World Allergy Forum Symposium: 
The Application of Monoclonal Therapies and 
Therapeutics to Asthma and Allergy

2008 AAAAI Annual Meeting 
Sunday, 16 March 2008 
10:45 a.m. – 12:00 p.m.

Philadelphia Convention Center 
Room 114, Street Level 
Philadelphia, PA, USA

Moderators: 
G. Walter Canonica, MD 
Thomas B. Casale, MD FAAAAI 

Designer Drug Design: How are Monoclonal Antibodies Made in 2008? 
Shyam S. Mohapatra, PhD FAAAAI 

Adverse Effects in the Application of Biotechnology 
Jean Bousquet, MD FAAAAI 

The Future Application of Monoclonal Antibodies to Asthma and Allergy 
Stephen T. Holgate, MD DSc FAAAAI 

The World Allergy Organization (WAO) is an international organization of 77 regional and national allergy and clinical immunology societies. WAO’s mission is to be a global resource and advocate in the field of allergy, advancing excellence in clinical care through education, research and training as a worldwide alliance of allergy and clinical immunology societies.
“The Application of Monoclonal Therapies and Therapeutics to Asthma and Allergy”

Program

Moderators:
G. Walter Canonica, MD
University of Genoa
Genoa, Italy

Thomas B. Casale, MD FAAAAI
Creighton University
Omaha, NE, United States

1. Welcome to the World Allergy Forum Symposium and Introduction to “The Application of Monoclonal Therapies and Therapeutics to Asthma and Allergy”
   G. Walter Canonica and Thomas B. Casale

2. Designer Drug Design: How are Monoclonal Antibodies Made in 2008?
   Shyam S. Mohapatra, PhD FAAAAI
   University of South Florida
   Tampa, FL, United States

2. Adverse Effects in the Application of Biotechnology
   Jean Bousquet, MD FAAAAI
   Hôpital Arnaud De Villeneuve
   Montpellier, France

3. The Future Application of Monoclonal Antibodies to Asthma and Allergy
   Stephen T. Holgate, MD DSc FAAAAI
   Southampton General Hospital
   Southampton, United Kingdom

Upon completion of this session, participants should be able to:
Discuss the basic science of the hybridoma technique;
Describe the reasons for failure of monoclonal antibody treatment;
Describe the potential application of monoclonal antibody technology to asthma and allergy.

2008-2009 World Allergy Forum Advisory Board

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ABOUT THE WORLD ALLERGY ORGANIZATION

WORLD ALLERGY ORGANIZATION (WAO)
The World Allergy Organization (WAO) is an international umbrella organization of 77 regional and national allergy and clinical immunology societies. By collaborating with member societies, WAO provides direct educational outreach programs, symposia and lectureships to WAO individual members around the globe.

THE WORLD ALLERGY ORGANIZATION MISSION
To be a global resource and advocate in the field of allergy, advancing excellence in clinical care through education, research and training as a world-wide alliance of allergy and clinical immunology societies.

PROGRAMS OF THE WORLD ALLERGY ORGANIZATION

GLORIA Global Resources in Allergy™
www.worldallergy.org/gloria
The GLORIA program promotes good practice in the management of allergic diseases through programs developed by panels of world experts. GLORIA educates medical professionals worldwide through regional and national presentations and local training initiatives. GLORIA educational modules promote the World Allergy Organization’s (WAO) mission – to optimize allergy care worldwide.

GLORIA MODULES
Module 1: Allergic Rhinitis
Module 2: Allergic Conjunctivitis
Module 3: Allergic Emergencies
Module 4: Immunotherapy
Module 5: Treatment of Severe Asthma
Module 6: Food Allergy
Module 7: Angioedema
Module 8: Anaphylaxis
Module 9: Diagnosis of IgE Sensitization
Module 10: Chronic Rhinosinusitis and Nasal Polyposis
Module 11: Drug Allergy

WORLD ALLERGY FORUM (WAF)
www.worldallergy.org/waf
WAF symposia are held at major international allergy meetings. Developed by international expert advisory panels, the symposia provide up-to-the-minute presentations on scientific and clinical developments in the field of allergic disease.

EMERGING SOCIETIES PROGRAM
www.worldallergy.org/esp
WAO offers advice on initiating and developing allergy societies throughout the world. This proactive initiative aims to expand and improve the specialty of allergy by supporting colleagues working in the field of allergy worldwide. Through sharing practical experiences and alerting new societies to the criteria required for WAO membership, ESM creates relationships with future World Allergy Organization member societies, and educates WAO’s leadership about the challenges and opportunities faced by colleagues in developing countries.

WORLD ALLERGY ORGANIZATION JOURNAL
www.waojournal.org
World Allergy Organization Journal is the official publication of the World Allergy Organization. An international online-only journal, World Allergy Organization Journal underscores WAO’s commitment to raising awareness and advancing excellence in clinical care, education, research and training in the field of allergy.
## WAO Member Societies

### National Member Societies

- Albanian Society of Allergology and Clinical Immunology
- American Academy of Allergy, Asthma and Immunology
- American College of Allergy, Asthma and Immunology
- Argentine Association of Allergy and Immunology
- Argentine Society of Allergy and Immunopathology
- Australasian Society of Clinical Immunology and Allergy
- Austrian Society of Allergology and Immunology
- Azerbaijani Society for Asthma, Allergy and Clinical Immunology
- Bangladesh Society of Allergy and Immunology
- Belgian Society of Allergology and Immunology
- Brazilian Society of Allergy and Immunopathology
- British Society for Allergy and Clinical Immunology
- Bulgarian National Society of Allergology
- Canadian Society of Allergy and Clinical Immunology
- Chilean Society of Allergy and Immunology
- China Allergology Society and Chinese Allergists
- (Chinese) Hong Kong Institute of Allergy
- Colombian Allergy, Asthma, and Immunology Association
- Croatian Society of Allergology and Clinical Immunology
- Cuban Society of Allergology
- Czech Society of Allergology and Clinical Immunology
- Danish Society for Allergology
- Egyptian Society of Allergy and Clinical Immunology
- Egyptian Society of Pediatric Allergy and Immunology
- Finnish Society of Allergology and Clinical Immunology
- French Society of Allergy and Clinical Immunology
- Georgian Association of Allergology and Clinical Immunology
- German Society for Allergy and Clinical Immunology
- Hellenic Society of Allergology and Clinical Immunology
- Hungarian Society of Allergology and Clinical Immunology
- Icelandic Society of Allergology and Immunology
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- Indonesian Society for Allergy and Immunology
- Israel Association of Allergy and Clinical Immunology
- Italian Association of Territorial and Hospital Allergists
- Italian Society for Allergology and Clinical Immunology
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- Peruvian Society of Allergy and Immunology
- Philippine Society of Allergy, Asthma and Immunology
- Polish Society of Allergology
- Portuguese Society of Allergology and Clinical Immunology
- Romanian Society of Allergology and Clinical Immunology
- Russian Association of Allergology and Clinical Immunology
- Association of Allergy and Clinical Immunology for Serbia and Montenegro
- Singapore Society of Immunology, Allergy & Rheumatology
- Allergy Society of South Africa
- Spanish Society of Allergology and Clinical Immunology
- Swiss Society of Allergology and Immunology
- Allergy and Immunology Society of Thailand
- Turkish National Society of Allergy and Clinical Immunology
- Ukrainian Association of Allergologists and Clinical Immunologists
- Uruguayan Society of Allergology
- Venezuelan Society of Allergy and Immunology
- Vietnam Association of Allergy, Asthma and Clinical Immunology
- Zimbabwe Allergy Society

### Associate Member Societies

- Ecuadorian Society of Allergology and Affiliated Sciences
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- Slovenian Association for Allergology and Clinical Immunology
- Allergy & Immunology Society of Sri Lanka
- Swedish Association for Allergology

### Regional Organizations

- The Asia Pacific Association of Allergology and Clinical Immunology
- Commonwealth of Independent States (CIS Society)
- European Academy of Allergology and Clinical Immunology
- Latin American Society of Allergy and Immunology

### Affiliate Organizations

- International Association of Asthma

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Dear Colleagues,

Welcome to Philadelphia, and to the 31st Symposium in the World Allergy Forum series! The longest-running educational program of the World Allergy Organization has been proud to be part of the AAAAI meeting every year since 1997, when Bill Busse and Larry Lichtenstein chaired our first symposium on the Cellular Mechanisms and Treatment of Allergic Rhinitis. Starting as a program with a focus on allergic rhinitis, World Allergy Forum has broadened over the years to encompass the scientific and clinical aspects of every major topic in allergy that is of interest to the practicing allergist.

Our topic today is the Application of Monoclonal Therapies and Therapeutics to Asthma and Allergy. We are starting to gain a good body of experience with the first licensed humanized monoclonal antibody against IgE, with a current indication for use in moderate to severe asthma in adults and adolescents. Will this therapy be safe for use in children, and if so, what considerations will inform our prescription of immunomodulators for pediatric patients? How amenable will other atopic diseases be to treatment with anti-IgE – will we see its application widened for use in allergic rhinoconjunctivitis, anaphylaxis, or eczema? How likely is it that humanized monoclonal antibodies such as anti-IL-5 and anti-TNFα, soluble IL-4 receptors, etc. will become part of our armamentarium, and what other monoclonals directed against the cytokines and mediators involved in allergic inflammation are in the pipeline? We are all excited by the theoretical concept of these new directions, but what are the potential problems associated with the clinical use of immunomodulators and biotechnology therapies?

Our excellent faculty will guide us through this evolving picture. Shyam Mohapatra will start the session by discussing how monoclonal antibodies are designed. Jean Bousquet will then give us an insight into the pitfalls that may influence the future availability and prescribing of these products. Stephen Holgate will conclude the symposium by considering the future application of monoclonal antibodies to asthma and allergy. It promises to be a great program, and we look forward to your comments and questions.

With best regards,

G. Walter Canonica, MD
President
World Allergy Organization

Thomas B. Casale, MD, FAAAAI
President
American Academy of Allergy, Asthma and Immunology
Since Paul Ehrlich, who first presented the concept that antibodies could be exploited in therapy, it took four decades before technological advances allowed the exploration of the potential of antibodies for immunotherapeutic applications. In the late 70s, Nobel laureates Kohler and Milstein introduced the monoclonal antibody technology, which was further revolutionized by advances in DNA technology, that led to the ability to tailor and manipulate the immunoglobulin molecule for specific functions and in vivo properties.

The ‘state of the art technology’ includes combinatorial chemistry, DNA mutagenesis, and the ability to fuse “display and selection” systems in the same setting; which allow physical linking of the mutated gene with its encoded proteins, making easier the recovery of the antibody with the desired properties and specificities. They enable conversion of a mouse-derived monoclonal complete antibody (Fc-(Fab)2) to different combinations or designs of “humanized” versions. These tools allow construction of antibodies comprising just the portion of the antibody with the antigen-binding property made up of both a heavy and light chain (Fv, single chain Fv – scFv) to larger engineered arrangements resulting from the multimerization of these Fv or scFv formats (diabodies, triabodies, tetrabodies). These same tools permit the construction of antibodies or antibody fragments having multiple specificity, which potentially favour the recognition of more than one antigen, the increase in the avidity of the antibody, the cross-activation of immune cells, or bridging an immune cell with its target. The engineering of these formats increase their affinity, stability and clearance time, reduce complications associated to undesirable immune reactions and make the new antibody versions carry drugs to their intended targets. Furthermore, current in vitro antibody generating systems utilize ribosomal display, phage display, yeast surface display, and mammalian cell display, which are capable of generating large and diverse libraries of clones (1010) expressing several combinations of Fv encoding genes. Depending on the display system, robotics or flow cytometry have facilitated the handling of many different clones to select those expressing the Fv with promising affinity properties. The Fv-encoding genes are mutated using directed mutagenesis approaches – CDR walking, windows mutagenesis, site-directed mutagenesis, hotspots CDR, and site saturation mutagenesis – or random mutagenesis approaches – error prone PCR, EvoGene, and DNA shuffling – or a combination of both. Moreover, a plethora of in vivo technologies allow antibody engineering at the level of single cells such as Escherichia coli, mammalian cells or whole animals. The success of an application of these technologies is evident from the recent approval by FDA of the Panitumumab, a fully human antibody directed against the epidermal growth factor receptor, which is obtained from transgenic mice expressing human antibody repertoires. These technological advances combined with the FDA fast-tracking policy are expected to expand the application of these new formats both in diagnostic kits for disease biomarkers and in therapeutic scenarios.
Designer antibodies: How are the monoclonal antibodies made to become drugs?

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History

- Since Paul Ehrlich, who first presented the concept that antibodies could be exploited in therapy, it took four decades before technological advances allowed the exploration of the potential of antibodies for immunotherapeutic applications.
- In the late 70s, Nobel laureates Kohler and Milstein introduced the monoclonal antibody technology.
- Advances in DNA technology and antibody engineering revolutionized our ability to tailor and manipulate the immunoglobulin molecule for specific functions and in vivo properties.
Foundations of Modern Technology to Produce Antibodies

- Display and Selection Systems
- Combinatorial Chemistry
- DNA Mutagenesis

Display and Selection Systems

A. Phage Display
B. Yeast / Cell Mammalian Display
C. Ribosomal Display

Key: To physically link the scFv or Fab of interest with the gene that encodes them.
Scheme Selection Amplification

Mutagenesis (Fine Tuning)

(a, b): Diversity mainly located at CDR3 (H3 and L3). Diversity is engineered by several means using directed (CDR walking [c, d], hot spot mutations [e, f]) and random mutagenesis (error-prone PCR [g, h] and DNA shuffling [i, j]). Moreover, in vivo mutagenesis could be done using E. coli mutator strains [k].

Robotics
Give me a hand

(a) Source of target molecules
- Recombinant proteins or synthetic compounds
- Expression systems (E. coli, yeast or mammalian cell culture)
- Yeast or E. coli (protoplasts, in vitro modifications)

(b) Phage library format and diversity
- Eukaryotic cells
- Low, medium or high complexity

(c) Phage selection and expression platforms
- Microtiter plates or magnetic particles
- Solution- or plate-based automated handling

(d) Phage propagation format
- On- or off-plates
- Large volume cultures
- Microtiter plates
Possibilities & Opportunities

- These technologies will synergize and spring forward the discovery and the production of new antibody combinations or design of "humanized" versions.

- These advances combined with the FDA fast-tracking policy are expected to expand:
  - Diagnostic kits – biomarkers
  - Therapeutic compounds
Adverse Effects in the Application of Biotechnology

Jean Bousquet & Marc Humbert
University Hospital and INSERM, Montpellier and University Hospital, Clamart Montpellier, France

Since the 1986 regulatory approval of muromonab-CD3, a mouse monoclonal antibody (MAb) directed against the T cell CD3ε antigen, MAbs have become an increasingly important class of therapeutic compounds in a variety of disease areas ranging from cancer, autoimmune, infectious and cardiac diseases as well as asthma. The limitation of murine MAbs due to immunogenicity was overcome by replacement of the murine sequences with their human counterpart leading to the development of chimeric, humanized, and human therapeutic MAb (1, 2). Remarkable progress has also been made following the development of the display technologies, enabling engineering of antibodies with modified properties such as molecular size, affinity, specificity, and valency. Moreover, antibody engineering technologies are constantly advancing to enable further tuning of the effector function and serum half life. Optimal delivery to the target tissue still remains to be addressed to avoid unwanted side effects as a result of systemic treatment while achieving meaningful therapeutic effect.

Other biological agents-like cytokines and fusion proteins as treatment modalities for a number of immune mediated and malignant diseases has also yielded great promise, but there are very few trials in asthma.

1- Efficacy of biologics: from concept to asthma

• Anti-IgE MAb

Immunoglobulin E (IgE) is increasingly recognized as a key component of asthma pathophysiology and contributes to both the early and late-phase inflammatory cascade of the airways by inhibiting allergen-induced activation of mast cells. Omalizumab binds free IgE and inhibits mast-cell degranulation. By reducing free IgE, omalizumab also downregulates FceRI on basophils and mast cells (3-5). These dual effects of omalizumab are important, as without FceRI downregulation almost complete removal of free IgE would be necessary to elicit functional consequences on mast cells and basophils. Moreover, unexpectedly, omalizumab considerably decreases the overall airways inflammation in asthma (6).

In patients with allergic asthma, omalizumab significantly reduces both the early phase and late phase asthmatic response to allergen challenge (7). A large number of randomized trials demonstrated that omalizumab is effective in reducing asthma symptoms and improving quality-of-life while reducing the need for inhaled corticosteroids (8). In patients with severe uncontrolled allergic asthma, omalizumab reduces severe exacerbations and hospitalizations (9-11), and the biologic is approved by both FDA and EMEA. Moreover, it has been suggested that the effect may persist after treatment (12).

• Anti-CD4 MAb

CD4+ T cells are likely to be involved as a source of pro-inflammatory cytokines in asthma. Keliximab, an anti-CD4 MAb, leads to a transient reduction in the number of CD4+ T cells and modulation of CD4+ receptor expression in severe asthmatics. The effects of keliximab may be mediated through a decrease in CD4+ surface expression and Tlymphocyte numbers, in addition to a reduction in allergen-induced lymphocyte numbers, in addition to a reduction in allergen-induced lymphocyte numbers. However, this clinical effects were modest (13) and the development of the MAb was stopped.

• Anti-IL-5 MAb

Eosinophils in atopic diseases and hypereosinophilic syndrome are often associated with a high expression of interleukin-5 (IL-5). IL-5 plays an important role in regulating the production, differentiation, recruitment, activation, and survival of eosinophils. Therefore, neutralizing IL-5 with an antibody was a promising therapeutic strategy in eosinophilic diseases (15). A very large number of animal studies suggested that anti-IL-5 MAb could be an effective asthma treatment (16). A first study using bronchial challenge did not show any efficacy in the late phase reaction and non-specific bronchial hyperreactivity following challenge (17). Mepolizumab treatment does not appear to add significant clinical benefit in patients with asthma with persistent symptoms despite inhaled corticosteroid therapy (18). These studies may indicate that eosinophil recruitment is not only driven by IL-5 (19) or that eosinophils do not have the major role proposed (20). However, the effects of IL-5 appear to be mainly in the circulation, inducing peripheral mobilization of eosinophils to the circulation without less effect on eosinophil mobilization in the lungs (21).

Moreover, the role of anti-IL-5 MAb may not be totally ruled out since it was found that remodeling may be reduced by anti-IL-5 MAb (22). Moreover, in patients with hypereosinophilic syndrome (23) and eosinophilic esophagitis, anti-IL-5 MAb resulted in an improvement of symptoms (15). Some patients with very high eosinophilic inflammation and nasal polyps may also benefit from anti-IL-5 MAb (24, 25). Further studies are needed to investigate the effect of mepolizumab on exacerbation rates, using protocols specifically tailored to patients with asthma with persistent airway eosinophilia.

• Anti-IL-4 biologics

Increases in T helper (Th) 2 cytokine concentrations have been seen in atopic asthma, with IL-4 and IL-13 thought to have a role in asthma and studies in animals suggest a role for this target (26). Although initial studies targeting IL-4 were disappointing (27, 28), a new study with a double inhibition may be more promising. Pitrakinra, an IL-4 variant that targets allergic Th2 inflammation by potently inhibiting the binding of IL-4 and IL-13 to IL-4Rα receptor complexes, was found to have some effect in phase 2 trials in asthma (29).
Adverse Effects in the Application of Biotechnology

**Anti-TNFα biologics**

It has been suggested that some of the features of severe asthma might be due to upregulation of the tumor necrosis factor-α (TNF-α) pathway. In support of this, studies have shown that severe asthma is associated with an increased presence of TNF-α within the airway and an increase in TNF-α expression on peripheral blood mononuclear cells. Moreover, TNF-α has the ability to induce several of the pro-inflammatory changes associated with severe asthma, including neutrophilic inflammation (30). Interest in the role of TNF-α in severe asthma has increased following a small crossover clinical trial (31) and an open study which have suggested that etanercept is effective in asthma (32). However, a large randomized clinical trial using golimumab in patients with severe asthma did not confirm these optimistic results.

**Safety of biologics**

Biological agents-like cytokines, MAb and fusion proteins can cause a great variety of adverse side-effects. Based on the peculiar features of biological agents a new classification of these adverse side-effects of biological agents was proposed - related but clearly distinct from the classification of side-effects observed with chemicals and drugs. This classification differentiated five distinct types, namely clinical reactions because of high cytokine levels (Type α), hypersensitivity because of an immune reaction against the biological agent (β), immune or cytokine imbalance syndromes (γ), symptoms because of cross-reactivity (δ) and symptoms not directly affecting the immune system (ε) (33) (Figure 1). This classification could help to better deal with the clinical features of these side-effects, to identify possible individual and general risk factors and to direct research in this novel area of medicine.

**Hypersensitivity reactions**

Monoclonal antibodies can be derived from several sources: murine antibodies (e.g. OKT-3), chimeric (e.g. infliximab), humanized (e.g. omalizumab) or human (e.g. adalimumab). Two forms of reactions have been identified: acute and delayed. They appear to be related to the presence of antibodies to the MAb (against murine (34) or human components), but many reactions do not appear to be anaphylactic (IgE mediated) (33, 35).

TNF-α antagonists (infliximab, etanercept, adalimumab) are widely used in rheumatoid arthritis and several other inflammatory diseases. Both immediate and delayed adverse reactions have been described, suggesting type I and T cell-mediated mechanisms (36). These reactions even occur in children (37). Mild to moderate reactions following injection occur in 29.3% patients with etanercept (38) and 15.3% with adalimumab (39, 40). Some studies describe histological findings of a cell-mediated Th1 reaction, with CD8+ T cells composing the majority of the dermal infiltrate (41). Histological features of eosinophilic cellulitis as a response to etanercept suggest a Th2-mediated phenomenon (42). Immediate positive skin tests against etanercept have only been demonstrated recently (43). Adalimumab, a human mAb against TNF-α, was not found to induce anaphylactic reactions and can be used in patients allergic to infliximab (44). However, delayed type reactions were observed using intradermal skin tests (43).

Like nearly all systemic cancer therapies, MAbs are associated with hypersensitivity reactions (45). Severe hypersensitivity reactions are rare, with an incidence of ≤ 5%. Reactions to taxanes and monoclonal antibodies are generally immediate, occurring during the first few minutes of the first or second infusion. However, 10%-30% of reactions to MAbs are delayed, and may occur in later infusions, indicating the importance of close observation of the patient following administration. Mild-to-moderate reactions can be managed by temporary infusion interruption, reduction of the infusion rate, and symptom management. Rechallenge should be considered after complete resolution of all symptoms. Severe reactions may require treatment discontinuation.

Cetuximab, a chimeric anti-epidermal growth factor receptor MAb currently used to treat metastatic colorectal cancer, is often associated with hypersensitivity reactions (46). Although cutaneous manifestations are the most common toxicities associated with cetuximab, they are rarely life-threatening. In a small controlled study, the reaction rate was 22% and significantly higher than the rate noted in any large published trial. In this study, all reactions occurred during the first dose and there was a strong relationship prior allergy history and chance of reaction (47).

**Figure 1:** Classification of adverse side effects of biological agents (33)
Risk of anaphylaxis was included in the US and EU prescribing information for omalizumab (anti-IgE MAbs), but the nature of these reactions needed further elaboration. A review of spontaneous postmarketing adverse event reports submitted to the US Food and Drug Administration’s Adverse Event Reporting System database and to the manufacturers of omalizumab and cases published in the literature was done through December 2006 (48). Diagnostic criteria for anaphylaxis outlined by the National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network were used to screen cases. 124 cases of anaphylaxis associated with omalizumab administration in 57,300 patients with asthma were identified (0.2%). Many cases had a delayed onset of symptoms beyond 2 hours after dose administration. Many cases were also characterized by a protracted progression, with individual signs and symptoms of anaphylaxis staggered over hours. Review of the case reports did not reveal any predictive risk factors for the delayed onset or protracted progression of anaphylaxis.

The American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma and Immunology Executive Committees formed the Omalizumab Joint Task Force (OJTF) with the purpose of reviewing the omalizumab clinical trials and postmarketing surveillance data on anaphylaxis and anaphylactoid reactions (49). Using the definition of anaphylaxis proposed at a 2005 multidisciplinary symposium (50), the Omalizumab Joint Task Force concluded that 35 patients had 41 episodes of anaphylaxis associated with omalizumab administration between June 1, 2003, and December 31, 2005. With 39,510 patients receiving omalizumab during the same period of time, this would correspond to an anaphylaxis-reporting rate of 0.09% of patients. Thus, omalizumab induces very few anaphylactic reactions by comparison to other biologics. Of those 36 events for which the time of reaction was known, 22 (61%) reactions occurred in the first 2 hours after one of the first 3 doses. Five (14%) of the events after the fourth or later doses occurred within 30 minutes. Considering the timing of these 36 events, an observation period of 2 hours for the first 3 injections and 30 minutes for subsequent injections would have captured 75% of the anaphylactic reactions.

These reactions have lead to a proposed modification of the administration of omalizumab (49). The OJTF report provides recommendations for physicians who prescribe Xolair (omalizumab) on the suggested wait periods after administration and patient education regarding anaphylaxis (Table 1) shown on next page.

• Serum sickness

Monoclonal antibodies which induce the formation of IgG antibodies can potentially activate complement and lead to serum sickness (51). Omalizumab does not usually activate complement and only one reaction of serum sickness has been described (52).

Immune imbalance

Biologics that suppress the immune system such as TNF-α blocking drugs are associated with immune imbalance and induce minor infections of the urinary tract, respiratory tract and sinuses, and with serious infections such as tuberculosis, sepsis (bacteria in the blood) and fungal infections (Table 2) shown on next page. Individuals with active infections should not be treated with TNF-α blocking biologics. Some patients who used TNF-α blocking biologics developed cancer. In a study in severe asthma, golimumab-treated patients experienced serious adverse events, infections were the most common adverse events. One patient died from sepsis and some malignancies occurred.

• Parasitic infections

Although the role of IgE in immunity against helminth parasites is unclear, there is concern that omalizumab may be unsafe in subjects at risk of helminth infection. An exploratory study was conducted in Brazil to investigate the safety of omalizumab in subjects with allergic asthma and/or allergic rhinitis at high risk of intestinal helminth infection (53). 137 subjects (12-30 years) at high risk of geohelminth infection received pre-study anthelmintic treatment, followed by 52 weeks’ treatment with omalizumab or placebo. Of the omalizumab subjects 50% experienced at least one intestinal geohelminth infection compared with 41% of placebo subjects, providing some evidence for a potential increased incidence of geohelminth infection in subjects receiving omalizumab. Omalizumab therapy was well tolerated, and did not appear to be associated with increased morbidity attributable to intestinal helminths as assessed by clinical and laboratory adverse events, maximal helminth infection intensities and additional anthelmintic requirements. Time to first infection was similar between treatment groups. Infection severity and response to anthelmintics appeared to be unaffected by omalizumab therapy.

3- Immune tolerance

Infliximab, a human-murine chimeric monoclonal IgG antibody against TNF-α effective in rheumatoid arthritis and other inflammatory diseases. With repeated infusions, however, the formation of neutralizing anti-infliximab antibodies becomes a problem, necessitating increased doses or more frequent drug administration and sometimes necessitating discontinuation of therapy because of secondary response failure and/or infusion-related side effects; this has been observed both in rheumatoid arthritis patients and in patients with other immunoinflammatory diseases. In clinical practice, however, patients with RA or any other chronic inflammatory disease treated with infliximab may differ considerably from the average patient in randomized clinical trials (54, 55). Trough serum infliximab levels after the first 2 intravenous infusions of infliximab at 3 mg/kg varied considerably between patients (range 0.22 g/ml). At this stage, only 13% of the patients were anti-infliximab antibody positive. With subsequent infusions, the frequency of antibody positivity rose to 30% and 44% (at 3 months and 6 months, respectively), accompanied by diminished trough levels of infliximab. Indeed, low infliximab levels at 1.5
Adverse Effects in the Application of Biotechnology

months predicted antibody development and later treatment failure. There were highly significant correlations between high levels of antibodies and later dose increases, side effects, and cessation of therapy. Adalimumab is well tolerated and appears to be effective in maintaining clinical remission in patients with bowel disease and lost response to infliximab [56, 57].

Certolizumab pegol is a pegylated humanized Fab’ fragment with a high binding affinity for TNF-α. Antinuclear antibodies developed in 8% of the patients in the certolizumab group; antibodies against certolizumab pegol developed in 9% of treated patients [58]. Such a tolerance has not been reported yet for omalizumab.

1- Costs

All biologics are very expensive treatments and many are not devoid of side effects. Thus, the patients treated should be perfectly characterized and biologics only administered if they can reduce severe symptoms and reduce hospitalizations. This is the case for omalizumab which is indicated in severe uncontrolled asthmatics despite the use of optimal therapy. However, even in these patients, the costs of treatment need to be scrutinized. It has been shown that omalizumab is cost-effective in some but not all studies [59-61]. However, since this treatment can reduce severe exacerbations and improve quality-of-life, it is indicated in patients with severe uncontrolled asthma despite optimal pharmacotherapy.

Table 1: Summary of AAAAI/ACAAI OJTF recommendations concerning omalizumab use (49)

| 1- Informed consent should be obtained from the patients after discussing the risks, benefits and alternatives to Xolair (omalizumab). |
| 2- The patients should be educated regarding the signs, symptoms, and treatment of anaphylaxis. |
| 3- Patients should be prescribed and educated on the proper use of the epinephrine autoinjector and advised to carry this before Xolair (omalizumab) administration and for the next 24 hr after Xolair (omalizumab) administration. |
| 4- An assessment of the patient’s current health status should be made before each injection to determine whether there were recent health changes that might require withholding treatment. This assessment should include vital signs and some measure of lung function (e.g. PEFR or FEV). |
| 5- The OJTF recommends that patients be kept under observation for 30 min after each injection. This time should be extended for 2 hr for the first 3 injections based on data reviewed by the OJTF, as well as suggested in the 2007 NHLBI Expert Panel Report 3 guidelines for the diagnosis and management of asthma. However, this could be modified based on a physician’s judgment after discussing risks with the patient. |

Table 2: Classification of side effects of anti-TNF-α biologics (33)

| Type β: hypersensitivity | Local and systemic urticaria, erythema, serum sickness and loss of efficiency (tolerance) |
| | Acute and delayed reactions after infusion |
| Type γ |
| Immunodeficiency | Tuberculosis, listeriosis, other granulomatous infectious diseases, neoplasia |
| Autoimmune/inflammatory | Interstitial pneunonpathy, acute fibrosis, systemic sclerosis, SLE, demyelinating diseases, pancytopenia, lichenoid skin reactions, psoriasis |
| Atopic/allergic | Atopic dermatitis |
| Type ε: non-immunologic effects | Heart insufficiency |
Adverse Effects in the Application of Biotechnology

References

20. O’Byrne PM. The demise of anti-IgE for asthma, or not. Am J Respir Crit Care Med. 2007 Dec;1;176(1):1059-60.
Adverse Effects in the Application of Biotechnology


Pitfalls and drawbacks in biologics

1- From the concept to treatment
2- Side effects
3- Cost-effectiveness

Pitfalls and drawbacks in biologics

1- From the concept to treatment
   - A success story

Problems for the development of new asthma treatment

- Asthma is associated with a complex pathway of pro- and anti-inflammatory mediators acting in association or not
- Corticosteroids block most of this complex set of mechanisms but they could not be developed today due to side effects
- New treatments should be safe
- Blocking one component of the pathway may be beneficial or have no effect
- Animal studies are not predictive of efficacy in man
Important mechanisms in asthma

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Th2</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tr</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL4/IL13</td>
<td>IL4: +++</td>
<td>+++</td>
<td>IL4/IL13: +++</td>
</tr>
<tr>
<td>IL5</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IL10</td>
<td>?</td>
<td>++</td>
<td>?</td>
</tr>
<tr>
<td>IL17</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>ILXX</td>
<td></td>
<td></td>
<td>Of course</td>
</tr>
<tr>
<td>IgE</td>
<td>Yes but</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>eosinophil</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Other chemokines</td>
<td>?</td>
<td>++</td>
<td>Of course</td>
</tr>
<tr>
<td>Any new idea</td>
<td>Of course</td>
<td>Of course</td>
<td>Very Important</td>
</tr>
</tbody>
</table>

Anti-IgE monoclonal antibody in asthma  
Fahy et al, AJRCCM 1997

**Free serum IgE levels**

![Graph](image1)

**Allergen challenge**

![Graph](image2)
Omalizumab reduces ICS dose needed

![Graph showing the reduction in ICS dose needed by Omalizumab compared to placebo across different studies.]

***p<0.001 vs placebo

---

Omalizumab downregulates IgE receptor expression in allergic rhinitis


![Graph showing the downregulation of IgE receptor expression by Omalizumab compared to controls.]

p<0.0022

---

Omalizumab reduces FceRI expression

Djukanovic et al, Am J Respir Crit Care Med 2004

![Images showing pre- and post-omalizumab FceRI expression.]

Pre-omalizumab  Post-omalizumab
Pitfalls and drawbacks in biologics

1- From the concept to treatment
   - A success story
   - An unsuccessful story

Anti-IL5 in blood eosinophils in asthma
Leckie et al, Lancet 2000

Anti-IL5 in sputum eosinophils and BHR after Allergen challenge in asthma
Leckie et al, Lancet 2000
Anti-IL5 in asthma symptoms
Flood Page et al, Am J Respir Crit Care Med 2007

![Graph showing the effect of different treatments on asthma symptoms over time.]

Anti-IL5 in humans
- Profound reduction of blood and sputum eosinophils
- Moderate reduction in tissue eosinophils
- Effect on remodelling?
- No effect in controlled trials of unselected asthmatics
- Some effect in hyper-eosinophilic syndrome
- Possible effect in nasal polyposis
- Might have a clinical benefit in some very well characterized asthmatics
- THUS ???????????

Pitfalls and drawbacks in biologics

1- From the concept to treatment
   - A success story
   - An unsuccessful story
   - Still more data are needed
Anti-IL4/IL-13 in bronchial challenge in asthma
Wenzel et al, Lancet 2007

Pitfalls and drawbacks in biologics
1- From the concept to treatment
2- Side effects
   - Classification

Classification of side effects of biologics
Pichler WJ, Allergy 2006
### Side effects of TNF-α

*Pichler WJ, Allergy 2006*

<table>
<thead>
<tr>
<th>Type of hypersensitivity</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I: Hypersensitivity</td>
<td>Local and systemic urticaria, erythema</td>
</tr>
<tr>
<td></td>
<td>Serum sickness</td>
</tr>
<tr>
<td></td>
<td>Loss of efficiency</td>
</tr>
<tr>
<td></td>
<td>Acute and delayed reactions after infusion</td>
</tr>
<tr>
<td>Type II: Immunodeficiency</td>
<td>Tuberculosis, listeriosis</td>
</tr>
<tr>
<td></td>
<td>Other granulomatous infectious diseases</td>
</tr>
<tr>
<td></td>
<td>Neoplasia</td>
</tr>
<tr>
<td>Type III: Autoimmunity</td>
<td>Interstitial pneumopathy</td>
</tr>
<tr>
<td></td>
<td>Acute fibrinolysis</td>
</tr>
<tr>
<td></td>
<td>Systemic sclerosis</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
</tr>
<tr>
<td></td>
<td>Demyelinating diseases</td>
</tr>
<tr>
<td></td>
<td>Pancreatitis</td>
</tr>
<tr>
<td></td>
<td>Lichenoid skin reactions, psoriasis</td>
</tr>
<tr>
<td>Type IV: Atopic/Allergic</td>
<td>Atopic dermatitis</td>
</tr>
<tr>
<td>Type V: Non immunologic</td>
<td>Heart insufficiency</td>
</tr>
</tbody>
</table>

### Pitfalls and drawbacks in biologics

1. From the concept to treatment
2. Side effects
   - Classification
   - Expected side effects

### Safety of anti-IgE in parasitosis

*Cruz et al, Clin Exp Allergy 2007*
Anti-IgE and parasitosis

- IgE was felt to be important in the fight against parasites
- A study with omalizumab did not show that parasitic infections or their duration were impaired by anti-IgE

Pitfalls and drawbacks in biologics

1- From the concept to treatment

2- Side effects
   - Classification
   - Expected side effects
   - Unexpected side effects

B and T cell epitopes in allergens

poly saccharides

allergen
tertiary structure

B cell epitope

T cell epitopes

primary structure
Interactions in T cell recognition of peptides
Kay and Larche 2001

Overlapping peptides of Fel d1
AllerVax® Cat

AVKDLCCDFAVGTLUNAOIGLIDMGNANGJINGDIAKERTYUIVBNXVBN

2 chains in Fel d1
deduction of the structure
study of major T-cell reactive epitopes
determination of 2 reactive peptide for chain 1
IPC-1 and IPC-2: 27 AA each
reduction in IL-4 release (Pène, J Allergy Clin Immunol 1998)

Efficacy and safety of Allervax® Cat
Norman et al, Am J Respir Crit Care Med 1996

Total symptom score

<table>
<thead>
<tr>
<th></th>
<th>Mean symptom score</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td></td>
</tr>
<tr>
<td>1 wk post</td>
<td></td>
</tr>
<tr>
<td>6 wk post</td>
<td></td>
</tr>
</tbody>
</table>

Side effects

<table>
<thead>
<tr>
<th></th>
<th>placebo</th>
<th>7.5 µg</th>
<th>75 µg</th>
<th>750 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>% patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Anti-IgE: safety

- overall excellent immunologic safety profile
- no development of IgE against anti-IgE
- no development of immune complexe disease (one case: Pilette)
- no apparent long term effects including cancer
- no effects on platelets in humans

Delayed onset and protracted anaphylaxis
Limb et al, J Allergy Clin Immunol 2007

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All anaphylaxis</td>
<td>124 (99%)</td>
</tr>
<tr>
<td>Immediate reaction</td>
<td>114 (91%)</td>
</tr>
<tr>
<td>Severe</td>
<td>80 (62%)</td>
</tr>
<tr>
<td>Minor</td>
<td>34 (27%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Hypotension or syncope</td>
<td>17 (13%)</td>
</tr>
<tr>
<td>Hypotension alone</td>
<td>13 (10%)</td>
</tr>
<tr>
<td>Previous history of anaphylaxis</td>
<td>29 (23%)</td>
</tr>
<tr>
<td>Time from react</td>
<td>9 (7%)</td>
</tr>
<tr>
<td>Fever</td>
<td>41 (32%)</td>
</tr>
<tr>
<td>Severe</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Site of reaction</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Ear</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Ear, nose, mouth</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Eye</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Nasal</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Oral</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Respiratory, skin, other</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Initial presentation</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Discharge</td>
<td>32 (25%)</td>
</tr>
<tr>
<td>Patient rechallenged with omalizumab</td>
<td>14 (11%)</td>
</tr>
</tbody>
</table>

- 124 case on 57,300 patients
- any dose
- immediate or delayed onset
- mechanisms unknown

SUMMARY OF AAAAI/ACAAI OJTF RECOMMENDATIONS

1. Informed consent should be obtained from the patient after discussing the risks, benefits, and alternatives to Xolair (omalizumab).
2. The patient should be educated regarding the signs, symptoms, and treatment of anaphylaxis (Table 1). "
3. Patients should be prescribed and educated on the proper use of the epinephrine autoinjector and advised to carry this before Xolair (omalizumab) administration and for the next 24 hours after Xolair (omalizumab) administration.
4. An assessment of the patient’s current health status should be made before each injection to determine whether there were any recent health changes that might require withholding treatment. This assessment should include vital signs and some measure of lung function (e.g., peak expiratory flow or FEV1)."
5. The OJTF recommends that patients be kept under observation for 30 minutes after each injection. This time should be extended for 2 hours for the first 3 injections based on the data reviewed by the OJTF, as well as suggested in the 2007 National Heart, Lung, and Blood Institute Expert Panel Report 3 “Guidelines for the diagnosis and management of asthma.” However, this could be modified based on a physician’s clinical judgment after discussing risks with the patient.
Pitfalls and drawbacks in biologics

1- From the concept to treatment
2- Side effects
3- Cost-effectiveness

Costs increase with asthma severity
Godard et al Eur Respir J 2002

![Bar chart showing costs increase with asthma severity]

How does the payer decide?

- Impact on health status (indirect + intangible costs)
- Impact on health care costs (direct costs)
- Survival QoL
- Hospitalisations
- Other Drugs Procedures, etc.
- Impact on health status
- Survival QoL
- Hospitalisations
- Other Drugs Procedures, etc.
- Impact on health care costs
Use of cost per QALY in decision making by NICE

<table>
<thead>
<tr>
<th>Cost per QALY</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; £3 000</td>
<td>Strongly support</td>
<td>Strongly support</td>
<td>Supported</td>
<td>Not proven</td>
</tr>
<tr>
<td>£3 000-20 000</td>
<td>Strongly support</td>
<td>Supported</td>
<td>Limited Support</td>
<td>Not proven</td>
</tr>
<tr>
<td>&gt;£20 000</td>
<td>Limited Support</td>
<td>Limited Support</td>
<td>Limited Support</td>
<td>Not proven</td>
</tr>
</tbody>
</table>

Cost-effectiveness for omalizumab add-on therapy vs standard therapy
Brown et al, Allergy 2007

Cost-effectiveness threshold

Probability cost-effectiveness (%)
Allergic diseases have reached epidemic proportions worldwide. An understanding of the cellular and soluble mediators that are involved in allergic inflammatory responses not only helps in understanding the mechanisms of current treatments, but is also important for the identification of new targets that are amenable to both small-molecule and biological interventions. It is the introduction of monoclonal antibodies and soluble cytokine receptors that is revolutionizing approaches to the treatment of asthma and allergy however, when compared to other areas of chronic inflammation; development of biologics in our field has been slow with the exception of immunotherapy. The successful introduction of omalizumab for severe allergic asthma has stimulated great interest in this approach, but even with this humanised monoclonal antibody, cost effectiveness analyses are restricting its use even though it has passed scrutiny by such agents as the National Institute of Health & Clinical Excellence in the UK. The need for 16 weeks therapy before a decision can be made to separate responders from non responders emphasises the need for biomarkers of response since patients vary greatly in their response to this treatment. Understanding the underlying mechanisms of allergic disease has stimulated the further development of a series of biologics targeted towards critical cells and molecules in the allergic cascade presented below. Because of the sentinel role that Th2 cytokines have in orchestrating allergic inflammation, they and their receptors are key therapeutic targets. Both IL-4 and IL-13 have a crucial role in the immunoglobulin isotype switching of B cells to produce IgE, whereas IL-4 alone is crucial for maintaining the Th2-cell phenotype, which makes both cytokines attractive therapeutic targets. A large number of animal studies have shown that blocking production or inhibiting the effects of IL-4 has profound effects on the allergic phenotype. A soluble, recombinant, human IL-4 receptor (altrakincept) consists of the extracellular portion of human IL-4 Rα and is non-immunogenic. A small proof-of-concept trial of nebulized inhaled altrakincept for 12 weeks in patients with mild to moderate asthma indicated efficacy by allowing withdrawal from treatment with inhaled corticosteroids without relapse, and this result was subsequently confirmed in a larger trial. However, a Phase III trial failed to confirm the efficacy of altrakincept for the treatment of asthma. This trial does not invalidate IL-4 as a target for the treatment of allergy and asthma, as there were concerns over the bioavailability of altrakincept in this study. Further Phase II studies are in progress using humanized IL-4-specific and IL-4Rα-blocking antibodies such as pascolizumab (SB240, 683). Two vaccines against IL-4 have been tested in mice, one in which IL-4 is chemically coupled to limpet haemocyanin and the other in which a 14-amino-acid peptide from IL-4 is inserted into variant hepatitis B virus core antigen. Both vaccines induced high antibody titres specific for mouse IL-4 and inhibited antigen-induced lung inflammation. However, using co-stimulation blockade in a mouse model of allergy to grass pollen, it was reported that the secondary IgE response is not T-cell dependent, thereby raising doubts over the usefulness of IL-4 blockade for treating established allergic disease. The numerous functions of IL-13 in regulating IgE production, eosinophilic inflammation, airway smooth-muscle hyperplasia, the induction of goblet-cell hyperplasia with mucus production, and the recruitment of monocytes, macrophages and T cells into the airway spaces make it a key therapeutic target in allergy and asthma. IL-13 binds to a low-affinity IL-13Rα1 subunit and a high-affinity complex comprised of IL-13Rα1 and IL-4Rα. Binding to this high-affinity complex leads to the phosphorylation-dependent activation of Janus kinase 1 (JAK1), JAK2 and STAT6. IL-4Rα also stabilizes the binding of IL-13 to its receptor to augment IL-13-mediated responses. However, a non-signalling, high-affinity IL-13-binding chain, IL-13Rα2, strongly inhibits the activity of IL-13. Selective blockade of IL-13 has been achieved in mice using a soluble form of IL-13Rα2, which competes for binding to IL-13 but not to IL4, and this led to the reversal of airway hyper-responsiveness and mucus production in allergen exposed sensitized mice. A soluble form of IL-13Rα2 that binds IL-13 with 100-fold greater affinity than does IL-13Rα1 is present in mouse but not human serum. Antagonizing the effects of IL-13 could also be achieved by administering soluble IL-13 receptors or IL-13R-specific monoclonal antibodies. In cynomolgus monkeys sensitized to Ascaris suum and then challenged with antigen from this nematode, a mouse antibody specific for human IL-13 (mAb13.2) and the humanized counterpart (IMA-638) inhibited eosinophil and neutrophil influx into the lungs as assessed by bronchialalveolar lavage. Phase I trials of the IL-13-specific monoclonal antibody CAT-354 in 34 mildly asthmatic patients have been successfully completed and Phase II trials are in progress. Subcutaneous or inhaled pitrakinra, a mutant IL-4 protein that inhibits the binding of IL-4 and IL-13 to IL-4Rα complexes, has recently shown efficacy in the treatment of allergen-induced asthma. A novel, recombinant IL-13 peptide-based vaccine has also been shown to reduce allergic inflammatory responses in mice. Rodent and non-human primate studies have indicated an important role for IL-5 in various models of asthma. Inhaled IL-5 modulates the number of eosinophil progenitors in both the airways and bone marrow of asthmatic individuals and induces local eosinophilia in non-asthmatic individuals. Two humanized, human-IL-5-specific monoclonal antibodies, Sch-55,700 and mepolizumab (SB-240,563), have been developed for the treatment of asthma. In a small double-blind trial, mepolizumab produced a rapid dose-dependent reduction in the number of circulating and sputum eosinophils that
persisted for 3 months but, surprisingly, this had no effect on either the late asthmatic response or on airway hyper-responsiveness. In a group of patients with severe persistent asthma, treatment with Sch-55,700 resulted in a decrease in the number of blood eosinophils, but over the course of 10 weeks it had no effect on any measures of asthma outcome, an observation that has recently been confirmed in a large trial with mepolizumab. A further study using mepolizumab confirmed the persistent suppression of eosinophils in blood, bone marrow and airway lavage, but in airway biopsies, there was only a 55% reduction in the number of tissue eosinophils. As a proportion of eosinophils in the airways of patients with asthma lack IL-5R, it was suggested that this might explain the apparent lack of clinical efficacy of targeting IL-5. IL-5 could have more subtle effects on asthmatic airways - for example, mepolizumab treatment decreases immunostaining for tenascin, lumican and procollagen III in the bronchial mucosal subepithelial basal lamina and in allergen challenged skin. In addition, IL-5-specific treatment resulted in a parallel decrease in the number of airway eosinophils expressing mRNA for TGFβ1 and of TGFβ1 levels in bronchoalveolar-lavage fluid, which indicates a possible role for IL-5 in airway remodelling. In contrast to asthma, mepolizumab is highly efficacious in the treatment of hypereosinophilic syndrome and eosinophilic oesophagitis, but not atopic dermatitis. A therapeutic DNA-based vaccine against IL-5 is also being developed. As asthma becomes more severe and aggressive it adopts a Th2 cytokine profile with enhanced production of IFNγ and TNFα. Both in mouse models and in 3 small clinical trials in severe asthma anti-TNF therapies (etanercept, infliximab) were reported to be efficacious. However in a large RCT involving over 300 patients with severe asthma, treated with golimumab (CNTO 148) for 52 weeks efficacy for baseline lung function and asthma exacerbations was not apparent. However, a subgroup analysis indicated that reversibility of lung function, late onset disease and concurrent sinusitis were predictors of efficacy and further trials in this subpopulation are now being undertaken. Blocking the actions of IL-9 reduces allergen-induced airway inflammation and airway hyper-responsiveness in mouse models. Two Phase I dose-escalation studies of an IL-9-specific monoclonal antibody (MEDI-528) in healthy volunteers have been completed without problems. Phase II trials are in progress for treating symptomatic, moderate to severe, persistent asthma. IL-2 is intimately involved in T cell activation and its α receptor (CD25) has been targeted with a monoclonal antibody Daclizumab that is efficacious in preventing renal transplant rejection. A phase IIa clinical trial in moderate-severe asthma has also revealed efficacy on the basis that activated T cells contribute to ongoing disease activity. As new targets for immunologics are discovered it is important that appropriate trial designs are used to test them taking account of markers of response and surrogate endpoints that include the identification of biomarkers. Some examples of novel targets that have been largely identified in mouse models of antigen driven allergic type inflammation include IL-15, IL-17A, IL-17E (or IL-25), IL-33, IL-31, IL-21 and thymic stromal lymphopoietin (TSLP) which are all proposed to enhance inflammatory responses. The engineering of monoclonal antibodies to include fully humanised molecules as well as antibody engineering to enhance certain properties such as antibody dependant cell cytotoxicity involving activation of NK cells created when the fucose-linked carbohydrate chain of the Fc portion of the antibody is removed, greatly enhances the potential of these agents as therapeutics so that whole cell populations can be eliminated rather than simply blunting their function.

References
The Future Application of Monoclonal Antibodies to Allergy and Asthma

Stephen T Holgate, IIFR Division, School of Medicine, University of Southampton.
sth@soton.ac.com

Known Cytokine Targets in Asthma


Environmental triggers

AIRWAY HYPERRESPONSIVENESS
AIRWAY NARROWING
ASTHMA SYMPTOMS

CHRONIC INFLAMMATION

eosinophils

Intact epithelium

Dendritic cells

Basophils

mast cells

Th-2 cells

AIRWAY HYPERRESPONSIVENESS
AIRWAY NARROWING
ASTHMA SYMPTOMS
Eosinophils Have an Important Inflammatory Role in Allergic Asthma

Anti-IL-5; A New Targeted Therapy For Asthma


- Anti-IL-5 administered as a single injection or repeated had a dramatic effect in depleting circulating eosinophils.
- Anti-IL-5 had no effect on the late asthmatic response to allergen challenge.
- Anti-IL-5 had no effect on baseline bronchial hyper-responsiveness.


The Effect of Anti-IL-5 Mab (Mepolizumab) on Chronic Asthma: Blood Eosinophils

(n=100-125 for Each Group)
Anti-IL-5 Treatment of Other Eosinophilic Disorders

  No effect of anti-interleukin-5 therapy (mepolizumab) on the atopy patch test in atopic dermatitis patients.

- Stein ML, Collins MH, Villanueva JM, Kushner JP, Putnam PE, Buckmeier BK, Filipovich AH, Assa’ad AH, Rothenberg ME.
  Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis.

  Anti-interleukin-5 (mepolizumab) therapy for hypereosinophilic syndromes.

Daclizumab

- Humanized monoclonal antibody specific for IL-2R alpha subunit (CD25)
- FDA (1987) and EMEA (1999) approved for renal allograft rejection (Zenapax®)
- Use in > 50,000 transplant patients
- 1 mg/kg IV x 5-doses (transplant induction)
- MS and asthma development
Daclizumab biological activity

- Binds to alpha chain of high affinity IL-2 receptor (CD25) and blocks IL-2 signaling
- Blocks IL-2Rα-mediated T- and B-cell activation
- Reduces allergen-induced lung eosinophilia in monkey asthma model


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Study Design

Primary endpoint

Randomization

Screening

Run-in

Run-in: 2 to 5 weeks
ICS taper/ rescue

Randomization (3:1)

DAC (8 doses)

Placebo (8 doses)

Treatment Period 1 - ICS stable phase: 12 weeks

Treatment Period 2 - ICS taper phase: 8 weeks

Follow-up: 16 weeks

---

Daclizumab prolongs time to asthma exacerbation

Exacerbation Free Survival

- Steroid stable
- Steroid taper
- Follow-up

Time to Systemic Corticosteroid Use (Days)

p=0.024

Baker et al, San Francisco AAAA March 18th 2004 will present
Preliminary Safety And Efficacy Of Daclizumab In The Treatment Of Patients With Moderate To Severe Chronic Persistent Asthma
Chemokine Biology

- Chemokines, a subfamily of cytokines that signal through 7TM G-protein coupled receptors.
- >20 chemokine receptors
- >40 chemokines have been characterised, with functional relevance to diseases such as HIV, atherosclerosis, rheumatoid arthritis, lymphoma, cancer and allergy.
- Th2 candidates: CCR3, CCR4 and CRTH2

Allergen challenge of asthmatic airways in vivo causes an influx of T cells

- Bronchial biopsy 24-hr after allergen challenge
- CD3+ cells/ Sq mm
  - Healthy – 274
  - Asthmatic – 4400
  - 98.5% IL-4 +ve

Allergen provocation in vivo induces TARC (CCL17) and MDC (CCL22) production
CCR4 expression: Th-2 > Th-0 > Th-1

Pacila Panina-Bordignon et al. JCI 2001

Application of Antibody-dependant Cell Cytotoxicity (ADCC)

ADCC activity is significantly increased by reducing fucose, a sugar chain naturally present in antibodies.

Structure of carbohydrate chain of Antibody:
- N-acetylglucosamine
- Galactose
- N-acetylglucosamine
- Fucose
- Sialic acid


ADCC Used to Target Cells which express Specific Surface Molecules

Methods for enhancing Antibody-Dependent Cellular Cytotoxicity (ADCC) by reducing fucose content in sugar chain

- Increase efficacy and potency
- Decrease clinical dosing
- Decrease product requirements
 Therapeutic antibody and ADCC

ADCC activity is critical to efficacy of many therapeutic antibodies including Herceptin and Rituxan on the market.

Th-2/CCR4+ cell lysis

Target cells

Antibody

Fc receptor

Effector cells

Cytotoxicity

Environmental triggers

Dendritic cells

Intact epithelium

Neutrophils

Eosinophils

Airway hyperresponsiveness
Airway narrowing
Asthma symptoms

Tumour Necrosis Factor

Th-2 cells

Mast cells

Tumor Necrosis Factor alpha (TNFα)

* Transmembrane protein
  - Cleaved by TACE on cell surface
* Active protein is trimeric
  - 157 amino acids / monomer
  - Unglycosylated
  - One intrachain Disulfide per monomer
* Binds p55 & p75 Receptors
* Receptors present on virtually all cells

TNF-α expression in the airways of patients with severe corticosteroid refractory asthma

Howarth PH et al. Thorax 2005; 60: 1012-8

Crossover-RCT of Etanercept in Corticosteroid Refractory Asthma


Antibody Generation with Transgenic Mice
