al. Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. Proc Natl Acad Sci USA. 1995; 92(11):5067-71; Stappert J, and Kemler R. A short core region of E-cadherin is essential for catenin binding and is highly phosphorylated. Cell Adhes Commun. 1994; 2(4): 319-27). When immunoprecipitation for HA-tagged beta-catenin was performed, CDH26 was also detected in the precipitates (FIG. 2B), indicating that ectopically expressed beta-catenin and CDH26 exist in the same complex in HEK 293T cells.

[0112] Beta-catenin interacts with alpha-catenin to link cadherin molecules indirectly to the actin cytoskeleton and thus support cell adhesion. We tested whether alpha-catenin exists in the same complex as CDH26 and found that CDH26 co-immunoprecipitated with alpha-catenin. As a positive control, beta-catenin was also observed to co-immunoprecipitate with alpha-catenin (FIG. 2C).

[0113] p120-catenin binds the juxtamembrane domain of the cytoplasmic portion of cadherin molecules and has been shown to function in maintenance of cadherin stability and localization to the cell surface. The primary amino acid sequence of the juxtamembrane domain of CDH1 was notably homologous (48%) to that in CDH26. We therefore tested whether CDH26 and p120-catenin could exist in the same protein complex. p120-catenin and CDH26 co-immunoprecipitated from lysates derived from transiently transfected HEK 293T cells (FIG. 2D).

[0114] We tested whether CDH26 could promote calcium-dependent cellular adhesion. Transduced HEK 293T cells were dispersed with trypsin in the presence of calcium, and single cells were incubated in buffer either lacking or containing CaCl₂. Neither control nor CDH26-overexpressing HEK 293T cells aggregated in the absence of calcium. Both cell types exhibited aggregation in the presence of calcium, with CDH26-overexpressing cells exhibiting a greater degree of aggregation compared to control cells (FIG. 2E). Transduced L929 cells, which lack endogenous cadherins, were used in the same aggregation assay. CDH26-transduced L929 cells, but not control cells, showed a high degree of aggregation in the presence of calcium (FIG. 2F).

CDH26 Engagement Initiates Intracellular Signaling

[0115] Cadherin engagement has been shown to initiate intracellular signaling including activation of MAPK family members (Pece S, and Gutkind J S. Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell-cell contact formation. J Biol Chem. 2000; 275(52):41227-33; Reddy P, et al. Formation of E-cadherin-mediated cell-cell adhesion activates AKT and mitogen activated protein kinase via phosphatidylinositol 3 kinase and ligand-independent activation of epidermal growth factor receptor in ovarian cancer cells. Mol Endocrinol. 2005; 19(10):2564-78). To test if CDH26 engagement activated the ERK pathway, a CDH26-hIgG1-Fc fusion protein was generated (FIG. 3A) and used to treat L929 cells. The fusion protein was recognized by antibodies directed against human IgG and CDH26 (FIG. 3B) and contained a low level of endotoxin. L929 cells overexpressing CDH26 were treated with the fusion protein or hIgG1 antibody. Protein lysates were collected kinetically following addition of the fusion protein, and western blot analysis was performed to detect phospho- and total p44/42 ERK1/2. Increased phosphorylated ERK signal was observed compared to the equivalent dose of control antibody or that observed when control cells were treated with the fusion protein (FIG. 3C).

[0116] We sought to determine if CDH26 engagement promoted activation of intracellular signaling pathways using an independent method. L929 cells transduced with either a control or CDH26 expression construct were grown to confluency and incubated in media lacking calcium to promote disengagement of cadherins on adjacent cells. The calcium concentration of the media was then increased to 1.8 mM to promote cadherin binding in trans. Phosphorylation of p44/42 ERK1/2 was then monitored kinetically after calcium restoration. We observed that at baseline and following incubation in calcium-free media, CDH26-overexpressing L929 clones had lower phospho-ERK levels compared to control clones. In control L929 cells, little if any ERK phosphorylation (1.5-fold increased at 5 min) was

observed upon calcium restoration. In contrast, cells that expressed CDH26 showed 11.6-fold increased phosphorylation of ERK upon calcium restoration; the phospho-ERK level subsequently diminished although with delayed kinetics compared to control cells (FIG. 3D).

CDH26 Impacts Epithelial Cell Gene Expression Profile

[0117] Since CDH26 engagement may have the capacity to induce intracellular signaling, including activation of the ERK pathway, we hypothesized that activation of these pathways could impact epithelial cell gene expression relevant to inflammation. We performed microarray analysis to compare global gene expression of TE-7 cells transduced with either a control or CDH26-overexpression construct that were cultured under conditions permissive for CDH26 engagement (i.e. $[Ca^{2+}] = 1.8$ mM). A panel of 86 transcripts was identified to have altered levels: 79 transcripts exhibited increased levels, and 7 showed decreased levels in CDH26-overexpressing cells compared to control cells ($P < 0.05$ and > 2 -fold change). Notable upregulated genes included CDH26 (positive control), members of protease (MMP7), protease inhibitor (SERPINB3, SERPINB13, SERPINB7), and cadherin (PCDH18, CDH10, PCDH9, PCDH7, FAT4) families. Gene ontology analysis was performed on the set of 2223 genes passing the criteria $P < 0.05$ (moderated t test) and ranked according to fold change after removing CDH26 from the list, and the top processes identified were homophilic cell adhesion ($P = 1.04 \times 10^{-5}$), cell adhesion ($P = 1.40 \times 10^{-4}$), and cell differentiation ($P = 4.04 \times 10^{-4}$). Interestingly, overexpression of CDH26 in an esophageal epithelial cell line was sufficient to promote changes in gene expression that partially overlapped with the transcripts altered in primary esophageal epithelial cells stimulated with IL-13 (Blanchard C, et al. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. *J Allergy Clin Immunol.* 2007; 120(6): 1292-300). Of the 86 differentially expressed genes, 16 (which included CDH26 as a positive control) were altered in IL-13-stimulated primary esophageal epithelial cells. Eight of these genes (SERPINB3, SERPINB13, CDH26, CYP1B1, DYPD, LOX, EHF, AASS) were upregulated in both TE-7 cells overexpressing CDH26 and IL-13-stimulated primary esophageal epithelial cells.

Cdh26 Enhances Eosinophil Transmigration

[0118] We hypothesized that an increased amount of CDH26 expressed on the surface of cells could impact the transmigration of eosinophils through such cells. Human peripheral blood eosinophils were placed in the upper chamber of transwells coated with HEK 293T cells transduced with either a control or CDH26 expression vector (FIG. 4, left); cells transduced with the CDH26 expression vector showed highly increased CDH26 expression, a portion of which localized to the cell surface. Eosinophils migrated through control cells toward eotaxin-1 in a dose-dependent manner. Migration was increased at each dose of eotaxin-1 through cells that overexpressed CDH26 compared to control cells (FIG. 4, right).

CDH26 binds $\alpha 4$ and αE integrins

[0119] Heterotypic binding of cadherins and integrins has been reported (Cepek K L et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature.* 1994 372(6502):190-3;

Whittard J D et al. E-cadherin is a ligand for integrin alpha2beta1. *Matrix Biol.* 2002; 21(6):525-32). There are a number of proteins known to bind integrins, including CDH1 as well as ICAM-1, MAdCAM-1, and fibronectin, for which 3D structures have been resolved and integrin binding sites have been localized (Cepek K L et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature.* 1994; 372(6502):190-3). To map these sites to CDH26, we generated a 3D model of CDH26 structure using homology modeling with the CDH1 structure (PDB ID 3Q2V) as a primary template. FIG. 5A shows the pairwise structure alignment of the model (dark gray) with other resolved structures (light gray) indicating putative integrin binding sites in CDH26. As can be seen, known integrin binding sites are located in unstructured loops of the extracellular domain and are negatively charged. D42 of MAdCAM-1 and E34 of ICAM-1 overlap with D98, E99, and E102 in CDH26. D1495 of fibronectin corresponds to E138 of CDH26. The presence of these negatively charged residues in unstructured loops on the surface suggests that CDH26 would bind integrins. However, while E31 of CDH1 coincides very well with N85 of CDH26, the latter is unlikely an integrin binding site, as a negatively charged residue is required, whereas CDH26 does not contain any Asp or Glu residues on the surface in close proximity to this location.

[0120] We tested whether L929 cells expressing high levels of CDH26 exhibited adhesion to $\alpha 4\beta 7$ compared to control cells. Indeed, L929 cells transduced with a CDH26 expression vector showed increased adhesion to $\alpha 4\beta 7$ -coated wells compared to control cells, in contrast to BSA-coated wells, which did not support adhesion of either cell type (FIG. 5B). Visualization of cellular binding revealed a marked increase of CDH26-expressing cells binding $\alpha 4\beta 7$ (FIG. 5C). To further prove that CDH26 could directly bind $\alpha 4\beta 7$, a solid phase adhesion assay was performed in which recombinant CDH26-hIgG1-Fc was added to wells that were coated with recombinant $\alpha 4\beta 7$. CDH26-hIgG1-Fc bound specifically to $\alpha 4\beta 7$ and not BSA, while negative control IgG1k did not bind either $\alpha 4\beta 7$ or BSA (FIG. 5D).

[0121] To further substantiate and address the specificity and molecular requirements for CDH26/ $\alpha 4\beta 7$ interaction, overexpression and co-immunoprecipitation studies were carried out using HEK 293T cells. When HA-tagged CDH26 (CDH26-HA) and integrin $\alpha 4$ were overexpressed in HEK 293T cells, integrin $\alpha 4$ co-immunoprecipitated with CDH26-HA, and the reciprocal was observed as CDH26-HA was found to co-immunoprecipitate with integrin $\alpha 4$. In addition to integrin $\alpha 4$, V5-tagged integrin αE (ITGAE-V5) was observed to co-immunoprecipitate with CDH26-HA. Integrin $\alpha 4$ did not co-immunoprecipitate with CDH1, although integrin αE co-immunoprecipitated with CDH1.

[0122] We next tested whether $\alpha 4$ bound to the only other beta subunit to which it is known to bind, integrin $\beta 1$, could mediate interaction with CDH26 by observing whether cells that express integrin $\alpha 4\beta 1$ adhered to recombinant CDH26-hIgG1-Fc. Jurkat cells, which express integrin $\alpha 4\beta 1$ but not $\alpha 4\beta 7$, adhered to CDH26-hIgG1-Fc to a significantly greater degree than they adhered to IgG control antibody (FIG. 6A). Pre-incubation of Jurkat cells with anti-integrin $\alpha 4$ antibody (HP2/1), but not an equivalent amount of control mIgG1, blocked their binding to CDH26-hIgG1-Fc in a dose-dependent manner. Jurkat cells stimulated with antibodies that activate integrin $\beta 1$ (clone TS2/16) adhered

to CDH26-Fc in an integrin $\alpha 4$ -dependent manner to a greater degree than they adhered to E-cadherin-Fc (FIG. 6B). We observed that adhesion of TS2/16-stimulated Jurkat cells to CDH26-Fc was synergistically enhanced by the presence of a minimal amount of the known integrin $\alpha 4\beta 1$ ligand VCAM-1-Fc; this enhanced binding was integrin $\alpha 4$ -dependent (FIG. 6C). Although CDH26-Fc did not mediate adhesion of primary human peripheral blood CD4+ T cells at the dose tested, synergistic binding was detected when both CDH26-Fc and a minimal dose of VCAM-1-Fc were present (FIG. 6D). A similar pattern of adhesion was observed for human peripheral blood eosinophils (FIG. 6E), where the combination of CDH26-Fc and a minimal amount of VCAM-1-Fc that alone did not mediate adhesion promoted adhesion of these cells that express both integrin $\alpha 4\beta 1$ and integrin $\alpha 4\beta 7$.

CDH26 Modulates CD4+ T Cell Activation

[0123] Since other $\alpha 4\beta 1$ ligands including VCAM-1 have been shown to co-stimulate CD4+ T cell activation, we sought to test the hypothesis that the putative integrin $\alpha 4$ ligand CDH26 had this property. Surprisingly, CDH26-Fc inhibited co-stimulation of isolated human peripheral blood CD4+ T cells activated by suboptimal stimulation of the T cell receptor (TCR) mediated by anti-CD3 antibodies (clone OKT3). After human peripheral blood CD4+ T cells were incubated for 48 h in the presence of plate-bound OKT3, an increased percentage of the cells expressed CD25 and CD69 at the cell surface, and the mean fluorescence intensity (MFI) of both molecules was increased (FIG. 7A-B). However, the presence of plate-bound CDH26-Fc inhibited both the increase in percentage of cells expressing CD25 and CD69 as well as the increased MFI of both molecules in a dose-dependent manner (FIG. 7A-B). We additionally tested whether CDH26-Fc inhibited CD4+ T cells co-stimulated with a suboptimal dose of OKT3 and VCAM-1-Fc. Indeed, as previously reported, VCAM-1-Fc co-stimulated CD4+ T cells treated with a suboptimal dose of OKT3; this co-stimulation was dramatically inhibited by CDH26-Fc as indicated by a decreased percentage of cells expressing CD25, CD69, and CD125 at the cell surface and by a decreased MFI of these molecules (FIG. 7A-C). Production of the cytokines IL-2 and IL-4 was inhibited by CDH26-Fc in a dose-dependent manner both in cells subjected to suboptimal TCR stimulation as well as in cells costimulated with VCAM-1-Fc (FIG. 7D).

Discussion

[0124] Herein we have elucidated the properties of CDH26, identifying it as a functional cadherin with unique features in that it is the only cadherin family member significantly upregulated in human allergic gastrointestinal tissue, where it localizes to epithelial cells in the inflamed esophagus and stomach. The Th2 cytokine IL-13, which we demonstrate is increased in allergic eosinophilic gastroenteropathy, is sufficient to drive CDH26 gene expression in epithelial cells in vitro. CDH26 mediates calcium-dependent cell adhesion, dimerizes/multimerizes, and interacts with alpha-, beta-, and p120-catenins. CDH26 engagement alters intracellular signaling including activation of ERK. Furthermore, epithelial cells overexpressing CDH26 display a distinct gene expression profile compared to control cells that includes transcripts related to cell adhesion and differentia-

tion, and about 20% (^{16/86}) of the identified transcripts overlap with those known to be regulated by IL-13 in primary esophageal epithelial cells. CDH26 also has the ability to impact leukocyte migration and adhesion including eosinophil transmigration through CDH26+ cells. Moreover, we present biochemical and functional evidence that CDH26-integrin interactions impact cellular adhesion; specifically, integrins $\alpha 4$ and αE co-immunoprecipitate with CDH26, recombinant CDH26-hIgG1-Fc binds recombinant $\alpha 4\beta 7$, CDH26-expressing cells adhere to integrin $\alpha 4\beta 7$, and Jurkat cells adhere to recombinant CDH26-hIgG1-Fc in a manner dependent on integrin $\alpha 4$. Besides uncovering a novel role for this molecule, we present evidence that it can be exploited to generate a potential therapeutic as CDH26-Fc is an immunosuppressive molecule. Taken together, we have identified a novel functional cadherin regulated during allergic inflammation, determined that it binds α integrins, and has immunomodulatory properties.

[0125] The fact that CDH26 was the only cadherin transcript differentially regulated in both EoE and EG suggests that it may modulate specific biological functions in Th2-associated conditions. Coordinated or aberrant changes in cadherin expression by particular cell types have been observed to impact parameters including motility, proliferation, and differentiation state such as during epithelial-mesenchymal and mesenchymal-epithelial transition (EMT/MET), which does occur in Th2 immune responses. It is notable that CDH26 overexpression in esophageal epithelial cells drove increased expression of several genes known to be involved in promoting or to reflect epithelial cell differentiation (e.g. KRT14, KRT5, KRT6A). In contrast to the upregulation of CDH26, another cadherin superfamily member, desmoglein-1, has recently been shown to be downregulated in EoE and to be responsible in part for induction of impaired barrier function, Th2 immune responses, and altered epithelial differentiation. These observations suggest appropriate coordinated expression of cadherin superfamily members is important in multiple facets of Th2 immune responses.

[0126] We additionally observed that IL-13 is sufficient to drive an increase in CDH26 transcripts in gastric and esophageal epithelial cells. The sequence several kilobases upstream of the transcription start site of CDH26 does contain two weak STAT consensus binding sequences, while the first intron contains a STAT6 consensus binding sequence. Furthermore, CDH26 is induced by IL-13 with similar kinetics compared to CCL26 (eotaxin-3), a direct target of IL-13 signaling. IL-13-mediated induction of CDH26 in TE-7 esophageal epithelial cells is inhibited by expression of dominant-negative STAT6. These factors suggest that increased CDH26 transcription by epithelial cells is directly mediated by IL-13 signaling and that in vivo CDH26 expression may be controlled at least in part by the observed increase in IL13 levels. This is consistent with previous in vitro evidence that Th2 cytokines including IL-4 and IL-13 induce CDH26 transcript expression by normal human bronchial epithelial cells. This common regulation of epithelial expression of CDH26 by Th2 cytokines may point to a conserved function of CDH26 in allergic inflammation extending beyond the gastrointestinal tract.

[0127] We observed that engagement of CDH26 resulted in ERK activation in CDH26-expressing cells. Several possibilities exist for the mechanism by which cadherin engagement impacts intracellular signaling. Cadherins can form

ligand-independent complexes with receptor tyrosine kinases (RTKs) including the epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), and vascular-endothelial growth factor receptor 2 (VEGFR2). Alternatively, CDH26 interaction with alpha-catenin and p120-catenin may regulate MAPK pathways; alteration of the activation state or localization of these molecules upon cadherin engagement could converge on MAPK signaling. ERK activation following CDH26 engagement could have diverse consequences including, but not limited to, alteration of gene expression important for proliferation and differentiation, as well as transcription-independent activation of pathways involved in cell motility.

[0128] We found that human eosinophils showed enhanced transmigration to eotaxin-1 through cells expressing high levels of CDH26. Although several explanations for the enhanced transmigration exist, we hypothesize that CDH26 expressed by the HEK 293T cells interacts with a cell surface protein expressed by eosinophils, such as an integrin, to enhance cell migration. More specifically, interaction between CDH26 expressed by epithelial cells and $\alpha 4$ integrin expressed by eosinophils may influence eosinophil localization, retention, or activation status within the allergic esophageal and gastric mucosa. Given our observation that CDH26 interacts with not only integrin $\alpha 4$ but also integrin αE , we further speculate that CDH26, which is expressed highly under inflammatory conditions, might have a function similar to E-cadherin to regulate localization or activation of leukocytes during allergic responses via interacting with leukocyte integrins. In addition to eosinophils, epithelial CDH26 may impact the localization or activation status of diverse $\alpha 4+$ and/or $\alpha E+$ cells (e.g. CD4+ T cells, mast cells) within the epithelium in the context of allergic inflammation.

[0129] We identified several lines of evidence that CDH26 interacts with alpha integrins and facilitates cellular binding to $\alpha 4$ -containing integrins, including both integrin $\alpha 4\beta 7$ and $\alpha 4\beta 1$. Biochemical assays suggested that the extracellular portion of CDH26 could directly bind the $\alpha 4$ -containing integrin $\alpha 4\beta 7$. Furthermore, we observed that cells expressing high levels of CDH26 adhered to recombinant integrin, and in the reciprocal situation, recombinant CDH26 was sufficient to mediate adhesion of Jurkat T cells in a manner dependent on integrin $\alpha 4$. Such interactions are consistent with the structural properties of CDH26, which include the presence of solvent-exposed acidic residues that could be critical to facilitate the CDH26/integrin interaction. We speculate that in addition to mediating adhesion, interaction of epithelial-expressed CDH26 with leukocyte integrins could initiate intracellular signaling in both the epithelial cell and the leukocyte. This could impact diverse processes such as alteration of gene expression, regulation of barrier function, or production of mediators by either cell type, serving to inhibit or augment allergic responses.

[0130] In summary, we have characterized the previously unstudied CDH26 and found that it is a functional cadherin with a potential role in promoting allergic responses in part through influencing epithelial gene expression and leukocyte adhesive and transmigration responses. This function could be carried out through interaction of epithelial-expressed CDH26 with counterligands expressed by inflammatory cells such as integrins. As such, CDH26 could drive Th2 inflammation by promoting $\alpha 4+$ and $\alpha E+$ immunocyte localization within the affected tissue. CDH26 is now the

second cadherin (besides CDH1) that has been shown to bind α -integrins, extending the paradigm of cadherin/integrin interactions.

Methods

Protein Extracts and Immunoprecipitation (IP)

[0131] Cell lysates were prepared from HEK 293T cells generally as described previously. Cells (approximately 2×10^6) were washed one time with PBS and incubated in IP buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2 mM EDTA, 1 mM dithiothreitol, 1% Nonidet P-40 [NP-40]; or 10 mM imidazole, 100 mM NaCl, 1 mM $MgCl_2$, 5 mM EDTA, 1% Triton X-100, pH 7.4) containing 1 \times cComplete protease inhibitor cocktail (Roche) for 10 min on ice. Cells were scraped from the plate and rotated at 4° C. for 10 min. Lysates were cleared by centrifugation at 20,000 \times g at 4° C. for 10 min. An equal amount of protein was added to total 500 microliters of IP buffer plus protease inhibitors (Roche). Antibodies (2 micrograms α -HA (Covance), α -myc (Covance), α -p120 (BD Transduction Laboratories), α -ITGA4 (Cell Signaling Technology, Inc.), mouse IgG1 control (AbD Serotec), or normal rabbit IgG control (R & D Systems)) were added and rotated overnight at 4° C. Subsequently, 20 microliters of protein A/G agarose beads (Santa Cruz Biotechnology, Inc.) were added per sample. After 2 h of rotation (4° C.), beads were washed 5 times in IP buffer containing protease inhibitors. 2 \times Laemmli buffer was added to the immunoprecipitates or total cell lysates saved prior to IP (input).

Biopsy Protein Extracts

[0132] Distal esophagus or gastric antrum biopsy specimens were transferred into 100 microliters of IP buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2 mM EDTA, 1 mM dithiothreitol, 1% Nonidet P-40 [NP-40], 1 \times protease inhibitors (Roche)) and sonicated. Lysates were cleared by centrifugation (20,000 \times g, 4° C., 10 min). Alternatively, protein was isolated from the organic fraction remaining after RNA isolation from biopsy specimens using the miRNeasy kit (Qiagen). DNA was precipitated by the addition of 0.3 volumes of ethanol followed by a 2,000 \times g spin. Protein was precipitated from the supernatant by addition of 3 volumes of acetone, pelleted by centrifugation (20,000 \times g, 10 min, 4° C.), dried, and solubilized in Laemmli buffer (2 \times).

Design and Production of CDH26-hIgG1-Fc Fusion Protein

[0133] To produce a chimeric CDH26-Fc protein composed of the extracellular domain of human CDH26 in frame with an Fc portion of a human IgG1, we first identified amino acids 1-613 as the putative extracellular domain of CDH26 using domain prediction based on analysis of the primary amino acid sequence. Next, we aligned the CDH26 primary amino acid sequence with that of human CDH1 using Clustal Omega to identify the CDH26 residues corresponding to those of CDH1 that had been included in the CDH1-Fc reported by Higgins et al. 1998 in the Journal of Cell Biology. The corresponding CDH26 residues were identified as amino acids 603-605. Three additional amino acids located immediately C-terminal of amino acids 603-605 were also included, so that the final portion of human CDH26 included in CDH26-Fc was amino acids 1-608.

[0134] For the Fc portion of the fusion protein, we used the human IgG1-Fc sequence from the expression plasmid pFUSE-hIgG1-Fc1 (Invitrogen).

[0135] The cloning strategy included a two-step PCR and traditional molecular cloning techniques to insert the nucleotides encoding human CDH26 amino acids 1-608 in frame with the nucleotides encoding the human IgG1-Fc region of pFUSE-hIgG1-Fc. The cloning strategy also involved placement of a Kozak sequence located immediately 5' prime of the sequence encoding the start codon of the fusion protein. The resulting plasmid is denoted pFUSE-CDH26-hIgG1-Fc. Because a stable cell line producing high levels of CDH26-Fc was desired, the sequence from the pFUSE-CDH26-hIgG1-Fc plasmid including the Kozak sequence and open reading frame of CDH26-Fc was transferred into the pMIRNA1-puro lentiviral vector to make a construct denoted pMIRNA1-puro-hCDH26-hIgG1-Fc. HEK 293T cells were transduced with lentivirus produced from pMIRNA1-puro-hCDH26-hIgG1-Fc and were placed under puromycin selection. Puromycin-resistant cells were dispersed, and limiting dilutions of the cells were plated. Single clones were isolated and assessed for their level of protein production. Clones that produced high levels of CDH26-Fc were expanded, and supernatant from the cells was subjected to protein G affinity chromatography to isolate CDH26-Fc.

[0136] HEK 293T cells (20x150 mm dishes) stably transduced with pMIRNA1-CDH26-hIgG1-Fc (described in Constructs) were grown in DMEM containing 10% ultra-low IgG FBS (Invitrogen). After 8 days, the supernatants from all dishes were combined. The supernatants were filtered (0.2 micron) and the resulting volume was diluted 1:1 in binding buffer (20 mM HEPES, 50 mM NaCl, 1 mM CaCl₂, pH 7.4) and then passed over a protein G column (GE Healthcare) that had been pre-washed with binding buffer. The column was then washed with binding buffer, and fusion protein was eluted in fractions using 100 mM glycine, pH 3.0. The eluates were immediately neutralized by the addition of an equal amount of 1 M Tris HCl, pH 8.0. Fractions were assayed by SDS-PAGE and Coomassie stain as well as western blot analysis to identify the fractions that contained the fusion protein. The pooled five fractions containing the most fusion protein were desalted using G20 columns (GE Healthcare) and binding buffer. Control hIgG1x antibody (low endotoxin, azide-free preparation) (Southern Biotech) was also desalted in a similar manner. Quantitation of LPS in the isolated proteins using the Limulus amoebocyte lysate (LAL) chromogenic endotoxin quantitation kit (Thermo Scientific) showed that the CDH26-Fc preparations had endotoxin concentrations of less than 0.2 endotoxin units (EU)/ml, which was lower than that detected in the control hIgG1x antibody.

Eosinophil Transmigration Assay

[0137] HEK 293T cells transduced with either pMIRNA1-control or -CDH26 were plated on Costar transwell inserts (polycarbonate, 6.5 mm diameter, 0.3 micron pore size) (Corning Inc.). Eosinophils (1.3×10^5 at a density of 1×10^6 cells/ml suspended in 1xHBSS plus 1 mM CaCl₂ plus 2% FBS) were applied to the top of the transwell, while the bottom chamber of the transwell contained 1xHBSS with 1 mM CaCl₂ and 2% FBS and the indicated concentration of chemoattractant (human eotaxin-1, Peprotech). Reactions were incubated at 37° C. for 1.5 h. Transwells were then subjected to Wright-Giemsa staining per the manufacturer's

protocol (Harleco, E M D Millipore) to confirm the confluency of the cells. The number of eosinophils present in the lower chambers was then assessed by hemocytometry.

Cell Adhesion to Recombinant Integrin Assay

[0138] Recombinant human $\alpha 4\beta 7$ (R&D Systems) was diluted to the indicated concentration in buffer (150 mM NaCl, 20 mM HEPES), and 100 microliters per well was added to half-well Costar ELISA plates (Corning) and incubated overnight at 4° C. The following day, wells were washed and coated with 5% BSA in buffer (150 mM NaCl, 20 mM HEPES) (3 h, 37° C.). L929 cell clones transduced with the indicated construct and grown to confluency were then dispersed and resuspended in assay buffer (150 mM NaCl, 20 mM HEPES, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM MnCl₂). For each cell type, 50,000 cells were added per well. The plate was spun at 10xg for 1 min and subsequently incubated at 37° C. for 1 h. Wells were washed by gravity one time by inverting the plate in a large beaker of assay buffer for 10 min. Two additional washes were performed by pipetting 100 microliters of assay buffer into the wells 4 times per wash. Fluorescence was measured prior to the washes and after each wash using a plate reader (ex/em 485/20 nm/528/20 nm) (BioTek). The results are expressed as the percent fluorescence remaining (fluorescence after the last wash/initial fluorescence) for each well.

Statistical Analysis

[0139] Data are expressed as mean \pm SEM or median \pm interquartile range. Statistical significance was determined using the unpaired t test (2 groups, normal distribution, equal variance), Mann-Whitney test (2 groups, nonparametric), 1-way ANOVA followed by the Tukey post-test (>2 groups), the Kruskal-Wallis test followed by Dunn's multiple comparison test (>2 groups, nonparametric), or 2-way ANOVA (calcium switch kinetic experiment) with Prism 5.0 software (GraphPad Software, Inc.). For microarray analysis, gene transcript levels were determined and statistical analysis was performed using algorithms in GeneSpring GX 7.3 software or GeneSpring 12.5 software (Agilent). More specifically, moderated t test, a modification of the unpaired t test that pools variation data for many genes instead of using single-gene estimated variances, was performed using GeneSpring 12.5. Gene ontology analysis was performed by submitting ranked gene lists to GOrilla, which uses an exact mHG p-value computation. A P-value threshold of 10⁻³ was set to identify significantly enriched terms, and multiple test correction was then applied (Benjamini and Hochberg method).

Expression Constructs

[0140] pCDNA3.1(-) was obtained from Promega. pLX304-hITGAE-V5 (ID HsCD00436970) was obtained from DNASU Plasmid Repository (The Biodesign Institute, Arizona State University). Expression plasmids were constructed by PCR amplification of the relevant open reading frame. PCR products were then ligated into the following restriction sites of pCDNA3.1(-): pCDH26-HA: EcoRI/KpnI, pCDH26-MYC: EcoRI/KpnI, pCDH26: EcoRI/NotI, pHA-CTNNB1: XbaI/KpnI, pCTNNB1-HA: XbaI/KpnI, pCTNNA1-HA: EcoRI/KpnI, pCTNND1: EcoRI/KpnI, pITGA4: XhoI/NotI, pITGB7, NotI/EcoRI, pCDH1: NotI/

HindIII. pMIRNA1-puro-control has been described previously and is referred to as "pMIRNA1-control" henceforth. [0141] pMIRNA1-CDH26 was constructed by introducing the CDH26 open reading frame into the EcoRI and NotI sites of pMIRNA1-control. pFUSE-CDH26-hlgG1-Fc was constructed by first amplifying nucleotides encoding the extracellular domain of CDH26 using primers 5067 and 5108 with pCDH26 as template. These primers introduced a region of nucleotide sequence complementary to the 5' portion of hlgG1-Fc at the 3' end of the PCR product. This PCR product was isolated and used as a primer along with primer 5109 in a second PCR reaction using pFUSE-hlgG1-Fc1 plasmid (Invitrogen) as template. The resulting PCR product was then digested with EcoRI and BsrGI restriction enzymes and ligated into the corresponding sites of pFUSE-hlgG1-Fc1 plasmid. This construct and pCDH26 were then digested with EcoRI and Sall, and the 1329 base pair restriction fragment from pCDH26 was ligated into the corresponding restriction sites of the pFUSE-based EcoRI/Sall-digested construct. Primers 5254 and JC650 were then used to amplify the CDH26-Fc open reading frame, and it was introduced into the NotI restriction site of pMIRNA1-control to make pMIRNA1-CDH26-hlgG1-Fc.

Lentivirus Production and Transduction of HEK 293T, TE-7, and L929 Cells

[0142] For pMIRNA1 constructs, lentivirus production was carried out by the CCHMC Viral Vector Core. HEK 293T cells were transduced by incubating lentivirus with the cells for 24 h in the presence of 5 micrograms per milliliter polybrene. Media were then changed, and after 24 h medium containing 2 micrograms per milliliter puromycin was added. After selection for 48 h in puromycin, cells were dispersed and plated to single cells in 96 well plates to obtain clones derived from single cells. A second round of dispersing, plating to single cells, and picking single colonies was done. CDH26 expression was verified by western blot analysis and FACS analysis. TE-7 and L929 cells were transduced in the same manner, except only one round of selection for clones was performed, and CDH26 expression was verified by western blot analysis.

Aggregation Assay

[0143] Calcium-dependent aggregation assays were carried out as described previously, with minor modifications. HEK 293T or L929 cells were treated with DMEM containing 0.1% trypsin at a final concentration of 5 mM CaCl₂ (30 min, 37° C.). Cells were washed once with DMEM containing 10% FBS and then twice with 1×HBSS containing 1% FBS. Cells were counted, and 2×10⁶ cells were aliquoted into 1.5-ml tubes (two tubes per cell type). Cells were spun down and resuspended in HEPES-buffered magnesium-free saline (HMF; 10 mM HEPES in saline) that either contained or lacked 1 mM CaCl₂. The initial particle number was counted, and then tubes were rotated at 37° C. for 30 min. The final particle number was then counted. The aggregation index was expressed as [(initial particle number-final particle number)/initial particle number].

Solid Phase Adhesion Assay

[0144] Recombinant human integrin α4β7 (R&D Systems) was diluted to the indicated concentration in buffer (150 mM NaCl, 20 mM HEPES); 100 microliters per well

was added to Costar half-well ELISA plates (Corning Inc.) and incubated overnight at 4° C. The following day, wells were washed and blocked with 5% BSA in buffer (150 mM NaCl, 20 mM HEPES) overnight at 4° C. The following day, the wells were washed, and IgG1× or CDH26-hlgG1-Fc diluted in assay buffer (150 mM NaCl, 20 mM HEPES, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM MnCl₂) containing 5% BSA was added to wells for 60 min at 37° C. Wells were washed 3×, and detection antibody (biotinylated anti-human IgG1 (Vector Laboratories, Inc.); 0.5 micrograms per milliliter in assay buffer with 5% BSA) was added for 2 h at room temperature. Wells were washed 3× with assay buffer with 5% BSA and streptavidin-HRP was added (1:200 in assay buffer with 5% BSA; R & D Systems). Wells were washed 3× with assay buffer with 5% BSA, and a 1:1 dilution of TMB substrate (BD Biosciences) was added. The reaction was stopped by the addition of 2N H₂SO₄. Absorbance at 450 nm and 900 nm was measured using a plate reader (BioTek).

Jurkat, CD4+ T Cell, and Eosinophil Adhesion Assay

[0145] CDH26-hlgG1-Fc, CDH1-Fc (R&D Systems), VCAM-1-Fc (R&D Systems), and/or hlgG1 (Southern Biotech) was diluted in buffer (150 mM NaCl, 20 mM HEPES) to appropriate concentrations so that the indicated amount of protein was added to Costar half-well ELISA plates in 50 microliters per well (Corning, Inc.) and incubated overnight at 4° C. The next day, Jurkat cells or isolated CD4+ T cells (1×10⁶/ml) were incubated in HEPES medium (132 mM NaCl, 6 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 1.2 mM KH₂PO₄, 20 mM HEPES, 5.5 mM glucose, 0.5% BSA) plus 4 micrograms per milliliter calcein-AM (Sigma-Aldrich) for 1 h at 37° C. Cells were washed 3× in HEPES medium. In the indicated cases, cells were incubated with 1.4 micrograms of either anti-integrin β1-activating antibodies (clone TS2/16, Santa Cruz Biotechnology) or an equivalent amount of mlgG1 (Southern Biotech). In the indicated cases, labeled cells were then pre-incubated with the indicated concentration (0.13 micrograms per milliliter to 0.026 micrograms per milliliter) of either mlgG1 (AbD Serotec) or anti-integrin alpha 4 (HP2/1; AbD Serotec) antibodies in assay buffer for non-TS2/16-treated cells (150 mM NaCl, 10 mM HEPES, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM MnCl₂) or HEPES medium for TS2/16-treated cells for 15 min at 4° C. prior to their addition to wells (50,000 cells/well). Plates were spun at 10×g for 1 min and then incubated at 37° C. for 45 min. Initial fluorescence per well was then measured using a plate reader ((ex/em 360/40 nm/460/40 nm); BioTek). Wells were washed by gravity one time by inverting the plate in a large beaker of assay buffer for 10 min. Two additional washes were performed by pipetting 100 microliters of assay buffer into the wells 4 times per wash. Final fluorescence was then measured. For eosinophil adhesion assays, isolated human peripheral blood eosinophils were pre-activated with 1.4 micrograms of either TS2/16 or mlgG1 control antibodies. The cells were then incubated with anti-CD32 antibodies (Stem Cell Technologies, Inc.). Eosinophils were then allowed to adhere to plates that had been coated with the indicated amount of protein(s) as described above for Jurkat and CD4+ T cell adhesion assays. Three washes were performed, and residual eosinophil peroxidase activity for each well was measured as a surrogate for the number of eosinophils that remained adhered to the wells after the washes.

T Cell Activation Assay

[0146] The indicated amounts of anti-CD3 (clone OKT3, eBioscience) antibodies, CDH26-hIgG1-Fc, CDH1-Fc (R&D Systems), VCAM-1-Fc (R&D Systems), and/or hIgG1× control antibody (Southern Biotech) were suspended in coating buffer (20 mM HEPES, 150 mM NaCl), added to wells of a 96 well cell culture plate, and incubated

overnight at 4° C. The following day, isolated human peripheral blood CD4+ T cells (150,000 per well) were added to the wells in RPMI supplemented with 10% FBS, 1% penicillin/streptomycin, and 200 mM glutamine. Cells were incubated for 48 h. Cells and supernatants were then collected for analysis by flow cytometry and ELISA, respectively.

SEQUENCE LISTING

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<223> OTHER INFORMATION: EC1

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Gly Leu Phe Ser Leu Glu Asp His Glu Asn Gly Arg Ile Tyr Val His
35 40 45

Arg Pro Val Asp Arg Glu Met Thr Pro Ser Phe Thr Val Tyr Phe Asp
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Phe Arg Val Asp Arg Leu Ser Gly Glu Ile Arg Leu Ser Gly Cys Leu
35 40 45

Asp Tyr Glu Thr Ala Pro Gln Phe Thr Leu Leu Ile Arg Ala Arg Asp

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          20          25          30
Glu Leu Asp Asn Thr Trp Gly Asn Ala Glu Asp Thr Trp Lys Leu Gly
          35          40          45
Arg Asn Trp Gly Gln Ser Val Glu Leu Leu Thr Leu Arg Ser Leu Pro
50          55          60
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Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
          35          40          45
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
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His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65          70          75          80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
          85          90          95
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
          100          105          110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
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Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
130          135          140
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145          150          155          160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
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Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
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Glu Thr Asp Asp Leu Thr Lys Gln Thr Lys Glu Lys Ile Tyr Gln Pro
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Leu Arg Arg Ser Lys Arg Arg Trp Val Ile Thr Thr Leu Glu Leu Glu
 50          55          60

Glu Glu Asp Pro Gly Pro Phe Pro Lys Leu Ile Gly Glu Leu Phe Asn
 65          70          75          80

Asn Met Ser Tyr Asn Met Ser Leu Met Tyr Leu Ile Ser Gly Pro Gly
 85          90          95

Val Asp Glu Tyr Pro Glu Ile Gly Leu Phe Ser Leu Glu Asp His Glu
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Val Asp Thr Ser Leu Ile Phe Asn Ile Arg Ile Ser Asp Val Asn Asp
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His Ala Pro Gln Phe Pro Glu Lys Glu Phe Asn Ile Thr Val Gln Glu
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Asp Glu Glu Asn Thr Pro Asn Ser Gln Val Leu Tyr Phe Leu Ile Ser
195          200          205

Gln Thr Pro Leu Leu Lys Glu Ser Gly Phe Arg Val Asp Arg Leu Ser
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Gly Glu Ile Arg Leu Ser Gly Cys Leu Asp Tyr Glu Thr Ala Pro Gln
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Phe Thr Leu Leu Ile Arg Ala Arg Asp Cys Gly Glu Pro Ser Leu Ser
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Pro Ala Phe Thr Gln Glu Asn Tyr Lys Val Gln Ile Pro Glu Gly Arg
275          280          285

Ala Ser Gln Gly Val Leu Arg Leu Leu Val Gln Asp Arg Asp Ser Pro
290          295          300

Phe Thr Ser Ala Trp Arg Ala Lys Phe Asn Ile Leu His Gly Asn Glu
305          310          315          320

Glu Gly His Phe Asp Ile Ser Thr Asp Pro Glu Thr Asn Glu Gly Ile
325          330          335

Leu Asn Val Ile Lys Pro Leu Asp Tyr Glu Thr Arg Pro Ala Gln Ser
340          345          350

Leu Ile Ile Val Val Glu Asn Glu Glu Arg Leu Val Phe Cys Glu Arg
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Gly Lys Leu Gln Pro Pro Arg Lys Ala Ala Ala Ser Ala Thr Val Ser
370          375          380

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Thr	Phe	Asn	Ala	Met	Asp	Pro	Asp	Ser	Gln	Ile	Arg	Tyr	Glu	Leu	Val
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His	Asp	Pro	Ala	Asn	Trp	Val	Ser	Val	Asp	Lys	Asn	Ser	Gly	Val	Val
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Leu	Leu	Thr	Leu	Arg	Ser	Leu	Pro	Arg	Gly	Asn	Tyr	Leu	Val	Pro	Leu
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Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
		660						665					670		
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
		675					680						685		
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
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Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
705					710					715					720
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
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Tyr	Thr	Leu	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	
		740						745					750		
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
		755				760						765			
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
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<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: segment 1 comprises or consists of the CDH26
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to serine 599 (Ser599)

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20           25           30
Ser Leu Met Tyr Leu Ile Ser Gly Pro Gly Val Asp Glu Tyr Pro Glu
35           40           45
Ile Gly Leu Phe Ser Leu Glu Asp His Glu Asn Gly Arg Ile Tyr Val
50           55           60
His Arg Pro Val Asp Arg Glu Met Thr Pro Ser Phe Thr Val Tyr Phe
65           70           75           80
Asp Val Val Glu Arg Ser Thr Gly Lys Ile Val Asp Thr Ser Leu Ile
85           90           95
Phe Asn Ile Arg Ile Ser Asp Val Asn Asp His Ala Pro Gln Phe Pro
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Glu Lys Glu Phe Asn Ile Thr Val Gln Glu Asn Gln Ser Ala Gly Gln
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 210 215 220
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 225 230 235 240
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Ala	Glu	Asp	Thr	Trp	Lys	Leu	Gly	Arg	Asn	Trp	Gly	Gln	Ser	Val	Glu	545	550	555	560
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Phe	Ile	Gly	Asp	Lys	Gln	Gly	Leu	Ser	Gln	Lys	Gln	Thr	Val	His	Val	580	585	590	
Arg	Ile	Cys	Pro	Cys	Ala	Ser	Gly	Leu	Thr	Cys	Val	Glu	Leu	Ala	Asp	595	600	605	
Ala	Glu	Val	Gly	Leu	His	Val	Gly	Ala	Leu	Phe	Pro	Val	Cys	Ala	Ala	610	615	620	
Phe	Val	Ala	Leu	Ala	Val	Ala	Leu	Leu	Phe	Leu	Leu	Arg	Cys	Tyr	Phe	625	630	635	640
Val	Leu	Glu	Pro	Lys	Arg	His	Gly	Cys	Ser	Val	Ser	Asn	Asp	Glu	Gly	645	650	655	
His	Gln	Thr	Leu	Val	Met	Tyr	Asn	Ala	Glu	Ser	Lys	Gly	Thr	Ser	Ala	660	665	670	
Gln	Thr	Trp	Ser	Asp	Val	Glu	Gly	Gln	Arg	Pro	Ala	Leu	Leu	Ile	Cys	675	680	685	
Thr	Ala	Ala	Ala	Gly	Pro	Thr	Gln	Gly	Val	Lys	Asp	Leu	Glu	Glu	Val	690	695	700	
Pro	Pro	Ser	Ala	Ala	Ser	Gln	Ser	Ala	Gln	Ala	Arg	Cys	Ala	Leu	Gly	705	710	715	720
Ser	Trp	Gly	Tyr	Gly	Lys	Pro	Phe	Glu	Pro	Arg	Ser	Val	Lys	Asn	Ile	725	730	735	
His	Ser	Thr	Pro	Ala	Tyr	Pro	Asp	Ala	Thr	Met	His	Arg	Gln	Leu	Leu	740	745	750	
Ala	Pro	Val	Glu	Gly	Arg	Met	Ala	Glu	Thr	Leu	Asn	Gln	Lys	Leu	His	755	760	765	
Val	Ala	Asn	Val	Leu	Glu	Asp	Asp	Pro	Gly	Tyr	Leu	Pro	His	Val	Tyr	770	775	780	
Ser	Glu	Glu	Gly	Glu	Cys	Gly	Gly	Ala	Pro	Ser	Leu	Ser	Ser	Leu	Ala	785	790	795	800

-continued

Ser	Leu	Glu	Gln	Glu	Leu	Gln	Pro	Asp	Leu	Leu	Asp	Ser	Leu	Gly	Ser
				805					810					815	
Lys	Ala	Thr	Pro	Phe	Glu	Glu	Ile	Tyr	Ser	Glu	Ser	Gly	Val	Pro	Ser
			820					825					830		

1. A fusion protein comprising two polypeptide segments, 1 and 2, segment 1 comprising or consisting of an extracellular region of the human cadherin 26 (CDH26) protein defined by EC1 (SEQ ID NO: 2), or fragment thereof, said fragment consisting of at least 3 contiguous amino acids of SEQ ID NO:2, and segment 2 comprising or consisting of a heavy chain constant region (Fc) of a human immunoglobulin (Ig) molecule.

2. The fusion protein of claim 1, wherein segment 1 comprises one or more additional extracellular cadherin repeat domains (EC) of human CDH26 selected from the group consisting of EC2, EC3, EC4, and EC5.

3. The fusion protein of claim 2, wherein segment 1 comprises or consists of SEQ ID NO: 10.

4. The fusion protein of claim 2, wherein the fragment of EC1 consists of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 2.

5. The fusion protein of claim 2, wherein segment 1 further comprises EC2 (SEQ ID NO: 3), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 3.

6. The fusion protein of claim 2, wherein segment 1 further comprises EC3 (SEQ ID NO: 4), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 4.

7. The fusion protein of claim 2, wherein segment 1 comprises or consists of EC4 (SEQ ID NO: 5), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 5.

8. The fusion protein of claim 2, wherein segment 1 comprises or consists of EC5 (SEQ ID NO: 6), or a contiguous amino acid fragment thereof, said fragment consisting of from 20 to 90 or from 20 to 50 contiguous amino acids of SEQ ID NO: 6.

9. The fusion protein of claim 1, wherein the Ig molecule is selected from the group consisting of IgG, IgE, IgM, IgD, IgA and IgY.

10. The fusion protein of claim 9, wherein the Ig molecule is an IgG or IgA molecule.

11. The fusion protein of claim 10, wherein the IgG or IgA molecule is selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2.

12. The fusion protein of claim 11, wherein the Ig molecule is selected from the group consisting of IgG1, IgG4, IgA1 and IgA2.

13. The fusion protein of claim 12, wherein the Ig molecule is IgG1.

14. The fusion protein of claim 13, wherein segment 2 comprises or consists of SEQ ID NO: 7.

15. The fusion protein of claim 1, further comprising a signal sequence.

16. The fusion protein of claim 15, wherein the signal sequence is selected from a signal peptide of any one of interleukin-2, CD5, immunoglobulin kappa light chain, trypsinogen, serum albumin, and prolactin, and functional fragments thereof.

17. The fusion protein of claim 1, further comprising a linker sequence between segments 1 and 2.

18. The fusion protein of claim 17, wherein the linker sequence comprises or consists of from 2 to 10, or from 10 to 20, or from 20 to 50 amino acids.

19. The fusion protein of claim 1, wherein the fusion protein comprises or consists of SEQ ID NO: 8.

20. A composition or pharmaceutical composition comprising the fusion protein of claim 1.

21. The composition or pharmaceutical composition of claim 20, further comprising a pharmaceutically acceptable carrier or excipient.

22. The composition or pharmaceutical composition of claim 21, which is formulated for topical administration.

23. The composition or pharmaceutical composition of claim 21, further comprising at least one additional active agent selected from an IL-13 inhibitor, a non-steroidal anti-inflammatory drug (NSAID), a cytokine inhibitor, an asthma medicine such as a bronchodilator, omalizumab, mepolizumab, or reslizumab, an immunosuppressive agent such as 6-mercaptopurine, or a steroid.

24. A method for treating an inflammatory disease, disorder, or condition in a subject in need thereof, the method comprising administering to the subject an amount of the pharmaceutical composition of claim 21.

25. A modified fusion protein according to claim 1, wherein the amino acid sequence of the modified fusion protein is from 90 to 99% identical to the unmodified sequence.

26. A modified fusion protein according to claim 1, wherein the sequence of segment 1 is modified to alter one or more of integrin binding, glycosylation, or homodimer formation.

27. A modified fusion protein according to claim 1, wherein the sequence of segment 2 is modified to improve the aqueous solubility, stability, avidity, and/or pharmacokinetics of the fusion protein.

28. The modified fusion protein of claim 27, wherein the sequence of segment 2 is modified to reduce complement binding or antibody dependent cytotoxicity.

29. The modified fusion protein of claim 26, wherein the sequence is modified by one or more amino acid insertions, substitutions or deletions.

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