New drugs for asthma

The latest conference on *New Drugs for Asthma* (IBC UK) held at the National Heart and Lung Institute (NHLI, London, UK) on the 16–17 June 1998 was opened by Peter Barnes (Professor of Thoracic Medicine at the Institute) with an overview of current therapy and the need for new drugs. Asthma is a chronic inflammatory disease of the airways characterized by a specific pattern of eosinophilic inflammation and enhanced activation of resident cells (Fig. 1). Cells in the airway produce multiple inflammatory mediators (>50 identified), but many are redundant, which explains why so many receptor antagonists of single mediators or inhibitors of mediator synthesis are disappointing as asthma treatments. Inhaled steroids are currently the first-line therapy in chronic asthma, they are used in high dose initially, but the dose is progressively reduced once asthma control is achieved. In spite of the safety and efficacy of existing asthma treatments, asthma mortality is increasing in most countries and asthma now affects >10% of children and 5% of adults in the Western World. The need for new treatments is therefore pressing and underlined by the fact that the cost of failing to treat severe asthmatics correctly is half of the total asthma costs in the USA ($5.8 billion).

Since the last conference, held three years ago, several potential drugs have moved to late Phase II and Phase III clinical trials. However, some have proved to be disappointing; for example, mast cell tryptase and inhibitors of type IV phosphodiesterases were not discussed at the conference.

Delays in drug development

The antileukotrienes are the only new class of anti-asthma therapy introduced into the clinic in 25 years of intense research. Neil Barnes (London Chest Hospital, UK) discussed how basic assumptions about the mechanism of action of leukotriene antagonists in the presence of steroids led to delays in clinical trials. Steroids were originally proposed to act via the induction of lipocortin-1 and subsequent inhibition of phospholipase A_2 and leukotriene formation. Hence, leukotriene antagonists were not tested in clinical trials as an adjunct to steroid therapy in severe asthmatics where they are in fact likely to be most prescribed and potentially most useful. The need for drugs to be tested in Phase I/II clinical trials to predict efficacy reliably at an early stage was a keynote theme of the conference. The early trials with leukotriene antagonists used a limited range of doses, but the effectiveness of leukotriene antagonists may have been recognized early if full dose-ranging studies before Phase III studies were performed. A particular dose may be used in drug development, but higher doses may lead to clinical benefit.

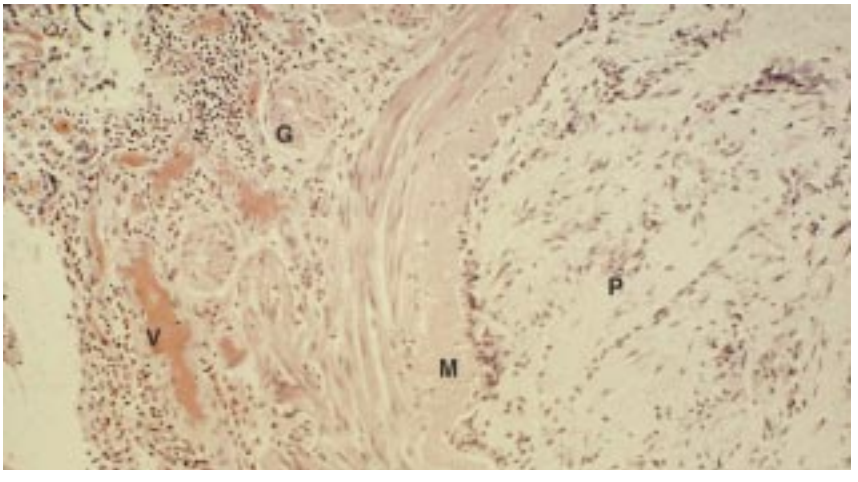

Figure 1. *Histology of an asthmatic patient showing occlusion of the lumen with a mucus plug (P) containing many inflammatory cells. G, submucosal gland; M, thickened basement membrane; V, blood vessel. reproduced with permission from Liu, Y.C., Khawaja, A.M. and Rogers, D.F. (1998) in* Asthma: Basic Mechanisms and Clinical Management *(3rd edn) (Barnes, P.J., ed.) pp. 205–227, Academic Press.*

Clinical study design

Trevor Hansel (NHLI) identified some clinical study designs to test candidate drugs. Severe asthma and, in particular, emergency asthma are potential areas for drug development – with the latter, the possibility for early registration in treating life-threatening asthma could lead to orphan drug status. Most clinical study designs require mild/moderate asthmatics but, perhaps, novel treatments should be tested in symptomatic asthmatics. The major challenge is to perform Phase I/II proof-of-concept studies with new asthma therapies to predict efficacy in the correct patient group, thus providing an early go/no-go step before large-scale Phase III trials. However, it requires the development of more relevant asthma models than allergen challenge and better surrogate markers of disease severity.

Anti-IgE therapy

Anti-IgE therapy is now at an advanced phase of drug development and Phase III clinical trials of the humanized recombinant monoclonal antibody (mAb)

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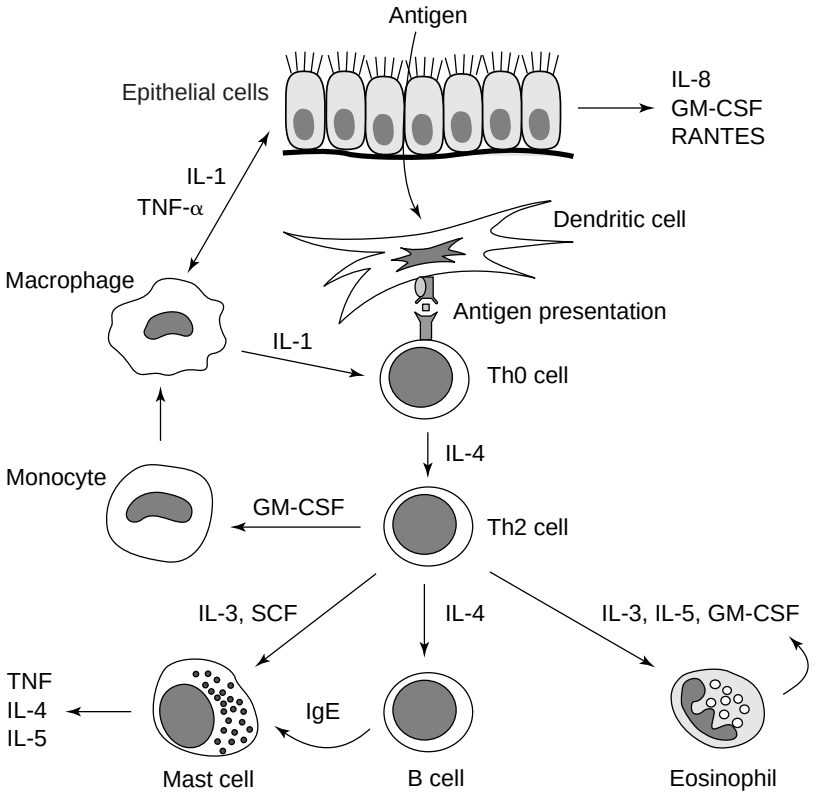

Figure 2. *Many cytokines are released from inflammatory cells and structural cells in the airway and orchestrate and perpetuate the inflammatory response. GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; SCF, stem cell factor; TNF, tumour necrosis factor. Redrawn from Barnes, P.J. (1998) in* Asthma: Basic Mechanisms and Clinical Management *(3rd edn) (Barnes, P.J., ed.) pp. 487–506, Academic Press.*

to IgE (rhuMAb-E25) are now underway. Robert Fick (Genentech, San Francisco, CA, USA) presented data from a study of 317 moderate to severe allergic asthmatics in a double-blind placebo-controlled trial of rhuMAb-E25. Patients receiving active treatment experienced a 99% mean fall in serum free IgE over 12 weeks. There were few adverse effects and none that were thought to be drug related. The antibody treatment did not appear to induce an anti-idiotypic response and the complexes produced were cleared slowly. Patients receiving active treatment showed significant increases in peak expiratory flow rate (31 l min^{-1} in those given the high dose of 14 ng kg^{-1} IU^{-1} ml^{-1}) and mean symptom score improvement of 42% in both high- and low-dose groups compared with a 25% increase in the placebo group. Anti-IgE therapy was also studied in patients with moderate to severe disease [mean forced expiratory volume in 1 s (FEV_1) was 71% of that predicted] who were also on oral corticosteroids (mean of 10 mg day^{-1}). Oral corticosteroid use decreased with continued treatment of rhuMAb-E25 for 20 weeks from baseline by 24% in placebo group, and 41% and 50% in the low- and high-dose groups, respectively. Although further studies are needed to understand anti-IgE treatment, the initial studies are encouraging.

Adhesion molecules

Adhesion molecules are increased in asthma, and targeting of these may be beneficial in reducing asthmatic inflammation. Paul Hellewell (University of Sheffield, UK) described the *in vivo* and *in vitro* models that have been developed to test cell adhesion. Several specific inhibitors have been revealed: E-selectin antagonists (sialyl-Lewis X and CGP69669A), anti-P-selectin mAb and several small-molecule inhibitors. These await evaluation in early Phase I trials. Inhaled anti-very late antigen 4 (VLA-4) mAb has been used in a sheep model of bronchial hyperresponsiveness where it blocks the late response to allergen and prevents bronchial hyperreactivity. Similar results have been achieved with an inhaled small-molecule inhibitor of VLA-4 that prevents VLA-4 binding to the CS-1 domain of fibronectin.

The efficacy of targeting adhesion molecules in inflammatory disease is provided by evidence for effectiveness of an anti-intercellular adhesion molecule 1 (ICAM-1) antibody in the treatment of rheumatoid arthritis and also in a small trial of subjects with ulcerative colitis. However, these are unlikely to be useful in asthma because of cost.

Cytokines in asthma

Cytokines play a key role in the chronic inflammation of asthma and appear to orchestrate, amplify and perpetuate the inflammatory process [Barnes, P.J. (1996) *Br. J. Clin. Pharmacol.* 42, 3–10; see Fig. 2]. Interleukin 4 (IL-4) and IL-5 are considered to be key mediators in specific allergic asthmatic inflammation. Importantly IL-4 directs the development of naive T cells towards the T helper 2 (Th2) subset, which appears to be the dominant phenotype in asthma. IL-4 is critical for the synthesis of IgE by B cells and is involved in eosinophil recruitment to the airways. Noel Snell (Bayer, Newbury, UK) presented the current position of anti-IL-4 treatment. Most work has been carried out with soluble truncated recombinant IL-4 receptors given by nebulization to mild atopic asthmatics in which they have been found to be long lasting and well tolerated. A Phase II placebo-controlled trial in moderately severe asthmatics demonstrated significant improvements in FEV_1, reductions in methacholine

hyperreactivity and nitric oxide. However, Snell identified several problems with the use of IL-4 modulators: although anti IL-4 therapy is a rational approach, IL-13 and IL-5 may act as alternative signals, and concomitant therapy may be required. Tolerance can also occur through compensatory increases in receptor density, and antibodies may develop to the treatment. Importantly, constitutively IL-4-deficient knockout mice show a reduced resistance to parasitic infestation.

Michael Minnicozzi (Schering Plough, Kenilworth, NJ, USA) presented data from experiments using a rat IgG1 antibody to murine IL-5 (TRFK-5) and a humanized antibody to human IL-5 (SCH55700) in rodent and primate models of allergic pulmonary inflammation. Blocking antibodies inhibit recruitment of eosinophils to the airway and enable airway hyperresponsiveness to return to normal levels in mice and monkeys. The effects are seen up to six months after a single injection of the highest dose given (1 mg kg^{-1}) and they appear to be dose dependent with regard to duration but not with respect to eosinophil recruitment to the lung. There were some possibilities of long-term modulation of immune response as the TRFK-5 treatment gave a rebound increase in airway T-cell numbers in the lung at 24 weeks. However, these effects were not related to changes in T-cell activation, which suggests that anti-IL-5 antibody treatment will have no long-term permanent modulation of the immune system and will allow recovery following treatment. The humanized antibodies are now undergoing clinical trials.

Anti-inflammatory effects

Some cytokines have anti-inflammatory effects in asthmatic inflammation. There is some evidence for a decrease or abnormal downregulation of anti-inflammatory cytokines in asthma and there is the possibility of restoring the anti-inflammatory/pro-inflammatory balance with exogenous therapeutic cytokines. Drugs may potentially activate anti-inflammatory receptors or stimulate specific downstream pathways to the same effect. Interferon γ (IFN-γ) inhibits Th2 cells and preferentially directs Th0 cells development towards the Th1 subset, which should theoretically reduce asthmatic inflammation. IL-12, an endogenous regulator of Th1 cells, determines the balance between Th1 and Th2 cells. In addition, it primes $CD4^+$ T cells to produce IFN-γ, which subsequently effects the eosinophilic inflammation in mice. The role of the cytokines was addressed by Fan Chung (NHLI) and Tony Coyle (Millennium Pharmaceuticals, Cambridge, MA, USA).

Recombinant human IL-12 is effective in a murine model in eliminating eosinophils in allergen-challenged animals with an associated decrease in bronchial hyperreactivity. These effects were concomitant with a decrease in IL-4 and IL-5 expression and an increase in IFN-γ levels. In animal models, IL-12 seems to be able to direct the immune response towards a Th1 phenotype when given as an adjuvant with allergens such as house dust mite. This is therefore a treatment for asthma that may reset a fundamental immunological 'switch' – it has important consequences regarding the desirability of sensitizing children or babies with house dust mite allergens and IL-12 in families with a history of asthma. Recombinant human IL-12 has been given to humans and appears to be safe at low dose. Recombinant human IL-12 is now undergoing Phase II clinical trials (Roche). Early results suggest that rhIL-12 may be ineffective in asthmatic subjects, although, this may be due to dosing problems.

IL-12 probably acts through the induction of IFN-γ. There are studies showing that 2.4 mg IFN-γ given to five asthmatic subjects over 19 days was well tolerated and produced a decrease in the proportion of bronchoalveolar lavage (BAL) eosinophils in four of the subjects. Disappointingly, there was no change in lung function or responsiveness in any subject.

Interleukin 10

Asthmatics have decreased levels of IL-10 released from BAL macrophages, and polymorphism in the IL-10 promoter leading to decreased expression of IL-10 are related to asthma severity. Murine models suggest that IL-10 should be of benefit. Clinical trials to show the effectiveness of IL-10 as a possible treatment of asthma are pending. One trial in 17 normal volunteers showed no adverse effects after rhIL-10 treatment up to 25 μg kg^{-1}. A transient neutrophilia and monocytosis was seen at six hours but lymphocytes decreased. In addition, mitogen-induced proliferation of peripheral blood mononuclear cells (PBMCs) was suppressed by 50% and the production of tumour necrosis factor α (TNF-α) and IL-1 was also decreased. Although treatment with IL-10 has advantages over treatment with IL-12 or IFN-γ, it augments major histocompatibility complex class II expression on B cells and also enhances IL-2 production.

Recombinant human IL-10 is effective in controlling inflammatory bowel disease and has been given in Phase I trials to healthy human volunteers with a reduction in lipopolysaccharide (LPS)-induced IL-1β and TNF-α release from PBMCs *ex vivo*. Other endogenous inhibitors of T-cell function include the natural antigen CTLA-4, which negatively regulates T-cell activation. The immunosuppressive agent CTLA-4Ig (Bristol-Myers Squibb) has been shown to inhibit IL-2 and IL-4 production in animals and is now in Phase III studies in transplantation and Phase II studies for allergic disease.

Administration of cytokines to an asthmatic presents theoretical problems as to the ideal route of administration, the length of treatment and the potential for altering immune function over time.

Novel receptor

Coyle presented data from experiments that have identified a novel receptor (T1/ST2) on Th2 cells, which is expressed after differentiation and has 30% homology to the IL-1 receptor. A blocking antibody to T1/ST2 has been used in animal models to decrease airway inflammation and BAL eosinophils. It has already proved to be effective in inhibiting *Leishmania* infection and

Th2-type cytokine release in mice. The development of antibodies against the human equivalent of T1/ST2 may be an effective therapy. Coyle proposed targeting of T-cell subset-specific transcription factors. In this case, antisense molecules have targeted GATA3, which is highly expressed in Th2 cells (but not in Th1 cells) and appears to control T-cell differentiation. Experiments in rats using adoptive transfer of T cells indicated that GATA3 antisense decreased IL-4, IL-5, IL-6, IL-10 and IL-13 expression and reduced bronchial hyperreactivity.

Chemotactic cytokines

The chemotactic cytokines (CC chemokines) act by attracting leukocytes (monocytes, basophils, eosinophils and lymphocytes) to sites of inflammation. They induce cell migration and activation by binding to specific G-protein-coupled cell surface receptors on target cells. These receptors belong to a family of nine related members (CCR1–CCR9). CC chemokines, especially eotaxin, monocyte chemoattractant protein 3 (MCP-3) and MCP-4, are highly potent in attracting eosinophils, acting through the CCR3 receptor. Claude Bernarde (Roche Bioscience, Palo Alto, CA, USA) discussed the potential of CCR3 blockade as a potential strategy for asthma therapy. Small-molecule CCR3 receptor antagonists are likely to be the most effective anti-eotaxin agents.

A specific CCR3 mAb (7B11; LeukoSite, Cambridge, MA, USA) has been developed that acts as a true CCR3 antagonist – it blocks eosinophil chemotaxis to CC chemokines and prevents Ca^{2+} influx. Further development of a humanized form of this antibody or of a small-molecule inhibitor are awaited for evaluation in animal models and Phase I trials. The potential for CCR3 receptor inhibition extends beyond eosinophils as they may also be used to target specific subsets of T cells expressing Th1 or Th2-specific CCR receptors.

Transcription factor activation

Anthony Manning (Signal Pharmaceuticals, San Diego, CA, USA) demonstrated how understanding regulation of the intracellular signalling pathways and inflammatory gene transcription of key pro- and anti-inflammatory cytokines is laying the foundation for a new era in anti-inflammatory drug discovery. Inflammatory gene transcription is regulated by a growing number of transcription factors of which AP-1 and NF-κB are two of the most important. These transcription factors are activated by specific kinases, inhibition of which may suppress the activation of an array of cytokine/chemokine genes. Signal Pharmaceuticals have recently identified a novel class of T-cell-specific inhibitors of AP-1 and NF-κB activity. The most potent of which (SP100030) inhibits AP-1- and NF-κB-dependent reporter gene expression in stably transfected Jurkat T cells with an IC_{50} of 30 nM. When these cells are stimulated, SP100030 inhibited transcription of IL-2, IL-8, TNF-α and granulocyte–macrophage colony-stimulating factor (GM-CSF) genes with a similar IC_{50}. These compounds are now being taken forward for preclinical drug testing – they hold great promise for the treatment of asthma.

Pharmacology of MAP kinase

Don Griswold (SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA) presented an update on the pharmacology of the mitogen-activated protein kinases (MAPK). The MAPK cascades act as the downstream intracellular signal-transduction pathways that lead to pro-inflammatory gene expression. Inhibiting these pathways are potential targets for powerful and specific nonsteroidal anti-inflammatory agents. Topical and oral drugs exist that appear to be safe and have no effect on cytochrome P450. Griswold presented data on the effect of a specific kinase inhibitor on contact sensitivity to the topical sensitizer oxazolone. Direct inhibition of IFN-γ and TNF-α were demonstrated with decreases in IL-2 and IL-4 probably secondary to reduced influx of $CD4^+$ T cells into the skin. The p38 inhibitors tested do not seem to inhibit T-cell proliferation in a model of rheumatoid arthritis and promise to be anti-inflammatory without being immunosuppressing. There are p38 inhibitors being tested that may impact on several pathways of asthma pathophysiology, including eosinophil survival, airway remodelling and T-cell function. The newer second-generation drugs inhibit more p38 isoforms, have oral availability and a high therapeutic index. These drugs need to be evaluated in animal models of asthma as well as early Phase I trials.

Novel glucocorticoids

Lars Thorsson and Anders Tunek (Astra Draco, Lund, Sweden) discussed the development of a new generation of glucocorticoids with increased airway targeting and decreased systemic activity. Various approaches were outlined including the use of prodrugs (budesonide-21-palmitate) and 'soft' steroids (GR215864). The possibility of designing drugs that separated the ability of the glucocorticoid receptor to switch genes on and off was also discussed. At present all of the new drugs are being tested *in vitro* and most fail to achieve the desired effect in either animal models or in early clinical trials (e.g. tipredane, butixicort). The presentation concluded that further improvement of glucocorticoid avidity and activity will require complex novel approaches.

Gene expression and proteomics

Peter Strong (Glaxo Wellcome, Stevenage, UK) proposed a radically new approach to the development of new drugs for asthma. Contemporary technology using differential gene expression and proteomics allows an open-minded approach to the study of asthma and provides a molecular characterization of the disease. Well-defined targets can then be identified and used to unravel the many unanswered questions that have not been answered by conventional hypothesis testing. Indeed, hypothesis-led drug development has often failed – as in the case of platelet-activating factor antagonist research. The new approach is proposed to work alongside conventional drug testing but aims to avoid the

misleading targets that have beset asthma drug development in the past. Asthma is a complex heterogeneous disease in humans and the current hypothesis of its aetiology have not been adequately tested by conventional methods. Finding the underlying molecular signature holds the promise of novel disease-modifying drugs for asthma or asthmatic diseases.

Summary

Overall the conference was a dynamic interface where academic clinicians and scientists from the pharmaceutical industry could meet. Each of the talks was followed by healthy discussion and in spite of the usual problems of disclosure of company information, the current position in asthma research was clearly expressed and perhaps the way forward more clearly defined.

Ian M. Adcock and John G. Matthews
Thoracic Medicine
Imperial College School of Medicine
at the NHLI
Dovehouse Street
London, UK SW3 6LY

Bioprospecting or biopiracy?

Biodiversity is dwindling at an alarming rate – it has been estimated that 50 species become extinct daily and it has been projected that by 2020, 15% of biodiversity will be lost (http://moby.ucdavis.edu/GAWS/122/1alfa/fpart1.htm). More than half of all plant and animal species live exclusively in the rainforests of the Third World. Deforestation through activities such as mining, timber harvesting, farming cash-crops and cattle ranching – short-term remedies to national debt – is therefore a major concern. The commercial potential of biodiversity has driven pharmaceutical and biotechnology companies to seek out and extract useful biological resources before it is too late.

More than half of the world's biodiversity resides in the rainsforests of the Third World. The exploitation of the knowledge and resources of indigenous communities must be based upon mutually agreed terms between the industrialized nations and developing countries.

Economic pressures

One economic trigger for the commercial viability of bioprospecting was provided by the decision of the US Supreme Court (*Diamond vs. Chakrabarty*, 1980) that Chakrabarty could patent a genetically engineered oil-eating bacterium because 'his discovery is not nature's handiwork, but his own'. On the strength of this decision, the US Patent and Trademark Office (PTO) began routinely granting patents on hybridized and recombinant organisms, and within a few years, micro-organisms, plants, animals, cell lines and genes were under private ownership. In 1995, the PTO issued a patent to the US National Institutes of Health (NIH) for an unmodified human cell line drawn from an indigenous person from Papua New Guinea. It was the first time such a patent has been granted and it caused international outrage.

While it is evident that intellectual property rights (IPR) are a prerequisite for businesses to protect their return on investment, some claim that the ethical issues of IPR over biodiversity, indigenous know-how and biological samples are perceived as secondary issues. The agricultural and pharmaceutical industries have pressured governments to implement the General Agreement on Tariffs and Trade (GATT) and other international trade agreements, including the Convention on Biological Diversity (CBD), and this has cemented their rights to patent the resources and

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