Abstract: Methods and compositions disclosed herein generally relate to methods of providing or enhancing a diagnosis of eosinophilic esophagitis (EoE), by identifying, validating, and measuring clinically relevant, quantifiable biomarkers of diagnostic and therapeutic responses for gastrointestinal tract dysfunction, particularly as those responses relate to EoE. In particular, the invention relates to identifying one or more biomarkers associated with EoE, obtaining a sample from a patient having at least one indication of EoE, then quantifying from the sample an amount of one or more of said biomarkers, wherein the level of said biomarker correlates with a predicted outcome. The invention further relates to diagnostic kits, tests, and/or arrays that can be used to quantify the one or more biomarkers associated with EoE.
NON-INVASIVE BIOMARKERS FOR EOSINOPHILIC ESOPHAGITIS

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

[0001] This invention was made with U.S. Government support under Grant Nos. AI080581 and DK067255 awarded by the National Institute of Health (NIH). The U.S. Government has certain rights in this invention.

CROSS REFERENCE TO RELATED APPLICATION

[0002] The present application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 61/621,781, NON-INVASIVE BIOMARKERS FOR EOSINOPHILIC ESOPHAGITIS, filed on April 9, 2012, which is currently co-pending herewith and which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The invention disclosed herein generally relates to diagnosis, treatment, and/or management of eosinophilic esophagitis and/or diseases, disorders, and/or conditions arising therefrom and/or related thereto.

BACKGROUND

[0004] All publications mentioned herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that can be useful in understanding the present subject matter. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed subject matter, or that any publication specifically or implicitly referenced is prior art.

[0005] Eosinophilic esophagitis (EoE, also abbreviated EE in some publications) is a painful and sometimes devastating inflammatory disease of the esophagus. EoE symptoms mimic gastroesophageal reflux disease (GERD) and include, but are not limited to, swallowing problems, vomiting, food refusal, food intolerance in infants, abdominal pain, dysphagia, and food impactions in adolescents and adults (see, e.g., Orenstein, S. et al. Am. J. Gastroenterol. 95:1422-30 (2000); Walsh, S. et al. Am. J. Surg. Pathol. 23:390-6 (1999); Liacouras, C. and Ruchelli, E. Curr. Opin. Pediatr. 16:560-6 (2004); SantAnna, A. et al. J. Pediatr. Gastroenterol. Nutr. 39:373-7 (2004)). Pediatric and adult EoE patients can develop


SUMMARY

[0007] Embodiments of the invention encompass methods of treating a patient with eosinophilic esophagitis (EoE), including: obtaining a sample from a patient, analyzing the sample to determine a level of one or more biomarkers associated with EoE, determining whether the level of the one or more biomarkers associated with EoE is up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE can result in the patient being diagnosed with EoE, and treating the patient with an appropriate therapeutic strategy based upon the diagnosis.

[0008] In some embodiments of the methods, the one or more biomarkers associated with EoE can include, for example, one or more cytokines, IL-15-responsive iNKT, T, and B cell receptors, chemokines, mediators, and IgE receptors, or the like. In
some embodiments, the one or more cytokines can include, for example, IL-5, IL-13, IL-15, INFy, and/or TGF-β, or the like. In some embodiments, the one or more IL-15 responsive iNKT, T, and B cell receptors can include, for example, IL-15Ra, γδ, αβ, CDId, Va24, γβ1, and/or Ja8, or the like. In some embodiments, the one or more chemokines can include, for example, CXCR6 and/or CXCL16, or the like. In some embodiments, the one or more mediators can include, for example, IgE, or the like. In some embodiments, the one or more IgE receptors can include, for example, FCeRI and/or FCeRII, or the like.

[0009] In some embodiments, the one or more biomarkers associated with EoE can include, for example, IL-5, IL-13, IL-15, INFy, TGF-β, IL-15Ra, γδ, αβ, CDId, Va24, γβ1, Ja8, CXCR6, CXCL16, IgE, FCeRI, and FCeRII, or the like. In some embodiments, the one or more biomarkers associated with EoE can include, for example, IL-15 and/or CXCL16, or the like. In some embodiments, the one or more biomarkers associated with EoE can include, for example, IL-15, or the like.

[0010] In some embodiments, the presence of an elevated level of, for example, γδ, αβ, Va24, CXCR6, FCeRI, and/or FCeRII, or the like, can result in the patient being diagnosed with EoE. In some embodiments, the presence of a reduced level of, for example, γβ1 and/or Ja8, or the like, can result in the patient being diagnosed with EoE.

[0011] In some embodiments, the mRNA level of the one or more biomarkers associated with EoE is determined. In some embodiments, the protein level of the one or more biomarkers associated with EoE is determined.

[0012] In some embodiments, the determination of whether the level(s) of the one or more biomarkers associated with EoE are elevated or reduced relative to a level of the one or more biomarkers measured in a normal individual can be combined with a determination of a level(s) of one or more additional biomarkers associated with EoE. In some embodiments, the one or more additional biomarkers associated with EoE can include an mRNA biomarker. In some embodiments, the one or more additional biomarkers associated with EoE can include eotaxin-3.

[0013] In some embodiments, the sample can be an esophageal tissue sample. In some embodiments, the sample can be a plasma or serum sample.

[0014] In some embodiments, the appropriate therapeutic strategy for a patient diagnosed with EoE can include, for example, allergen removal, steroid treatment, dietary management, proton pump inhibitor (PPI) therapy, administration of one or more topical glucocorticoid, administration of one or more humanized antibody against one or more
relevant cytokines and/or mediators, administration of one or more small molecule inhibitors of an eosinophil and/or allergic disease activation pathway, administration of one or more small molecule inhibitors capable of modulating levels of one or more biomarkers associated with EoE, and/or any combination thereof, or the like. In some embodiments, the topical glucocorticoid can include, for example, fluticasone, budesonide, and/or ciclesonide, or the like. In some embodiments, the humanized antibody against one or more relevant cytokines and/or mediators can include, for example, an antibody targeting one or more biomarkers associated with EoE, or the like. In some embodiments, the one or more biomarkers associated with EoE can include, for example, IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CD1d, Va24, νβ1, Jal8, CXCR6, CXCL16, IgE, FcεRI, and/or FcεRII, or the like. In some embodiments, the humanized antibody against one or more relevant cytokines and/or mediators can be, for example, anti-IL-15, anti-IgE, anti-CD1d, anti-Va24Jal8, anti-CXCL16, and anti-IL-15Ra, or the like.

[0015] Embodiments of the invention also encompass methods of treating a patient with eosinophilic esophagitis (EoE), including: obtaining a sample from a patient, analyzing the sample to determine a level of one or more biomarkers associated with EoE, determining whether the level of the one or more biomarkers associated with EoE is up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE results in the patient being diagnosed with EoE, and treating the patient with an appropriate therapeutic strategy based upon the diagnosis, further including a determination of eosinophilic esophagitis or GERD. In some embodiments, the presence of reduced level of, for example, Vail and Jal8, or the like, in combination with a non-elevated or non-reduced level of one or more additional biomarkers associated with eosinophilic esophagitis can result in the patient being diagnosed with GERD.

[0016] Embodiments of the invention also encompass methods of diagnosing a patient with eosinophilic esophagitis (EoE), including: obtaining a sample from a patient, analyzing the sample to determine a level of one or more biomarkers associated with EoE, and determining whether the level of the one or more biomarkers are up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE can result in the patient being diagnosed with EoE.
Embodiments of the invention also encompass diagnostic kits, tests, or arrays, including materials for quantification of at least two analytes, wherein the at least two analytes can be, for example, biomarkers associated with eosinophilic esophagitis (EoE). In some embodiments, the at least two analytes can include, for example, one or more cytokines, IL-15 responsive iNKT, T, and B cell receptors, chemokines, mediators, and IgE receptors, or the like. In some embodiments, the one or more cytokines can include, for example, IL-5, IL-13, IL-15, INFγ, and/or TGF-β, or the like. In some embodiments, the one or more IL-15 responsive iNKT, T, and B cell receptors can include, for example, IL-15Ra, γδ, αβ, CD1d, Va24, Vβ11, and/or Jα18, or the like. In some embodiments, the one or more chemokines can include, for example, CXCR6 and/or CXCL16, or the like. In some embodiments, the one or more mediators can include, for example, IgE, or the like. In some embodiments, the one or more IgE receptors can include, for example, FcεRI and/or FcεRII, or the like.

In some embodiments, the diagnostic kit, test, or array can include a gene chip. In some embodiments, the gene chip can include a low density array. In some embodiments, the diagnostic kit, test, or array can include a surface with a DNA array.

BRIEF DESCRIPTION OF THE DRAWINGS

Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

Figures 1A-D depict representative photomicrographs for the study of IL-15 induction in human EoE. Figures 1A-B demonstrate that there were no IL-15-positive cells in the esophageal biopsies of non-EoE patients. Figure 1A provides the photomicrograph under the original magnification of 10x; Figure 1B provides the photomicrograph under 400x magnification. Figures 1C-D demonstrate that a number of IL-15-positive cells were detected in EoE patient biopsies. Figure 1C provides the photomicrograph under the original magnification of 10x; Figure 1D provides the photomicrograph under 400x magnification. IL-15-positive cells are indicated with arrows.

Figures 2A-D depict patterns resulting from staining a representative EoE patient esophageal biopsy with various media. Figure 2A shows the cell nuclei through 4',6-diamidino-2-phenylindole (DAPI) staining. Figure 2B shows T cells through anti-CD3 staining. Figure 2C shows NK cells through anti-CD56 staining. Figure 2D shows invariant natural killer T (iNKT) cells through co-localization of anti-CD3/anti-CD56 staining.
[0022] Figure 3 depicts eosinophil counts and kinetics after exposing rtTA-CC 10-IL-15 bitransgenic mice to 4, 8, and 12-weeks of a normal diet or a diet of food impregnated with doxycycline (DOX).

[0023] Figure 4 depicts results from fluorescence-activated cell sorting (FACS) analysis conducted on freshly isolated bone marrow cells (BMCs) from naive mice cultured with and without IL-15 (20 ng/ml) and stem cell growth factor (SCF) (10 ng/ml) for 3 weeks at 37°C and 5% CO₂; around 65% of the anti-Fc8RIa positive cell subsets of the culture developed into MCs (Fc8RI/C-kit⁺), while 35.3% developed into basophils (Fc8RI/C-kif).

[0024] Figures 5A-B depict enzyme-linked immunosorbent assay (ELISA) analyses of rtTA-CC 10-IL-15 bitransgenic mice treated with 6 weeks of a diet of normal food or a diet of food impregnated with DOX. Figure 5A depicts esophageal B cell levels. Figure 5B depicts serum IgE levels.

[0025] Figures 6A-B depict representative flow cytometric histograms after exposing purified B cells to different concentrations of IL-15 in vitro. Figure 6A depicts IL-15 (20 ng/ml)-induced activation of B cells, analyzed using anti-CD69 antibody. Figure 6B depicts dose-dependent B cell proliferation, analyzed by thymidine incorporation.

[0026] Figure 7 depicts a quantitative morphometric analysis of mast cell (MC) numbers in the esophagi of rtTA-CC 10-IL-15 mice following an 8-week regimen of a diet of normal food or a diet of food impregnated with DOX.

[0027] Figures 8A-C depict results from bromodeoxyuridine (BrdU) incorporation analysis in saline- and allergen-challenged wild type (WT) and MC-deficient (WW⁺) mice. Figure 8A depicts BrdU⁺ cells, as indicated by arrows, found in the epithelium of saline-challenged mice. Figure 8B depicts BrdU⁺ cells, as indicated by arrows, found in both the epithelium and muscularis mucosa of allergen-challenged WT mice; EP indicates the epithelium, and MM indicates the muscularis mucosa. Figure 8C depicts a morphometric quantitative analysis indicating a significant increase of BrdU⁺ cells in the muscularis mucosa of allergen-challenged WT mice compared to allergen-challenged WW⁺ mice.

[0028] Figures 9A-C depict the detection of anti-CD19⁺ B cells in the esophageal biopsies of EoE patients compared to normal individuals. Figure 9A depicts a lack of B cells from esophageal biopsies of normal individuals. Figure 9B depicts B cells, as indicated by arrows, and IgE⁺ cells, as indicated by arrows, detected in the biopsies of EoE patients. Figure 9C depicts the quantification of IgE⁺ cells in the esophageal biopsies of normal (NL) and EoE patients; data are expressed as mean ± standard deviation (SD), with p<0.001.
Figure 10 depicts a representative photograph showing esophageal stricture in mice following stomach IL-5 transgene-induced EoE, as detected by feeding the mice barium, then subsequently analyzing the gastrointestinal tract x-ray; esophageal stricture is indicated by the rectangle.

Figure 11 depicts eosinophil numbers analyzed in the esophagi of IL-15Ra-deficient mice following anti-myelin basic protein (MBP) immunostaining.

Figure 12 depicts eosinophil numbers analyzed in the esophagi of CD1d null mice following anti-MBP immunostaining.

Figures 13A-B depict the protein and mRNA levels of IL-15 and CXCL16 in the blood and blood leukocytes of EoE patients compared to those of normal individuals. Figure 13A depicts blood IL-15 protein levels. Figure 13B depicts blood mRNA expression levels of iNKT-specific chemokine CXCL16.

Figures 14A-J depict real-time PCR analyses to detect receptor mRNA levels in the blood of normal, EoE, and GERD patients. Figure 14A depicts the relative expression of FcsRI mRNA levels. Figure 14B depicts the relative expression of FcsRII mRNA levels. Figure 14C depicts the relative expression of γ T cell receptor mRNA levels. Figure 14D depicts the relative expression of δ T cell receptor mRNA levels. Figure 14E depicts the relative expression of β T cell receptor mRNA levels. Figure 14G depicts the relative expression of CXCR6 mRNA levels. Figure 14H depicts the relative expression of v β1 mRNA levels. Figure 14I depicts the relative expression of Jq18 mRNA levels. Figure 14J depicts the relative expression of Va24 mRNA levels. Data are normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).
DETAILED DESCRIPTION OF THE INVENTION

[0034] All references cited herein are incorporated by reference in their entirety. Also incorporated herein by reference in their entirety include: United States Patent Application No. 61/474,775, IN Variant Natural Killer T Cell And Its Chemokine CXCL16 Are Target Molecules For The Treatment Of Eosinophilic Esophagitis, filed on April 13, 2011.

[0035] Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0036] As used herein, the term "non-invasive biomarker" refers to a molecule measured in blood whose concentration reflects the severity or presence of some disease state.

[0037] As used herein, "blood" can include, for example, plasma, serum, whole blood, blood lysates, and the like.

[0038] As used herein, the term "assessing" includes any form of measurement, and includes determining if an element is present or not. The terms "determining," "measuring," "evaluating," "assessing" and "assaying" can be used interchangeably and can include quantitative and/or qualitative determinations.

[0039] As used herein, the term "diagnosing or monitoring" with reference to a disease state or condition refers to a method or process of determining if a subject has or does not have a particular disease state or condition or determining the severity or degree of the particular disease state or condition.

[0040] As used herein, the terms "treatment," "treating," "treat," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect can be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or can be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a subject, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease and/or relieving one or more disease symptoms. "Treatment" can also encompass delivery of an agent or administration of a therapy in order to provide for a pharmacologic effect, even in the absence of a disease or condition. The term "treatment" is used in some embodiments to refer to administration of a
compound of the present invention to mitigate a disease or a disorder in a host, preferably in a mammalian subject, more preferably in humans. Thus, the term "treatment" can include includes: preventing a disorder from occurring in a host, particularly when the host is predisposed to acquiring the disease, but has not yet been diagnosed with the disease; inhibiting the disorder; and/or alleviating or reversing the disorder. Insofar as the methods of the present invention are directed to preventing disorders, it is understood that the term "prevent" does not require that the disease state be completely thwarted (see Webster's Ninth Collegiate Dictionary). Rather, as used herein, the term preventing refers to the ability of the skilled artisan to identify a population that is susceptible to disorders, such that administration of the compounds of the present invention can occur prior to onset of a disease. The term does not mean that the disease state must be completely avoided.

[0041] As used herein, the terms "modulated" or "modulation," or "regulated" or "regulation" and "differentially regulated" can refer to both up regulation (i.e., activation or stimulation, e.g., by agonizing or potentiating) and down regulation (i.e., inhibition or suppression, e.g., by antagonizing, decreasing or inhibiting), unless otherwise specified or clear from the context of a specific usage.

[0042] As used herein, the term "marker" or "biomarker" refers to a biological molecule, such as, for example, a nucleic acid, peptide, protein, hormone, and the like, whose presence or concentration can be detected and correlated with a known condition, such as a disease state. It can also be used to refer to a differentially expressed gene whose expression pattern can be utilized as part of a predictive, prognostic or diagnostic process in healthy conditions or a disease state, or which, alternatively, can be used in methods for identifying a useful treatment or prevention therapy.

[0043] As used herein, the term "expression levels" refers, for example, to a determined level of biomarker expression. The term "pattern of expression levels" refers to a determined level of biomarker expression compared either to a reference (e.g. a housekeeping gene or inversely regulated genes, or other reference biomarker) or to a computed average expression value (e.g. in DNA-chip analyses). A pattern is not limited to the comparison of two biomarkers but is more related to multiple comparisons of biomarkers to reference biomarkers or samples. A certain "pattern of expression levels" can also result and be determined by comparison and measurement of several biomarkers as disclosed herein and display the relative abundance of these transcripts to each other.
As used herein, a "reference pattern of expression levels" refers to any pattern of expression levels that can be used for the comparison to another pattern of expression levels. In some embodiments of the invention, a reference pattern of expression levels is, for example, an average pattern of expression levels observed in a group of healthy or diseased individuals, serving as a reference group.


Most EoE patients have evidence of food and aeroallergen hypersensitivity, and a relatively large fraction of the patient population has food anaphylaxis (Fox, V. et al. Gastrointest. Endosc. 56:260-70 (2002)). Food allergies affect an estimated 6% of children and 3.7% of adults in the US (see, e.g., Waldmann, T. and Tagaya, Y. Annu. Rev. Immunol. 17:19-49 (1999); Kim, H. et al. J. Exp. Med. 201:41-7 (2005)). Recent literature on pediatric patients with EoE confirms that nearly all patients respond to an elemental diet, with resolution of symptoms and normalization of biopsies (Spergel, J. Curr. Opin. Allergy Clin. Immunol. 7:274-8 (2007)) but that food reintroduction causes symptoms to recur and esophageal eosinophilia to return. Food allergies are therefore considered to be the cause of EoE in humans (Spergel, J. et al. Ann. Allergy Asthma Immunol. 95:336-43 (2005)).

In addition to the EoE condition itself, mastocytosis, induced IgE, and esophageal pathological abnormalities, such as rings, furrows, and strictures, have been

[0048] A direct link has been demonstrated between the development of EoE in a mouse model of experimental EoE and indoor insect allergy (cockroach and dust mites), as well as food allergy (peanut and corn) (Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010)); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010)). It has also been demonstrated that -30% of EoE patients have insect hypersensitivity, and 40-50% have peanut or corn hypersensitivity (Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010)); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010)).

[0049] Therapies for esophageal inflammation are primarily based on food antigen elimination trials (allergen removal), anti-inflammatory approaches (steroid treatment), dietary management, the combination of steroid treatment and dietary management, and physical dilatation when strictures are present. For example, EoE therapies include the use of proton pump inhibitors (PPIs), topical glucocorticoids, such as fluticasone or budesonide, humanized antibodies against relevant cytokines, such as etoxacin-3, IL-13, and IL-5, and small molecule inhibitors of an eosinophil and/or allergic disease activation pathway, such as a prostaglandin D2, IL-4, or IL-13 antagonist. However, none of these therapies is ideal. Improved diagnosis and management of EoE are vitally important for future advances in therapeutic interventions.

[0050] Physical dilatation, which has been the strategy reported for adults, temporarily reduces symptoms of dysphagia. However, this treatment involves a significant risk of rupture and hemorrhage, needs to be repeated regularly, and does not reduce the underlying inflammation (Kawano, T. et al. Science 278:1626-9 (1997)).

[0051] An antigen elimination approach in sensitized individuals (e.g. aeroallergen avoidance and food elimination diets) is typically unsatisfactory or practically difficult, such as when patients are sensitized to many allergens. A diet consisting exclusively of an elemental (amino acid-based) formula frequently improves symptoms and normalizes esophageal pathology (Teng, M. et al. J. Immunol. 183:191 1-20 (2009); Teng, M.

[0052] Systemic steroids are used to treat acute exacerbations, and topical glucocorticoids are used to provide long-term control (Thomas, S. et al. J. Immunol. 171:2571-80 (2003); Geissmann, F. et al. PLoSBiol 3:el 13 (2005); Faubion, W. et al. J. Pediatr. Gastroenterol. Nutr. 27:90-3 (1998); Arora, A. et al. Mayo Clin. Proc. 78:830-5 (2003)). Glucocorticoid treatment shows a significant effect in reducing esophageal eosinophilia, and newer glucocorticoids with decreased systemic effects can improve the care of EoE patients. Although some treatments have been shown to be effective in EoE, the molecular mechanisms involved in the remission have heretofore not been established and remain unclear.

[0053] Humanized antibody therapy designed to block IL-5 and IL-13 has been explored, given that IL-5 and IL-13 have been identified as having key roles in eliciting esophageal eosinophilia (Mishra, A. et al. J. Immunol. 168:2464-9 (2002); Mishra, A. J. Ped. Gastro. Nut. 45:383-5 (2007)) and tissue remodeling (Mishra, A. et al. Gastroenterology 134:204-14 (2008)). Clinical trials using humanized anti-IL-5 and anti-IL-13 for EoE treatment have demonstrated some promise (Stein, M. et al. J. Allergy Clin. Immunol. 118:1312-9 (2006)); however, early clinical results have not been as strikingly positive as hoped, and EoE returns in most patients as soon as the therapy is withdrawn; therefore, this therapy is not promising. This can be because IL-5 and IL-13 are secondary products of activated T cell subsets, as opposed to products that contribute to the growth and survival of T cell subsets like, invariant natural killer T (iNKT) or γδ T cells; these secondary products elevate, rather than initiate, EoE pathogenesis.

[0054] EoE diagnosis criteria have been recommended by an expert panel established as part of the First International Group of EoE Researchers (FIGERS) (Liacouras, C. et al. J. Allergy Clin. Immunol. 128:3-20e6 (2011)). EoE diagnosis requires endoscopy, which is an invasive and inconvenient procedure, followed by biopsy analysis to record the characteristic histological features of esophageal mucosal eosinophilia and epithelial proliferative changes. There are few diagnostic biomarkers for EoE, other than determination
of eosinophil counts/high power field (hpf) from esophageal biopsies; there is also no proven permanent EoE treatment strategy.


[0056] Non-invasive techniques for the diagnosis of EoE, such as biomarker detection methods, would be preferable to endoscopic techniques. Determination of target molecules that have potential use in EoE diagnosis and future therapy would therefore be beneficial, as would innovative fundamental studies which uncover new possibilities for diagnostic and therapeutic interventions. The identification of reliable, non-invasive EoE biomarkers would advance EoE treatment, as it would allow for more accurate and timely detection of changes resulting from therapy administration. The ability to avoid repeated endoscopies will not only have a positive impact in the medical care of the patients, but it will also reduce the possibility of complications related to the invasive nature of the procedure and the cost of patient care. The discovery of a biomarker for EoE would therefore change the approach to the diagnosis and management of patients with EoE and would have broad applicability on clinical practice and patient health.

[0057] A number of invasive diagnostic and monitoring molecules have been proposed as biomarkers for EoE. However, most biomarkers proposed for EoE diagnosis rely on endoscopic and histological analysis of patient esophageal biopsies, and a number of questions remain regarding their ability to diagnose EoE. In addition, none of the heretofore established non-invasive biomarkers has been proven to differentiate EoE from GERD, a closely related esophageal disease. Moreover, some EoE patients can have eosinophil counts below the FIGURE recommended eosinophil levels (<15 eosinophils/hpf), making diagnosis difficult; there are also clear cases of GERD with biopsies that are difficult to differentiate from EoE, as described in a recent group of PPI-responsive EoE patients (Liacouras, C. et al.)
J. Allergy Clin. Immunol. 128:3-20e6 (2011)). These observations can lead to a delay in diagnosis or, in the worst-case scenario, to the inappropriate use of therapy.

[0058] A few non-invasive EoE biomarkers have been proposed, such as absolute blood eosinophilia, eosinophil-3, and eosinophil-derived neurotoxins; however, many of these molecules have either not yet been tested or fail to differentiate EoE from GERD. Thus, there is a need for specific, noninvasive biomarkers for EoE that are capable of differentiating EoE from GERD.

[0059] As described herein, mRNA levels of T cell and B cell receptors, namely CXR6, Va24, γδ, αβ, FceRI, and FceRII, were measured in the blood of EoE patients. The experiments described herein demonstrate that these compounds can be used as non-invasive biomarkers for EoE and can differentiate EoE from GERD.

EoE Pathogenesis

[0060] Eosinophils and IgE-associated mast cell (MC)/basophil activation are involved in various steps of EoE pathogenesis. As immunoglobulin (Ig) class switching has been shown to be involved in the pathogenesis of allergic diseases (Shimoda, K. et al. Nature 380:630-3 (1996); Punnonen, J. et al. Proc. Natl. Acad. Sci. U.S.A. 90:3730-4 (1993)), IL-15-induced IgE therefore can be involved in promoting esophageal pathological abnormalities in EoE.


IL-13 mRNA has been demonstrated to be induced in EoE patients compared with normal individuals (Blanchard, C. et al. J. Allergy Clin. Immunol. 120:1292-300 (2007)), and murine models have also demonstrated that IL-13 promotes esophageal eosinophilia (Mishra, A. and Rothenberg, M. Gastroenterology 125:1419-27 (2003)) and remodeling (Zuo, L. et al. J. Immunol. 185:660-9 (2010)) in mice. These data elucidate the relationship between IL-13 and human EoE and demonstrate that murine IL-13-induced esophageal transcripts resemble the human EoE transcriptome.


Allergen-induced IL-15 and its responsive and non-responsive T cell subsets have been shown to have a role in the initiation and progression of the pathogenesis of human and experimental EoE ((Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. Proc. Natl. Acad. Sci. Under review (2011)), and IL-15, iNKT cells, MCs, and IgE are all found to be elevated in human EoE (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. J. Allergy Clin. Immunol. In press (2012); Vicario, M. et al. Gut 59:12-20 (2010)). In addition, iNKT cells have been shown to be involved in an experimental model of food allergen- and aeroallergen-induced EoE (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012); Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010)). Accordingly, very high numbers of accumulated T cells in the esophageal mucosa of human EoE were found to be iNKT cells (Figure 2).

As described herein, chronic IL-15 expression was studied to determine whether it leads to IgE-associated EoE pathogenesis, and the mRNA and protein expression pattern of the specific surface molecules of IL-15 and IL-15 responsive iNKT cells (IL-15Ra,
CD4), T cell surface receptors (CXCR6, Va24, γδ T, and αβ T cells), chemokines (CXCL16), mediators (IgE), and receptors of IgE (FcsRI and FcsRII) were investigated for their potential as diagnostic and therapeutic targets for EoE. These studies have served to elucidate the mechanism of IL-15-induced IgE-associated EoE pathogenesis and to determine non-invasive biomarkers that differentiate EoE from GERD as well as potential therapeutic targets.

**IL-15 Expression in EoE Pathogenesis**

[0067] IL-15 is an allergen-induced cytokine that mediates diverse biological responses, ranging from proliferation and differentiation to protection from apoptosis. IL-15 binds to a trimeric receptor complex consisting of IL2RP, IL2Ry, and IL15Ra chains. This cytokine shares the IL2RP chain with IL2 and shares the IL2Ry chain with other cytokines (such as IL2, IL4, IL9, IL13, and IL21); IL15Ra is a specific receptor subunit for IL15 (Vasilopoulos, S. and Shaker, R. *Curr. Gastroenterol. Rep.* 3:225-30 (2001)).


[0069] IL-15 is found to be increased in experimental EoE and in blood and esophageal biopsies of pediatric and adult EoE patients and is thus correlated with esophageal
eosinophilia in human EoE (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Figures 1A-D). As IL-15 and IL-15Ra gene expression are induced in human and experimental EoE and have a significant role in the pathogenesis of allergen-induced experimental EoE (Zhu, X. et al. Gastroenterology 139:82-193 (2010)), IL-15 can have a key role in the initiation and progression of EoE.


[0071] The role of iNKT cells in the induction of experimental EoE has been described in several ways. Firstly, the mRNA/protein of IL-15 and IL-15 responsive iNKT and γδ T cell surface molecules and receptors cells have been studied in the esophageal biopsies of humans with EoE induced by food (peanut and corn) allergens and aeroallergens and have been demonstrated to promote iNKT cell-dependent EoE in an allergen-sensitized murine model of EoE (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012); Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec. 26, In Press (2010)); experimental EoE was achieved by inducing eosinophil active cytokines, such as IL-5 and IL-13, and chemokines, such as eotaxin-3. Secondly, in vivo iNKT cell activation has been shown to be sufficient to promote EoE in mice, and iNKT cell-deficient CD1d null mice have been shown to be protected from food or aeroallergen-induced experimental EoE (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012); Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec. 26, In Press (2010)). iNKT cells are selected and restricted by CD1d, a non-classical MHC I-like prototypical iNKT cell ligand and specific Va24Jal8 receptor (Kawano, T. et al. Science 278:1626-9 (1997)).

[0072] IL-15-responsive iNKT cells have been shown to activate B cells to produce IgE and IgG in both innate and acquired immunity (Kim, H. et al. J. Exp. Med. 201:41-7 (2005); Taniguchi, M. et al. Annu. Rev. Immunol. 21:483-513 (2003)). Both IgE and B cells have been demonstrated to be induced in human EoE (Vicario, M. et al. Gut 59:12-20 (2010)). IgE binds and activates eosinophils, MCs, and basophils via its receptor

Blood and esophageal biopsies from normal individuals and EoE patients have also been used to show the significance of IL-15 and iNKT cells in the initiation and progression of the disease (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012)). In addition, iNKT cell-specific chemokine CXCL16 has been shown to be induced and correlated with esophageal eosinophilia in human EoE (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012)).

As described herein, most of the T cells accumulated in the esophagi of EoE patients are iNKT cells (Figures 2A-D); therefore, IL-15 is a growth and survival factor for iNKT and γδT cells. IL-15 also promotes proliferation and activation of B cells and MCs (Figures 6A-B), and chronic IL-15 in vivo expression induces B cells and IgE in mice (Figures 5A-B). Esophageal IL-15 overexpression in mice was found to increase MCs in the esophagus and promote epithelial and muscle cell hyperplasia in MCs and B cell proliferation and activation, including Ig class switching in the esophagus (Figures 5-8). These results are consistent with the finding that EoE patients have increased B cell counts and increased expression of Ig class switch genes in esophageal biopsies (Vicario, M. et al. Gut 59:12-20 (2010)). It has also been shown that esophageal functional impairment develops in EoE (Mavi, P. et al. Am. J. Physiol. Gastrointest. Liver Physiol. Online published (2012)).

Transgenic mice designed to overexpress IL-15 were found to develop esophageal eosinophilia, MC hyperplasia, local B cell activation and proliferation, and
increased blood IgE and eosinophil levels following 8 weeks of doxycycline (DOX) exposure, indicating that IL-15 promotes IgE induction and Ig class switching (Figures 3-7).

[0077] Due to the increased levels of MCs found in transgenic mice overexpressing IL-15 (Figure 7), the role of MCs in IL-15-mediated esophageal pathological abnormalities was studied. The consequences of IL-15-induced, IgE-associated EoE pathogenesis were then studied, such as the development of chronic IL-15-induced esophageal pathological abnormalities (e.g. esophageal stricture), including comparison of a series of broad immunologic and pathologic characteristics of experimental EoE with the respective control (Figure 10).

[0078] Experimental modeling establishes the role of iNKT cells in the induction of EoE (Figure 12). As iNKT stimulation is sufficient to induce EoE in mice (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012)), IL-15 and iNKT cells can have a prominent role in initiating immunological responses in EoE and production of IL-5 and IL-13, which further elevate esophageal inflammation in experimental and human EoE.

[0079] These data indicate that IL-15 and iNKT cell singling are important in initiating EoE, and IL-13 or IL-5 produced in response to their singling further facilitate the disease pathogenesis and tissue survival of eosinophils. This supports previous findings that indicates that IL-15 interaction is involved in IL-5- and IL-13-mediated induction and progression of experimental as well as human EoE (Mishra, A. and Rothenberg, M. Gastroenterology 125:1419-27 (2003); Mishra, A. et al. J. Immunol. 168:2464-9 (2002)).

[0080] The detailed mechanistic studies described herein examined the role of most of the cell types influenced by IL-15 signaling and their responses in EoE pathogenesis. The evidence presented demonstrates that EoE is an IL-15-induced and iNKT cell-mediated esophageal disorder and that chronic IL-15 expression leads to the development of IgE-associated EoE pathogenesis. The data presented herein (Figures 4-7) demonstrate that an IL-15 overexpressed mouse model has similar characteristics to a human EoE model and can be used to better understand the mechanism of the induction of B cells, MCs, and IgE production in EoE.

[0081] These pre-clinical observations can be extrapolated into human EoE, as the mechanisms operational in experimental mouse models also occur in human EoE. The murine and translational studies described herein elucidate aspects of chronic IL-15-induced IgE-associated EoE pathogenesis and identify promising molecules for EoE diagnosis and target therapy. The role of IL-15 and iNKT cells in the initiation and progression of EoE
pathogenesis has also been established. The data presented herein establish the significance of IL-15 and its responsive cells in the pathogenesis, diagnosis, and treatment of EoE and provide a rationale for investigating the surface molecules of IL-15, IL-15-responsive cells, chemokines, and mediators as potential diagnostic and therapeutic target molecules for human EoE.

[0082] Based on the above, several non-invasive diagnostic biomarkers and therapeutic target molecules for human EoE, such as IL-15 and surface molecules of IL-15 responsive γδT and iNKT cell receptors (IL-15Ra, γδ, αβ, Va24, Jα18, νβ1), chemokines (CXCL16 and CXCR6), mediators (IgE), and IgE receptors (FCeRI and FCeRII), were identified. The mRNA and protein expression patterns of the IL-15 and cell surface molecules and mediators, including chemokines, of IL-15 responsive iNKT cells were therefore screened in order to determine novel diagnostic and therapeutic interventions for human EoE, as described below.

Identification of Diagnostic Biomarkers for Human EoE

[0083] As stated previously, EoE patients have induced IL-15, iNKT cells, B cells, and IgE in the blood and esophagus, and their role in the initiation and progression of EoE pathogenesis has been demonstrated (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. J. Allergy Clin. Immunol. In press (2012); Vicario, M. et al. Gut 59:12-20 (2010)); similar observations have been made in mice that over-express IL-15, resulting in induction of B cells and IgE (Figures 3, 5A-B, and 7). These observations are in accordance with an earlier clinical report that demonstrates local B cell and IgE induction in human EoE (Vicario, M. et al. Gut 59:12-20 (2010)).

[0084] IL-15 is a growth and survival factor for iNKT cells (Ohteki, T. Curr. Mol. Med. 2:371-80 (2002)), and the studies described herein have demonstrated its promotion of the proliferation and activation of B cells and MCs (Figures 5A-B and 7). IL-15 has been shown to promote IgE induction in IL-15 overexpressed mice, and IgE levels are elevated in human EoE (Mavi, P. et al. Gastroenterology Online published on Feb. 21 (2012)). Based on this information and the findings described herein, IL-15 and the cell surface molecules and chemokines of IL-15-responsive cells (including iNKT, γδT and B cells) therefore can be studied in order to determine potential non-invasive diagnostic biomarkers for human EoE that also differentiate EoE from GERD.

[0085] This approach is based on several related facts. First, iNKT cell-specific chemokines (e.g. CXCL16) are highly expressed in the esophageal mucosa of EoE patients

As described herein, blood mRNA and protein levels of several IL-15-induced and IL-15-responsive candidate biomarkers (namely, IL-15, IL-15 responsive γδT and iNKT cell receptors such as IL-15Ra, γδ, αβ, CDld, Va24, ν βι 1, Jal8, chemokines such as CXCR6 and CXCL16, mediators such as IgE, and IgE receptors such as FcεRI and FcεRII) were determined in normal individuals, GERD patients, and EoE patients; these compounds were investigated with the aim of identifying non-invasive biomarkers of EoE disease activity that could be used to monitor esophageal inflammation without the need for invasive serial surveillance endoscopies. Statistical correlations between esophageal eosinophilia and mRNA and protein levels of different molecules of IL-15 responsive cells were also analyzed. The GERD patient blood analysis was used to establish the specificity of these molecules for EoE diagnosis. The most attractive marker proteins for EoE are proteins that are not only elevated in esophageal biopsies but can be also detected in the peripheral blood of EoE patients. These studies allowed for the identification of non-invasive biomarkers of EoE disease activity that can be used to monitor esophageal inflammation without the need for serial surveillance endoscopies.

IL-15 protein and mRNA of an iNKT-specific chemokine (CXCL16) were found to be induced in the blood of EoE patients (Figures 13A-B). The mRNA and protein levels of several other potential diagnostic biomarker molecules were then evaluated in the blood or blood cells of normal individuals and patients with EoE or GERD in order to establish a panel of non-invasive biomarkers for human EoE; IL-15-responsive cell surface molecules, including receptors, mediators, and chemokines, were found to be non-invasive diagnostic biomarkers for EoE capable of differentiating EoE from GERD (Figures 14A-J).

As described herein, blood mRNA levels several molecules, including T cell receptors CXCR6, Va24, γδ T, and αβ T, and IgE receptors FcεRI and FcεRII, were
found to be significantly altered in EoE patients compared to normal individuals. The mRNA levels of most of these molecules in GERD patients were found to be comparable to those of normal individuals and were significantly altered relative to EoE patients. Certain T cell receptors, including Vail and Jq18, were found to be reduced in EoE patients compared to normal individuals; however, these reduced levels were similar in EoE and GERD patients. These findings establish a panel of T cell receptors and IgE receptors whose blood mRNA levels can be used in a non-invasive diagnostic biomarker panel which is capable of diagnosing EoE, as well as differentiating EoE from GERD.

[0089] Taken together, the studies described herein allow for the determination of a molecule, or a combination of molecules, that will be able to predict a diagnosis of EoE in patients before the endoscope evaluation. These molecules can also serve to differentiate EoE from GERD.

[0090] Additional molecules are studied in the same fashion order to determine their potential as non-invasive biomarkers for EoE capable of differentiating EoE from GERD. For example, IL-15, IL-15Ra, IL-5, IL-13, INFγ, CXCL16, and TGF-β protein and mRNA levels can be compared between EoE patients, GERD patients, and normal individuals.

[0091] IL-15 and IL-15Ra mRNA levels can be subsequently compared to those of other Th1 and Th2 cytokines, such as IL-5, IL-13, and INFγ, which are growth and survival factor for eosinophils and MCs/basophils. Both types of inflammatory cells are the source of producing TGF-β, which has been shown to be induced in EoE patients and is a key cytokine in the development of esophageal remodeling and fibrosis (Aceves, S. et al. J. Allergy Clin. Immunol. 119:206-12 (2007); Mishra, A. et al. Gastroenterology 134:204-14 (2008); Zuo, L. et al. J. Immunol. 185:660-9 (2010)). Furthermore, IL-15 overexpressed mice and human EoE have induced MCs in the esophagus and are a rich source of TGF-β (Abonia, J. et al. J. Allergy Clin. Immunol. 126:140-9 (2010)). Therefore, these molecules can be potential non-invasive biomarkers for EoE and can potentially differentiate EoE from GERD.

Identification of Target Molecules for EoE Therapy

[0092] Given the involvement of IL-15 and iNKT cells in EoE pathogenesis, as described herein, IL-15 and iNKT cells therefore can be studied in order to determine potential target molecules for EoE therapy. IL-15 and surface molecules, mediators and chemokines of IL-15 responsive cells are targeted for EoE diagnosis and therapy by
combining innovative experimental approaches using EoE experimental models and human samples.

[0093] Potential therapeutic target molecules are tested in the food allergen- and aeroallergen-induced murine model of experimental EoE using specific humanized neutralizing monoclonal antibodies. Allergen-induced murine models of EoE have most of the characteristic features observed in human EoE, such as intraepithelial eosinophils, epithelial hyperplasia, and esophageal remodeling (Denburg, J. et al. Int. Arch. Allergy Immunol. 117:155-9 (1998); McCloskey, N. et al. J. Biol. Chem. 282:24083-91 (2007)). Blocking of endogenous IL-15 in vivo with a soluble IL-15Ra (sIL-15Ra) antagonist has previously been shown to be a very efficient treatment in inflammatory, collagen-induced arthritis and OVA-induced pulmonary inflammation (Ohteki, T. Curr. Mol. Med. 2:371-80 (2002); Ruckert, R. et al. J. Immunol. 174:5507-15 (2005); Ohteki, T. et al. Nat. Immunol. 2:1138-43 (2001)), and IL-15Ra gene-deficient mice are shown to be protected from the induction of experimental EoE (Figure 11).

[0094] In addition, anti-CD lδd neutralizing antibody has been successfully used to neutralize iNKT cells in tumor repression (Teng, M. et al. J. Immunol. 183:191 1-20 (2009); Teng, M. et al. J. Immunol. 182:3366-71 (2009)). CD lδd gene-deficient mice are shown to be protected from experimental EoE (Figure 12). Therefore, neutralizing iNKT cells with monoclonal anti-CD lδd or anti-Va24Jα8 or its chemokine anti-CXCL16 can protect mice from developing food allergen- or aeroallergen-induced EoE.

[0095] As iNKT cells home in the tissue via the interaction of the chemokine CXCL16 and its receptor CXCR6 (Jiang, X. et al. J. Immunol. 175:2051-5 (2005)), neutralization of the iNKT cell-specific chemokine CXCL16 can provide protection from experimental EoE. CXCL16, a CXCR6-specific chemokine, has unique structural properties, and CXCR6 is expressed at high levels on iNKT cells as compared with other lymphocytes under physiological conditions (Thomas, S. et al. J. Immunol. 171:2571-80 (2003); Geissmann, F. et al. PLoSBiol 3:el 13 (2005)). As CXCR6+ T cells and CXCL16 have been shown to be induced in human EoE (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012)), treatment with neutralizing CXCL16 antibody (R&D Systems) can protect mice from food allergen- and aeroallergen-induced, iNKT cell-mediated EoE.

[0096] Esophageal IL-15 overexpression in mice induces MCs and IgE levels. Therefore, experimental EoE-induced esophageal pathological abnormalities through IL-15-induced IgE neutralization is studied in order to determine whether allergen-induced, IL-15-
mediated EoE pathogenesis, such as epithelial and muscle cell hyperplasia and stricture, is dependent on IgE and thus prevented by anti-IgE treatment in mice.

[0097] Because IL-15 and iNKT cells are involved in initiation and progression of EoE, IL-15-responsive iNKT cell surface molecules, such as CDld, IL-15Ra, and IL-15-induced IgE, and iNKT-specific chemokine CXCL16 are investigated as potential target molecules for EoE therapy by using their respective neutralizing monoclonal antibodies in individual experiments. The anti-IL-15, anti-IgE, anti-CDld, anti-Va24Jal8, anti-CXCL16, and anti-IL-15Ra neutralized and non-neutralized mice are tested for EoE pathogenesis following the food allergen- or aeroallergen-induced experimental EoE protocol (Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010); Mishra, A. et al. J. Clin. Invest. 107:83-90 (2001)). Success using neutralizing antibodies in in vivo experimentation in mice has been previously demonstrated (Mishra, A. et al. J. Clin. Invest. 107:83-90 (2001); Blanchard, C. et al. Clin. Exp. Allergy 35:1096-103 (2005)).

[0098] The experiments described herein serve to establish a therapeutic strategy for EoE. The proposed neutralizing antibody therapy in a mouse model of experimental EoE provides an experimental framework to support clinical trials of these molecules in human EoE.

Treatment Based on Biomarker Levels

[0099] Embodiments of the invention are directed to methods of treating EoE in a patient, wherein the methods comprise analyzing the sample from a patient to determine a level of one or more biomarkers associated with EoE, determining whether the level of the one or more biomarkers associated with EoE is up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE results in the patient being diagnosed with EoE, and treating the patient with an appropriate therapeutic strategy based upon the diagnosis. In some embodiments, the methods can be used to distinguish EoE from GERD in a subject.

[00100] Embodiments of the invention are also directed to methods of diagnosing a patient with EoE, wherein the methods comprise analyzing the sample from a patient to determine a level of one or more biomarkers associated with EoE, and determining whether the level of the one or more biomarkers are up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an
elevated or reduced level of one or more biomarkers associated with EoE results in the patient being diagnosed with EoE.

[00101] In embodiments of the invention, the one or more biomarkers associated with EoE include one or more cytokines, IL-15-responsive iNKT, T, and B cell receptors, chemokines, mediators, and IgE receptors. In some embodiments, the one or more biomarkers associated with EoE include IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII.

[00102] In some embodiments, at least 2 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 3 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 4 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 5 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 6 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 7 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 8 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 9 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 10 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 11 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 12 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, νβi, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some
embodiments, at least 13 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 14 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 15 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, at least 16 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, all of the biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII.

[00103] In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, or 9 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, 10, 11, 12, 13, 14, 15, or 16 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, anywhere between 1 to 10 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, anywhere between 1-17 biomarkers associated with EoE are selected from IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CDld, Va24, κβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, the one or more biomarkers associated with EoE are measured using one or more methods and/or tools, including for example, but not limited to, Taqman (Life Technologies, Carlsbad, CA), Light-Cycler (Roche Applied Science, Penzberg, Germany), ABI fluidic card (Life Technologies), NanoString® (NanoString Technologies, Seattle, WA), NANODROP® technology (Thermo Fisher Scientific (Wilmington, DE), fluidic card, and the like. The person of skill in the art will recognize such other formats and tools, which can be commercially available or which can be developed specifically for such analysis.
[00106] Determination of the biomarker level(s) as described herein can be combined with determination of the levels of one or more additional biomarkers associated with EoE. For example, determination of the biomarker level(s) as described herein can be combined with determination of the levels of one or more genes of the EoE transcriptome. Such a determination can include measurement of the gene DNA or RNA, or the gene product. Such genes can include, for example, eotaxin-3, and the like.

EoE Therapies

[00107] Certain embodiments of the invention involve administering EoE therapies, including allergen removal, steroid treatment, dietary management, proton pump inhibitor (PPI) therapy, administration of one or more topical glucocorticoid, administration of one or more humanized antibody against one or more relevant cytokines and/or mediators, administration of one or more small molecule inhibitors of an eosinophil and/or allergic disease activation pathway, administration of one or more small molecule inhibitors capable of modulating levels of one or more biomarkers associated with EoE, and/or any combination thereof. Topical glucocorticoids that can be used as EoE therapies include, for example, fluticasone, budesonide, ciclesonide, and the like. Humanized antibodies against one or more relevant cytokines and/or mediators can include, for example, antibodies targeting one or more biomarkers associated with EoE. The one or more biomarkers associated with EoE can include, for example, IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CD1d, Va24, v βi 1, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII. In some embodiments, the humanized antibody against one or more relevant cytokines and/or mediators can include, for example, anti-IL-15, anti-IgE, anti-CD1d, anti-Va24Jal8, anti-CXCL16, and anti-IL-15Ra, and the like.

[00108] The example targeting strategies and compounds presently provided are intended to be representative. One of skill in the art will recognize that different compounds from those listed above can be used to achieve a comparable outcome and how to identify such compounds.

[00109] Heretofore unknown therapeutics against specific biomarkers associated with EoE can be developed by the screening of various compounds. Compounds that can be screened to determine their utility as therapeutics against specific biomarkers associated with EoE include for example, but are not limited to, libraries of known compounds, including natural products, such as plant or animal extracts, synthetic chemicals, biologically active materials including proteins, peptides such as soluble peptides, including but not limited to
members of random peptide libraries and combinatorial chemistry derived molecular libraries made of D- or L-configuration amino acids, or both, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries), antibodies (including, but not limited to, polyclonal, monoclonal, chimeric, human, anti-idiotypic or single chain antibodies, and Fab, F(ab′)_2 and Fab expression library fragments, and epitope-binding fragments thereof), organic and inorganic molecules, and the like.

[00110] In addition to the more traditional sources of test compounds, computer modeling and searching technologies permit the rational selection of test compounds by utilizing structural information from the ligand binding sites relevant proteins. Such rational selection of test compounds can decrease the number of test compounds that must be screened in order to identify a therapeutic compound. Knowledge of the sequences of relevant proteins allows for the generation of models of their binding sites that can be used to screen for potential ligands. This process can be accomplished in several manners known in the art. A preferred approach involves generating a sequence alignment of the protein sequence to a template (derived from the crystal structures or NMR-based model of a similar protein(s), conversion of the amino acid structures and refining the model by molecular mechanics and visual examination. If a strong sequence alignment cannot be obtained then a model can also be generated by building models of the hydrophobic helices. Mutational data that point towards residue-residue contacts can also be used to position the helices relative to each other so that these contacts are achieved. During this process, docking of the known ligands into the binding site cavity within the helices can also be used to help position the helices by developing interactions that would stabilize the binding of the ligand. The model can be completed by refinement using molecular mechanics and loop building using standard homology modeling techniques. (General information regarding modeling can be found in Schoneberg, T. et. al. Molecular and Cellular Endocrinology 151:181-93 (1999); Flower, D. Biochimica et Biophysica Acta 1422:207-34 (1999); and Sexton, P. Current Opinion in Drug Discovery and Development 2:440-8 (1999).)

[00111] Once the model is completed, it can be used in conjunction with one of several existing computer programs to narrow the number of compounds to be screened by the screening methods of the present invention, like the DOCK program (UCSF Molecular Design Institute, San Francisco, CA). In several of its variants it can screen databases of commercial and/or proprietary compounds for steric fit and rough electrostatic
complementarity to the binding site. Another program that can be used is FLEXX (Tripos Inc., St. Louis, MO).

Administration

[00112] The compounds and antibodies used as therapeutic targets or agents as described above can be administered via oral or parenteral delivery routes (subcutaneous or intravenous). Such therapeutics can be administered by any pharmaceutically acceptable carrier, including, for example, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional medium or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Routes of administration include for example, but are not limited to, intravenous, intramuscular, and oral, and the like. Additional routes of administration include, for example, sublingual, buccal, parenteral (including, for example, subcutaneous, intramuscular, intraarterial, intradermal, intraperitoneal, intracisternal, intravesical, intrathecal, or intravenous), transdermal, oral, transmucosal, and rectal administration, and the like.

[00113] Solutions or suspensions used for appropriate routes of administration, including, for example, but not limited to parenteral, intradermal, or subcutaneous application, and the like, can include, for example, the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfate; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates, or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose, and the like. The pH can be adjusted with acids or bases, such as, for example, hydrochloric acid or sodium hydroxide, and the like. The parenteral preparation can be enclosed in, for example, ampules, disposable syringes, or multiple dose vials made of glass or plastic, and the like.

[00114] Pharmaceutical compositions suitable for injectable use include, for example, sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion, and the like.
For intravenous administration, suitable carriers include, for example, physiological saline,
bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS), and the like. In all cases, the composition should be fluid to the extent that easy syringability exists. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof, and the like. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, such as, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it can be preferable to include isotonic agents, such as, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride, and the like, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption such as, for example, aluminum monostearate and gelatin, and the like.

[00115] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00116] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets, for example. For oral administration, the agent can be contained in enteric forms to survive the stomach or further coated or mixed to be released in a particular region of the gastrointestinal (GI) tract by known methods. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, or the like. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be
included as part of the composition. The tablets, pills, capsules, troches, and the like can contain any of the following exemplary ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel®, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring, or the like.

[00117] For administration by inhalation, the compounds can be delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer, or the like.

[00118] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives, and the like. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00119] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00120] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems, and the like. Biodegradable, biocompatible polymers can be used, such as, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid, and the like. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811, which is incorporated herein by reference in its entirety.
[00121] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "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 details for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals. Such details are known to those of skill in the art.

[00122] Certain embodiments of the invention include using quantification data from a gene-expression analysis and/or from a mRNA analysis, from a sample of blood, urine, saliva, esophageal tissue, or the like. Embodiments of the invention include not only methods of conducting and interpreting such tests but also include reagents, kits, assays, and the like, for conducting the tests.

[00123] The correlations disclosed herein, between EoE biomarker levels and/or mRNA levels and/or gene expression levels, provide a basis for conducting a diagnosis of EoE, or for conducting a stratification of patients with EoE, or for enhancing the reliability of a diagnosis of EoE by combining the results of a quantification of an EoE biomarker with results from other tests or indicia of EoE. For example, the results of a quantification of one biomarker could be combined with the results of a quantification of one or more additional biomarker, cytokine, mRNA, or the like. Thus, even in situations in which a given biomarker correlates only moderately or weakly with septic shock, the correlation can be one indicium, combinable with one or more others that, in combination, provide an enhanced clarity and certainty of diagnosis. Accordingly, the methods and materials of the invention are expressly contemplated to be used both alone and in combination with other tests and indicia, whether quantitative or qualitative in nature.

[00124] While no animal model can completely mimic human disease, the model described herein can provide an experimental framework to define mechanisms that may be operational in human EoE. The significance of the EoE model described herein in human EoE has been demonstrated by a number of translational studies that demonstrate that a similar mechanism is operational in experimental and human EoE, as the EoE model used has previously provided an experimental system that successfully analyzed anti-IL-5 and anti-IL-13 for human EoE therapy. Therefore, therapeutic target molecules can be predicted based
on the data obtained using murine model of EoE. Murine experimental models are thought to have the potential to uncover the mechanisms operational in the development of esophageal pathological abnormalities in human EoE. The allergen-induced experimental EoE models described herein are relevant to human EoE, as seasonal aeroallergen sensitization and food allergens are implicated in the induction of EoE in humans (Mishra, A. et al. J. Clin. Invest. 107:83-90 (2001); Onbasi, K. et al. Clin. Exp. Allergy 35:1423-31 (2005); Plaza-Martin, A. et al. Allergol. Immunopathol. (Madr) 35:35-7 (2007)).

[00125] The disclosure, figures, and tables herein make mention of statistical significance and "p values." While p values below 0.05 are considered to be statistically significant, it is within the scope of embodiments of the present invention to make use of correlations having a reported p value above 0.05 as well as below 0.05. For example, in a study having a small sample size but a genuine correlation, a p value can be above 0.05, such as, for example, 0.06, 0.07, 0.08, 0.09, 0.10, 0.15, or more. Since p value is affected by sample size, two studies can have the same proportion of outcomes, and a study with a smaller sample size can have a p value above 0.05, while the study with the larger sample size can have a p value below 0.05, even though the correlation is proportionally the same. Thus, while a p value below 0.05, for any sample size, is a strong indication of a statistically significant correlation, a genuine correlation can exist, that is tested with a small sample size, and the p value of such a test can be above 0.05.

[00126] Having described the invention in detail, it will be apparent that modifications, variations, and equivalent embodiments are possible without departing the scope of the invention defined in the appended claims. Furthermore, it should be appreciated that the illustrative embodiments described in the detailed description, drawings, and claims in the present disclosure are provided as non-limiting examples.

EXAMPLES

[00127] The following non-limiting examples are provided to further illustrate embodiments of the invention disclosed herein. It should 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 should, 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.

EXAMPLE 1
IL-15 IS INDUCED IN HUMAN EoE

[0012] IL-15 is increased in the blood and esophageal biopsies of EoE patients (Zhu, X. et al. Gastroenterology 139:182-93 (2010)). Therefore, IL-15 immunoreactivity was tested on non-EoE and EoE patient esophageal biopsy samples.

[0012] EoE patient esophageal biopsies were shown to contain a number of IL-15-positive cells. There were no IL-15-positive cells in the esophageal biopsies of non-EoE patients (Figures 1A-B), whereas a number of IL-15-positive cells were detected in EoE patient biopsies (Figures 1C-D). These data indicate that infiltrating cells are the source of IL-15 and that IL-15 blood levels can be used as a non-invasive diagnostic biomarker for human EoE.

EXAMPLE 2
EoE PATIENTS HAVE INCREASED NUMBERS OF iNKT CELLS

[0013] IL-15 is a growth and survival cytokine for iNKT cells. Therefore, a study was conducted to determine whether esophageal induction of IL-15 causes accumulation of iNKT cells in the esophageal mucosa of EoE patients.

[0013] Esophageal biopsies of normal individuals and EoE patients were stained with various media (Figures 2A-D) to show the cell nuclei of a representative EoE patient esophageal biopsy. Figure 2A shows the cell nuclei through 4',6-diamidino-2-phenylindole (DAPI) staining. Figure 2B shows T cells through anti-CD3 staining. Figure 2C shows NK cells through anti-CD56 staining. Figure 2D shows iNKT cells through co-localization of anti-CD3/anti-CD56 staining.

[0013] These data indicate that iNKT cells are increased in the esophageal mucosa of EoE patients as compared to non-deductible anti-CD3/anti-CD56 double-positive cells in normal individuals. These findings are further confirmed by staining the biopsies with iNKT-specific receptor anti-Jal8/Va24 (Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012)). These data indicate that iNKT cells or iNKT cell receptors can be used as targets for EoE therapy.
EXAMPLE 3
EXPRESSION OF IL-15 mRNA IN THE ESOPHAGUS IS INDUCED BY DOXYCYCLINE IN IL-15 BITRANSGENIC MICE

[00133] IL-15 has a role in EoE pathogenesis and the induced expression of IL-15 mRNA and protein in human EoE (Zhu, X. et al. Gastroenterology 139:182-93 (2010)). Therefore, the mechanism of IL-15-induced EoE pathogenesis can be examined by generating DOX-regulated rtTA-CClO-IL-15 bitransgenic mice.

[00134] An inducible dual-construct expression system was employed to externally regulate IL-15 expression in the lungs and esophagus. Esophageal IL-15 overexpressing bitransgenic mice were developed using rtTA (activator) and CClO-IL-15 (responder) constructs. The rtTA-CClO-IL-15 transgenic mice generated to chronically over-express IL-15 in the esophagus were either exposed to a doxycycline (DOX) diet or a DOX-free diet at 8 weeks old and were then kinetically examined for esophageal B cell activation and proliferation, the induction of Ig class switching genes, and serum levels of IgE and IgG.

[00135] DOX-induced IL-15 was detectable within 4 weeks of continued DOX treatment in the lungs and esophagi of the mice. Initial examination after 4, 8, and 12-weeks of DOX or no-DOX treatment showed a time dependent increase of esophageal eosinophilia in DOX-regulated IL-15 bitransgenic mice, along with the kinetics (Figure 3). These mice can be used to examine EoE pathogenesis induced by chronic, postnatal IL-15.

EXAMPLE 4
IL-15 IS A GROWTH AND SURVIVAL FACTOR FOR MCS/BASOPHILS

[00136] Increased MC counts are found in the esophagi of rtTA-ClO-IL-15 bitransgenic mice. Therefore, IL-15 can be a growth factor for MCs/basophils.

[00137] Freshly isolated bone marrow cells (BMCs) from naive mice were cultured with and without IL-15 (20 ng/ml) and stem cell growth factor (SCF) (10 ng/ml) for 3 weeks at 37°C and 5% CO₂. Fluorescence-activated cell sorting (FACS) analysis indicated that ~65% anti-FcsRLIa positive cell subsets of the culture developed into MCs (FcsRI/C-kit+), while 35.3% developed into basophils (FcsRI/C-kif) (Figure 4). No cells developed into MCs/basophils without IL-15/SCF.

EXAMPLE 5
CHRONIC IL-15 EXPRESSION INDUCES ESOPHAGEAL B CELL AND SERUM IGE LEVELS

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[0013] Local B cell proliferation and serum IgE are promoted following DOX exposure in rtTA-CClO-IL-15 bitransgenic mice. Accordingly, total esophageal cells were isolated, and B cell numbers were examined by flow cytometric analysis, as previously described (Zhu, X. et al. Am. J. Physiol. Gastrointest. Liver Physiol. 297:G550-8 (2009)). Serum IgE levels were determined by performing enzyme-linked immunosorbent assay (ELISA) analyses of mice treated with 6 weeks of a diet of normal food or a diet of food impregnated with DOX.

[0013] Induced B cells and serum IgE were noticeably increased in the DOX-treated mice (Figures 5A-B). These results confirm that increased IL-15 expression in vivo induces esophageal B cell and serum IgE levels.

EXAMPLE 6
IL-15 INDUCES B CELL ACTIVATION AND PROLIFERATION IN VITRO

[0100] Blood and esophageal IL-15, IgE, and B cell levels are increased in EoE patients. Therefore, IL-15 can directly activate and cause proliferation of B cells.

[0101] Purified B cells were exposed to different concentrations of IL-15 in vitro in order to determine the dose-dependent activation and proliferation of B cells in response to IL-15. Flow cytometry was used to determine IL-15 (20 ng/ml)-induced activation of B cells, analyzed using anti-CD69 antibody (Figure 6A), and dose-dependent B cell proliferation, analyzed by thymidine incorporation (Figure 6B).

[0102] IL-15 is shown to have B cell stimulatory activity for B cell activation and proliferation. These results indicate that EoE pathogenesis is B cell-induced and IgE-mediated.

EXAMPLE 7
MC NUMBERS ARE INCREASED IN IL-15 BITRANSGENIC MICE

[0103] Levels of MCs have been shown to be increased in human EoE (Vicario, M. et al. Gut 59:12-20 (2010); Straumann, A. Schweiz Rundsch. Med. Prax. 95:191-5 (2006)). Therefore, IL-15 exposure to BMCs can produce MCs.

[0104] The impact of esophageal IL-15 overexpression on increased esophageal MC numbers was studied. MC numbers in the esophagi of rtTA-CClO-IL-15 mice were examined following an 8-week regimen of a diet of normal food or a diet of food impregnated with DOX. Quantitative morphometric analysis indicated an approximately 3-fold increase in MCs in DOX-exposed rtTA-CClO-IL-15 mice (Figure 7).
EXAMPLE 8
MCS ARE INVOLVED IN EoE PATHOGENESIS

[0105] MCs are localized in the esophageal lamina propria and muscularis mucosa following the induction of EoE in mice. Therefore, induced MCs can promote esophageal muscle cell hyperplasia and hypertrophy in EoE.

[0106] Bromodeoxyuridine (BrdU) incorporation analysis was performed in saline- and allergen-challenged wild type (WT) and MC-deficient (WW) mice. BrdU+ cells were detected in the epithelium of saline-challenged mice (Figure 8A), whereas allergen-challenged mice showed BrdU+ cells in both the epithelium and muscularis mucosa of WT mice (Figure 8B). The morphometric quantitative analysis indicated a significant increase of BrdU+ cells in the muscularis mucosa of allergen-challenged WT mice compared to the allergen-challenged WW mice (Figure 8C).

EXAMPLE 9
B CELLS, MCS, AND IGE+ CELLS ARE INDUCED IN HUMAN EoE

[0107] IgE, B cells, MCs and IgE+ cells have been shown to be induced in the blood and/or esophageal biopsies of EoE patients (Vicario, M. et al. Gut 59:12-20 (2010)). Anti-CD19+ B cells were detected in the esophageal biopsies of EoE patients compared to normal individuals. Significant numbers of B cells were not detected in the esophageal biopsies of normal individuals (Figure 9A); however, a number of B cells and IgE+ cells were detected in the biopsies of EoE patients (Figure 9B). IgE+ cell numbers in the esophageal biopsies of normal and EoE patients were quantified (Figure 9C).

EXAMPLE 10
ESOPHAGEAL STRUCTURE DEVELOPS IN EXPERIMENTAL AND HUMAN EoE


[0109] Esophageal stricture following chronic eosinophilic esophageal inflammation in mice was examined in an experimental murine model of EoE. Esophageal stricture was detected by feeding the mice barium, then subsequently analyzing the gastrointestinal tract x-ray. Esophageal stricture is demonstrated in mice following stomach
IL-5 transgene-induced EoE (Figure 10). This esophageal abnormality was not observed in age- and sex-matched WT mice.

EXAMPLE 11

**IL-15Ra GENE-DEFICIENT MICE ARE PROTECTED FROM EoE INDUCTION**

[0110] IL-15 mRNA and protein production are induced in the esophagus following the induction of experimental and human EoE. Therefore, IL-15-deficiency can protect against induction of EoE in mice.

[0111] Experimental EoE was induced in WT and IL-15Ra-deficient mice following a previously described allergen-based EoE protocol (Mishra, A. *et al.* *J. Clin. Invest.* 107:83-90 (2001)). Eosinophil numbers were then counted in the esophagus after anti-myelin basic protein (MBP) immunostaining (Mishra, A. *et al.* *J. Clin. Invest.* 107:83-90 (2001)). IL-15Ra-deficient mice were shown to have significantly reduced esophageal eosinophilia compared to WT mice in the allergen-induced EoE model (Figure 11). These data indicate that IL-15Ra can be used as a target molecule for EoE therapy.

EXAMPLE 12

**iNKT CELL-DEFICIENT MICE ARE PROTECTED FROM EoE**

[0112] CD1d is an iNKT-specific cell surface molecule, and fluorescence-activated cell sorting (FACS) analysis shows an increase of CD1d-tetramer-positive iNKT cells in the esophagus following intranasal allergen challenge (Rajavelu, P. *et al.* *J. Allergy Clin. Immunol.* In Press (2012)). Therefore, CD1d null mice can be protected from the induction of allergen-induced EoE.

[00140] Experimental EoE was induced in WT and CD1d null mice per an established protocol (Mishra, A. *et al.* *J. Clin. Invest.* 107:83-90 (2001)). Eosinophil numbers were then analyzed in the esophagus following anti-MBP immunostaining (Mishra, A. *et al.* *J. Clin. Invest.* 107:83-90 (2001)).

[0113] CD1d null mice were shown to have significant reductions in EoE compared to WT mice (Figure 12). These data indicate that CD1d or iNKT cell-specific or cell surface molecules, such as Jal8, can be used as targets for EoE therapy.
EXAMPLE 13

ANALYSIS OF BLOOD IL-15 AND CXCL16 LEVELS IN HUMAN EoE

[0114] IL-15 and CXCL16 are induced in the esophageal biopsies of human EoE (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. Proc. Natl. Acad. Sci. Under review (2011)). Therefore, ELISA and real time PCR analysis were performed to determine the protein and mRNA levels of IL-15 and CXCL16 in the blood and blood leukocytes of EoE patients.

[0115] The levels of IL-15 protein (Figure 13A) and mRNA expression of iNKT-specific chemokine CXCL16 (Figure 13B) were found to be significantly induced in the blood of EoE patients compared to those of normal individuals. This indicates that the surface molecules, mediators, and chemokines induced in the esophageal biopsies will also be detected in the blood of EoE patients. Examination of a number of other proposed molecules for mRNA and protein levels in the blood, and blood leukocytes of normal, EoE, and GERD patients establishes possible non-invasive biomarkers for human EoE.

EXAMPLE 14

DETERMINATION OF NON-INVASIVE BIOMARKERS FOR HUMAN EoE

[0116] The blood from patients with EoE typically has a small population of infiltrating mononuclear cells consistent with lymphocytes (Bullock, J. et al. J. Pediatr. Gastroenterol. Nutr. 45:22-31 (2007)), but these cells have not been extensively characterized. Therefore, blood leukocyte subsets of normal individuals and GERD or EoE patients were investigated following flow cytometric and qPCR analysis, in order to determine whether EoE patients have increased levels of IL-15-responsive iNKT cells and B cells, as well as increased mRNA and protein levels of cell-specific receptors, such as IL-15Ra, γδ, Va24, Jal8, and CXCR6, in the blood leukocytes compared to normal individuals or GERD patients in order to determine their potential as non-invasive diagnostic biomarkers for human EoE that also differentiate EoE from GERD. Most of the molecular candidates for monitoring EoE pathogenesis were selected based on their significant roles in EoE, as reported in previous studies (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rajavelu, P. et al. J. Allergy Clin. Immunol. In Press (2012); Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010)) and the results described above.
The blood cells were stained with aGalCer-CD1d-tetramer to identify iNKT cells, anti-B220/CD19 for B cells, anti-γδ for γδ T cells, anti-CD45R for B cell receptors, and anti-CXCR6 for NKT receptors by FACS analysis, as previously described (Zhu, X. et al. Am. J. Physiol. Gastrointest. Liver Physiol. 297:G550-8 (2009)).

Blood RNA was isolated from normal individuals, EoE patients, and GERD patients, and real time quantitative PCR analysis for mRNA levels of the proposed T cell and B cell surface receptor biomarker molecules were examined and normalized with respective mRNA levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as previously described (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010); Zhu, X. et al. Am. J. Physiol. Gastrointest. Liver Physiol. 297:G550-8 (2009)). Potential biomarkers were then compared for their capacity to differentiate EoE from GERD in order to establish solid differential diagnostic parameters for EoE and GERD. The nCounter Analysis System™ (Nanostring Technology, Seattle, WA) was used if necessary, using a novel digital technology that is based on direct multiplexed measurement of gene expression and offers high levels of sensitivity (<1 copy per cell) and precision. The technology uses single-molecule imaging and high numbers of unique transcripts in a single reaction. Such technology permits using the small amount of RNA available from the blood for monitoring the patient disease status following the treatment. Another advantage of the nCounter Analysis™ is that design of the diagnosis chips can be custom designed based on the genes of interest.

Real time PCR analysis was performed to detect mRNA levels of FCERI, FCERII, γδ and αβ T cell receptors (TCRs), CXCR6, ν βi t, Jal8, and Va24 in the blood of normal, EoE, and GERD patients. The relative expression of mRNA levels of these potential biomarkers were found to be either significantly induced or significantly reduced in the blood of EoE patients compared to GERD patients and normal individuals. The mRNA levels of the IgE receptor FcsRII were found to be significantly reduced (Figure 14A), while the mRNA levels of FcsRII (Figure 14B) were found to be significantly induced in the blood of EoE patients compared to GERD patients and normal individuals. The mRNA levels of the γδ T cell receptor were also studied, and both γ and δ were found to be significantly reduced in the blood of EoE patients compared to GERD patients and normal individuals (Figures 14C-D). The mRNA levels of the αβ T cell receptor were also studied, and both a and β were found to be significantly reduced in EoE patients compared to normal individuals and GERD patients (Figures 14E-F). The mRNA levels of the CXCR6 T cell receptor were also found to be
significantly reduced in the blood of EoE patients compared to GERD patients and normal individuals (Figure 14G). The mRNA levels of v β1 and Jα18 were found to be reduced in the blood of EoE patients compared to normal individuals, but these levels did not differentiate GERD patients from EoE patients (Figures 14H-I). The mRNA levels of Va24 were found to be significantly reduced in the blood of EoE patients compared to normal individuals and EoE patients (Figure 14J).

[0120] These data indicate that transcript levels of FCeRI, FCeRII, γδ, αβ, CXCR6, v β1, Jalβ, and Va24 can be used as non-invasive biomarkers for EoE. Further, transcript levels of FCeRI, FCeRII, γδ, αβ, CXCR6, and Va24 can be used to distinguish EoE from GERD.

EXAMPLE 15

DETERMINATION OF ADDITIONAL NON-INVASIVE BIOMARKERS FOR HUMAN EoE

[0121] The relative levels of IL-15 and IL-15Ra mRNA are measured in the blood leukocytes and IL-15 protein in the serum of normal individuals and patients with EoE or GERD, in order to determine whether IL-15 and IL-15Ra mRNA and protein levels in the blood can be used as non-invasive diagnostic biomarkers for EoE capable of differentiating EoE from GERD.

[0122] The IL-15 and IL-15Ra mRNA levels are subsequently compared to those of other Tγ1 and Tγ2 cytokines, such as IL-5, IL-13, and INFγ. The relative expression of cytokine mRNA is quantified by qPCR using the LightCycler instrument (Bio-Rad, Philadelphia, PA), as previously described (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Zhu, X. et al. Am. J. Physiol. Gastrointest. Liver Physiol. 297:G550-8 (2009); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010)). The results are normalized by amplified GAPDH of same cDNA and expressed as fold induction.

[0123] Additionally, IL-5, IL-13, IL-15, and INFγ protein and mRNA levels are examined in serum from normal individuals and patients with EoE or GERD using a commercially available ELISA kit (R&D Systems, Minneapolis, MN). Increased or decreased levels of IL-15 and IL-15Ra mRNA and protein (compared to other Tγ2 cytokines) in the blood of EoE patients compared to GERD patients or normal individuals allow these molecules to be used as non-invasive biomarkers for EoE.
The mRNA and protein levels of CXCL16 are also examined in blood obtained from normal individuals and EoE or GERD patients by performing qPCR, as previously described (Zhu, X. et al. Gastroenterology 139:182-93 (2010); Zhu, X. et al. Am. J. Physiol. Gastrointest. Liver Physiol. 297:G550-8 (2009); Rayapudi, M. et al. J. Leukoc. Biol. 88:337-46 (2010)), in order to determine whether EoE patients have increased mRNA and protein levels of the iNKT-specific chemokine CXCL16 compared to normal individuals and GERD patients. The soluble CXCL16 is subsequently measured in the blood using a commercially available ELISA kit (R&D Systems).

Soluble TGF-β in the blood of these patients is also measured in blood obtained from normal individuals and EoE or GERD patients by qPCR, in order to determine whether EoE patients have increased mRNA and protein levels of TGF-β compared to normal individuals and GERD patients.

EXAMPLE 16
USE OF IL-15 AND INKT CELL MOLECULES AS DIAGNOSTIC AND THERAPEUTIC INTERVENTIONS FOR EoE

Based on the above findings, IL-15 and iNKT cells can be studied in order to determine potential target molecules for therapeutic interventions. The proposed molecules are tested in the food allergen- and aeroallergen-induced murine model of experimental EoE using specific humanized neutralizing monoclonal antibodies.

A neutralizing antibody therapy found to be successful in a mouse model of experimental EoE provides an experimental framework to support clinical trials of these molecules in human EoE. The anti-IL-15, anti-IgE, anti-CD1d, anti-Va24Jal8, anti-CXCL16, and anti-IL-15Ra neutralized and non-neutralized mice are tested for EoE pathogenesis following the food allergen- or aeroallergen-induced experimental EoE protocol (Rajavelu, P. et al. AJP-Gastroenterology Online published on Dec 26, In Press (2010); Mishra, A. et al. J. Clin. Invest. 107:83-90 (2001)). Food allergen- and aeroallergen-induced experimental EoE is induced in mice neutralized with anti-IL-15 and anti-IL-15Ra neutralized or non-neutralized mice, along with isotype-matched IgGs, as previously described (Mishra, A. et al. J. Clin. Invest. 107:83-90 (2001); Blanchard, C. et al. Clin. Exp. Allergy 35:1096-103 (2005)), in order to determine whether neutralizing IL-15 or IL-15Ra antibody treatment prior to aeroallergen challenge can protect mice from developing allergen-induced experimental EoE.

Human clinical trials using the humanized antibodies identified to be effective in the murine model are therefore conducted. As an alternative to single antibody treatment, a combination of antibodies, such as a combination of antibodies to the chemokine CXCL16 and iNKT cell-specific molecules in experimental EoE, can also be utilized.

All experimental data are expressed as mean ± standard deviation (SD). Statistical significance was performed with GraphPad Prism for Windows version 4.0 (GraphPad Software, San Diego, CA). Studying approximately 40 individuals each of EoE patients, GERD patients, and normal individuals provides a power of 80% to detect a difference of at least 40% among the groups. The chi-square test was used to compare the proportion of positive markers between EoE patients, GERD patients, and normal individuals. Disease activity was correlated with biomarker levels obtained from extracts of blood or biopsy samples. Statistical significance comparing different treatments or groups was determined by the Student t test (normal distribution equal variance), Welch t test (normal distribution, unequal variance), Mann-Whitney U test (nonparametric test, two groups), ANOVA (parametric), or Kruskal-Wallis and Dunn's multiple comparison tests (nonparametric test, multiple groups).

The various methods and techniques described above provide a number of ways to carry out the application. Of course, it is to be understood that not necessarily all objectives or advantages described can be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art will recognize that the methods can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages.
as taught or suggested herein. A variety of alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several features, while others specifically exclude one, another, or several features, while still others mitigate a particular feature by inclusion of one, another, or several advantageous features.

[0132] Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be employed in various combinations by one of ordinary skill in this art to perform methods in accordance with the principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

[0133] Although the application has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the embodiments of the application extend beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and modifications and equivalents thereof.

[0134] In some embodiments, the numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, used to describe and claim certain embodiments of the application are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the application are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable.

[0135] In some embodiments, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be
performed in any suitable order unless otherwise indicated herein or otherwise clearly
contradicted by context. The use of any and all examples, or exemplary language (for
example, "such as") provided with respect to certain embodiments herein is intended merely
to better illuminate the application and does not pose a limitation on the scope of the
application otherwise claimed. No language in the specification should be construed as
indicating any non-claimed element essential to the practice of the application.

[0136] Preferred embodiments of this application are described herein, including
the best mode known to the inventors for carrying out the application. Variations on those
preferred embodiments will become apparent to those of ordinary skill in the art upon reading
the foregoing description. It is contemplated that skilled artisans can employ such variations
as appropriate, and the application can be practiced otherwise than specifically described
herein. Accordingly, many embodiments of this application include all modifications and
equivalents of the subject matter recited in the claims appended hereto as permitted by
applicable law. Moreover, any combination of the above-described elements in all possible
variations thereof is encompassed by the application unless otherwise indicated herein or
otherwise clearly contradicted by context.

[0137] All patents, patent applications, publications of patent applications, and
other material, such as articles, books, specifications, publications, documents, things, and/or
the like, referenced herein are hereby incorporated herein by this reference in their entirety
for all purposes, excepting any prosecution file history associated with same, any of same that
is inconsistent with or in conflict with the present document, or any of same that may have a
limiting affect as to the broadest scope of the claims now or later associated with the present
document. By way of example, should there be any inconsistency or conflict between the
description, definition, and/or the use of a term associated with any of the incorporated
material and that associated with the present document, the description, definition, and/or the
use of the term in the present document shall prevail.

[0138] In closing, it is to be understood that the embodiments of the application
disclosed herein are illustrative of the principles of the embodiments of the application.
Other modifications that can be employed can be within the scope of the application. Thus,
by way of example, but not of limitation, alternative configurations of the embodiments of
the application can be utilized in accordance with the teachings herein. Accordingly,
embodiments of the present application are not limited to that precisely as shown and
described.
CLAIMS

What is claimed is:

1. A method of treating a patient with eosinophilic esophagitis (EoE), the method comprising:

   obtaining a sample from a patient;
   analyzing the sample to determine a level of one or more biomarkers associated with EoE;
   determining whether the level of the one or more biomarkers associated with EoE is up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE results in the patient being diagnosed with EoE; and
   treating the patient with an appropriate therapeutic strategy based upon the diagnosis.

2. The method of Claim 1, wherein the one or more biomarkers associated with EoE are selected from the group consisting of one or more cytokines, IL-15-responsive iNKT, T, and B cell receptors, chemokines, mediators, and IgE receptors.

3. The method of Claim 2, wherein the one or more cytokines comprise IL-5, IL-13, IL-15, INFγ, and/or TGF-β.

4. The method of Claim 2, wherein the one or more IL-15 responsive iNKT, T, and B cell receptors comprise IL-15Ra, γδ, αβ, CD1d, Va24, ν βι 1, and/or Jal8.

5. The method of Claim 2, wherein the one or more chemokines comprise CXCR6 and/or CXCL16.

6. The method of Claim 2, wherein the one or more mediators comprise IgE.

7. The method of Claim 2, wherein the one or more IgE receptors comprise FcεRI and/or FcεRII.

8. The method of Claim 1, wherein the one or more biomarkers associated with EoE comprise IL-5, IL-13, IL-15, INFγ, TGF-β, IL-15Ra, γδ, αβ, CD1d, Va24, ν βι 1, Jal8, CXCR6, CXCL16, IgE, FcεRI, and FcεRII.

9. The method of Claim 1, wherein the one or more biomarkers associated with EoE
comprise IL-15 and/or CXCL16.

10. The method of Claim 1, wherein the one or more biomarkers associated with EoE comprise IL-15.

11. The method of Claim 8, wherein the presence of an elevated level of γδ, αβ, Va24, CXCR6, FCeRI, and/or FCeRII results in the patient being diagnosed with EoE.

12. The method of Claim 8, wherein the presence of a reduced level of νβαί and/or Jα18 results in the patient being diagnosed with EoE.

13. The method of Claim 1, wherein the mRNA level of the one or more biomarkers associated with EoE is determined.

14. The method of Claim 1, wherein the protein level of the one or more biomarkers associated with EoE is determined.

15. The method of Claim 1, wherein the determination of whether the level(s) of the one or more biomarkers associated with EoE are elevated or reduced relative to a level of the one or more biomarkers measured in a normal individual is combined with a determination of a level(s) of one or more additional biomarkers associated with EoE.

16. The method of Claim 15, wherein the one or more additional biomarkers associated with EoE comprises an mRNA biomarker.

17. The method of Claim 15, wherein the one or more additional biomarkers associated with EoE comprises eotaxin-3.

18. The method of Claim 1, wherein the sample is an esophageal tissue sample.

19. The method of Claim 1, wherein the sample comprises a plasma or serum sample.

20. The method of Claim 1, wherein the appropriate therapeutic strategy for a patient diagnosed with EoE comprises allergen removal, steroid treatment, dietary management, proton pump inhibitor (PPI) therapy, administration of one or more topical glucocorticoid, administration of one or more humanized antibody against one or more relevant cytokines and/or mediators, administration of one or more small molecule inhibitors of an eosinophil and/or allergic disease activation pathway, administration of one or more small molecule inhibitors capable of modulating levels of one or more biomarkers associated with EoE, and/or any combination thereof.
21. The method of Claim 20, wherein the topical glucocorticoid comprises fluticasone, budesonide, and/or ciclesonide.

22. The method of Claim 20, wherein the humanized antibody against one or more relevant cytokines and/or mediators comprises an antibody targeting one or more biomarkers associated with EoE.

23. The method of Claim 22, wherein the one or more biomarkers associated with EoE comprise IL-5, IL-13, INFy, TGF-β, IL-15Ra, γδαβ, CDld, Va24, νβγδ, Jal8, CXCR6, CXCL16, IgE, FCeRI, and/or FCeRII.

24. The method of Claim 22, wherein the humanized antibody against one or more relevant cytokines and/or mediators is selected from the group consisting of anti-IL-15, anti-IgE, anti-CDld, anti-Va24Jal8, anti-CXCL16, and anti-IL-15Ra.

25. The method of Claim 1, further comprising a determination of eosinophilic esophagitis or GERD.

26. The method of Claim 25, wherein the presence of reduced level of Vail and Jal8 in combination with a non-elevated or non-reduced level of one or more additional biomarkers associated with eosinophilic esophagitis results in the patient being diagnosed with GERD.

27. A method of diagnosing a patient with eosinophilic esophagitis (EoE), the method comprising:

   obtaining a sample from a patient;

   analyzing the sample to determine a level of one or more biomarkers associated with EoE; and

   determining whether the level of the one or more biomarkers are up-regulated or down-regulated relative to a level of the one or more biomarkers measured in a normal individual, wherein the presence of an elevated or reduced level of one or more biomarkers associated with EoE results in the patient being diagnosed with EoE.

28. A diagnostic kit, test, or array, comprising materials for quantification of at least two analytes, wherein the at least two analytes are biomarkers associated with eosinophilic esophagitis (EoE).

29. The diagnostic kit, test, or array of Claim 28, wherein the at least two analytes are selected from the group consisting of one or more cytokines, IL-15 responsive iNKT, T, and
B cell receptors, chemokines, mediators, and IgE receptors.

30. The method of Claim 29, wherein the one or more cytokines comprise IL-5, IL-13, IL-15, INFγ, and/or TGF-β.

31. The method of Claim 29, wherein the one or more IL-15 responsive iNKT, T, and B cell receptors comprise IL-15Ra, γδ, αβ, CD1d, Va24, νβ1, and/or Jα18.

32. The method of Claim 29, wherein the one or more chemokines comprise CXCR6 and/or CXCL16.

33. The method of Claim 29, wherein the one or more mediators comprise IgE.

34. The method of Claim 29, wherein the one or more IgE receptors comprise FcεRI and/or FcεRII.

35. The diagnostic kit, test, or array of Claim 29, wherein the diagnostic kit, test, or array comprises a gene chip.

36. The diagnostic kit, test, or array of Claim 35, wherein the gene chip comprises a low density array.

37. The diagnostic kit, test, or array of Claim 29, wherein the diagnostic kit, test, or array comprises a surface with a DNA array.
Fig. 13

RNA: CCL16/GADDH
Relative Expression

EE
Normal

50
40
30
20
10
0
IL-1B (pg/ml)

A

10
8
6
4
2
0

B

9/15
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; A61K 39/395 (2013.01)
USPC - 435/6.17; 424/142.1

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68; A61K 39/395 (2013.01 )
USPC: 435/6.17, 6.1, 4; 424/142.1 , 173.1

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>US 2012/004205 A1 (ROTHENBERG, ME) January 5, 2012; abstract; paragraphs [0007], [0009], [0012], [0013], [0016]-[0018], [0026], [0032], [0046], [0048]</td>
<td>1-3, 8, 13-21, 25, 27-30, 35, 37</td>
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<tr>
<td>Y</td>
<td>US 2009/0269774 A1 (ROTHENBERG, ME et al.) October 29, 2009, abstract; paragraph [0017], Table 1</td>
<td>4, 5, 9, 10, 31, 32</td>
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<td>Y</td>
<td>WO 2011/034597 A1 (FIEBIGER, EE et al.) March 24, 2011; abstract; page 4, line 25 to page 5, line 6; page 24, line 20 to page 25, line 3; page 25, lines 4-5</td>
<td>6, 7, 11, 22-24, 33, 34</td>
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Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search
22 July 2013 (22.07.2013)

Date of mailing of the international search report
29 JUL 2013

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