like charybdotoxin. The ability to mimic the CD4 binding activity of gp120 in a relatively small peptide is impressive, and there may be some therapeutic potential in AIDS.

### Toxins to probe therapeutic targets

Toxins can be very useful as probes of potential therapeutic targets. They can be used at the molecular level to reveal differences between different subtypes of receptors or ion channels. This approach was demonstrated on actions of brevetoxins and ciguatoxins on different types of $Na^+$ channels by presentations by Richard Lewis (CDDD) and David Adams (University of Queensland, Brisbane, Australia). As well as providing a better understanding of the properties of tetrodotoxin-sensitive and -resistant $Na^+$ channels, this work explores the therapeutic potential of agents that might act selectively on different types of $Na^+$ channels.

The ability of dendrotoxins from mamba snake venoms to distinguish different subtypes within the Kv1 family of $K^+$ channels was used in a study by Alan Harvey and colleagues (Strathclyde University, Glasgow, UK) aimed at localizing different $K^+$ channels in the brain and looking for selective changes with ageing and in Alzheimer's disease. Use of the toxins revealed changes in subunit expression in different conditions, leading to speculation that there may be scope for therapeutic intervention to improve cognitive function by targeting particular subtypes within the Kv1 family of $K^+$ channels.

### Future prospects

The conference highlighted the rapid progress being made in understanding the structure and the pharmacological effects of some known toxins. Many contributions indicated the scope for finding additional toxins with novel structures and potentially useful activity. However, the conference also emphasized the considerable amount of work and the long time required to go from venom to drug. There is hope that ziconotide will be rapidly approved for therapeutic use so that it will act as a catalyst for other projects in the area of 'venoms to drugs'.

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# Conquering airway inflammation in the 21st century

Inflammation of the lower airways is central to the pathophysiology of several severe lung diseases, in particular asthma and chronic obstructive pulmonary disease (COPD). The first meeting, held last year, on *Conquering Airway Inflammation in the 21st Century* focused upon airway inflammation, asthma and current therapy ($\beta_2$-adrenoceptor agonists, glucocorticosteroids and theophylline), and introduced several new therapeutic targets and 'anti-inflammatory' molecules [Rogers, D.F. and Giembycz, M.A. (1998) *Trends Pharmacol. Sci.* 19, 160–164]. This year's meeting, held at the National Heart & Lung Institute (London, UK) on the 14–16 September 1998, extended discussion of inflammation to airways remodelling, and had an increased emphasis on new targets and molecules.

In several instances, pharmaceutical moieties, which last year had demonstrated encouraging effects in preclinical evaluation, had now entered clinical trial. It was interesting to note that airways remodelling, although critical to development of the irreversible component of impaired lung function in asthma and COPD, was not an endpoint target for evaluation of drug efficacy. This is understandable, as remodelling is not an easy parameter to quantify. However, inhibition and/or reversal of airways remodelling may affect the overall value of any therapy for asthma and COPD. The following account focuses upon the newer targets and molecules discussed at the meeting, in particular where chemical structures and clinical data were presented.

### Phosphodiesterase inhibitors

Elevation of cyclic AMP, either by $\beta_2$-adrenoceptor agonists or as a result of one of the possible mechanisms of action of theophylline, is a central therapeutic recommendation in guidelines on the management of asthma. Cyclic AMP can also be elevated by inhibiting the enzyme(s), termed phosphodiesterase (PDE), that degrade it. Although PDEs comprise a ten-member superfamily, it is PDE4 that is the predominant isoenzyme in immune and pro-inflammatory cells, and it is a major contributor to cyclic AMP metabolism in airways smooth muscle.

First generation PDE4 inhibitors such as rolipram are active across a broad spectrum of disease models. However, their therapeutic utility is limited by unwanted side effects, predominantly nausea, vomiting and gastric acid secretion. Fortunately, there are currently two options to reduce side effects. The first is development of compounds that are selective for one of the four gene families (PDE4A, B, C or D), which may selectively promote the desirable, rather than the deleterious, effects of PDE

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inhibitors. The second approach is based upon the ability of PDE4 to adopt at least two non-interconvertible, or slowly interconvertible, conformations, for which rolipram has high (PDE4H) and low affinity (PDE4L). In its simplest form, it is proposed that inhibition of PDE4H promotes many of the unwanted side effects (vomiting, gastric acid secretion) inherent in non-selective, first-generation PDE inhibitors, whereas inhibition of PDE4L is associated with certain beneficial effects including suppression of cytokine generation and release.

Thus, a PDE inhibitor selective for a specific gene product and the low affinity rolipram binding site could, at least in theory, have therapeutic potential in airways inflammation. Ariflo (SB207499; see Fig. 1), presented by Ted Torphy (SmithKline Beecham, King of Prussia, PA, USA), is equipotent with rolipram against PDE4L but 100-fold less potent against PDE4H, and it has a tenfold selectivity for PDE4D over the other PDE gene families. An additional property of Ariflo is that it is charged at physiological pH, which reduces penetration across the blood brain barrier and, consequently, lowers the potential for CNS-derived side effects. Successful preclinical and Phase I clinical data indicated Phase II trials. Interestingly, in trials of COPD it was found in a group of patients with moderate disease [mean forced expiratory volume in one second ($FEV_1$) of 47% predicted], $FEV_1$ improved by ~130 ml (~13% of their initial $FEV_1$) over a six-week treatment period. Studies are now under way to establish if PDE4D is functionally the most important isoform in human neutrophils, which might explain, at least in part, its clinical efficacy. Moreover, these results in COPD should rekindle enthusiasm for PDE4 inhibitors as a treatment for asthmatic inflammation which, thus far, has been disappointing.

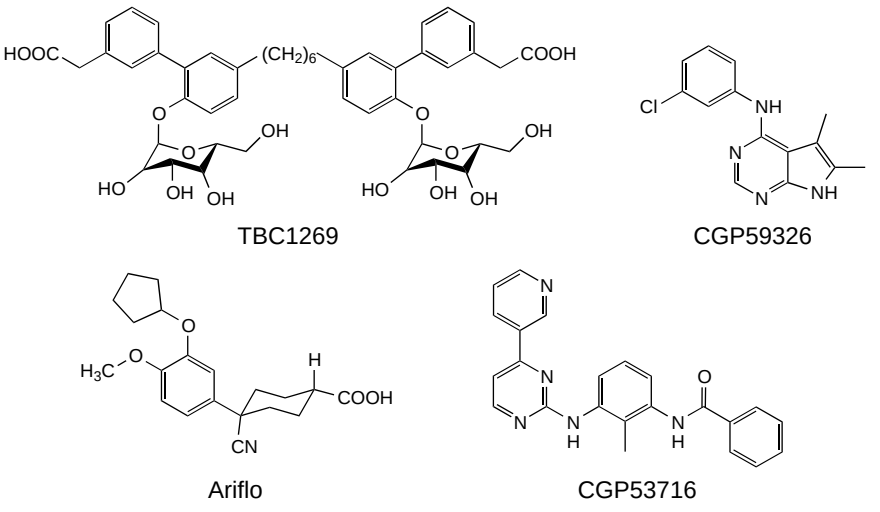


***Figure 1.*** *Structures of some novel compounds with potential for treatment of airways inflammation. TBC1269 is an E-selectin antagonist; Ariflo is a PDE-4 inhibitor; CGP59326 is an inhibitor of epidermal growth factor receptor protein kinase; CGP53716 is an inhibitor of platelet-derived growth factor receptor protein kinase.*

## Adhesion molecule inhibition

Inhibitors of cell adhesion molecules (CAMs) are being developed to inhibit leukocyte migration into the airways, with consequent suppression of inflammation. Although it is unclear which leukocyte to target, and which endothelial, epithelial or matrix CAM is most relevant and also which is the most effective route of administration, inhibitors of $\alpha_4$ integrins and selectins are in clinical trial in asthma.

CY9701 (Mariano Elices, Cytel, San Diego, CA, USA), and TBC1269 {1,6-bis [3-(3-carboxymethylphenyl)-4-(2-α-D-mannopyranosyloxy)phenyl]hexane; Fig. 1} (Ian Scott, Texas Biotechnology, Houston, TX, USA) are first-generation small-molecule peptidomimetic and non-oligosaccharide, glycomimetic antagonists, respectively. CY9701 is an antagonist of the integrin, very late antigen-4 (VLA-4; $\alpha_4\beta_1$), while TBC1269, a simplified analogue of the sialyl Lewis X tetrasaccharide, is an E-selectin antagonist. Scott described how enhanced potency was achieved through the synthesis of dimeric compounds that capitalize on additional interaction sites available at the carbohydrate/selectin interface which are used by more complex ligands such as dimeric sialyl Lewis X. Both drugs inhibit antigen-induced late-phase responses in allergic sheep and mice, and the associated pulmonary eosinophilia. Importantly, administration of the drugs after antigen challenge is still effective. In addition, CY9701 is reported to reduce eotaxin, interleukin 4 (IL-4) and IL-5 expression in the airways of mice, which indicates an effect on Th2 cell-driven inflammation.

TBC1269 has now entered Phase II clinical trial where it was given intravenously over 15 min to 21 mild to moderate asthmatics. Preliminary data indicate that it inhibited the late-phase response to allergen, and reduced sputum eosinophils. Second generation molecules such as TBC2019, which has a sufficiently long duration of action to be given the day prior to challenge, and TBC427, which is orally active, are under preclinical evaluation, and third generation molecules are in development. Texas Biotech also have an $\alpha_4\beta_1$ program and have developed molecules (e.g. TBC772) that prevent binding to vascular CAM that are active in several *in vivo* models of inflammation.

## Cytokine inhibition

A wide variety of approaches to inhibiting the effects of cytokines were

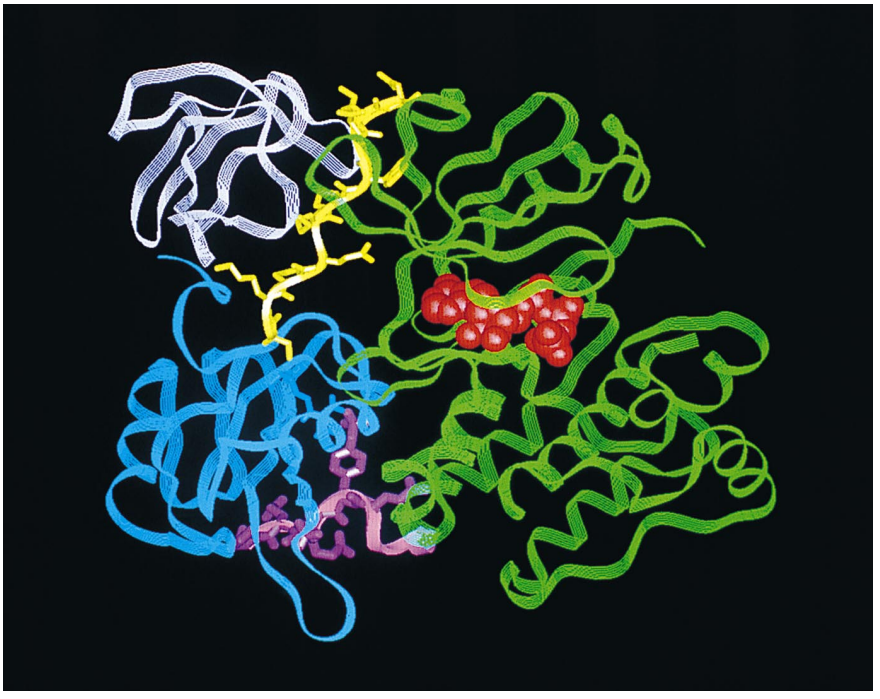

***Figure 2.*** *Structure of the tyrosine kinase c-src. Ribbon diagram illustrating the structure and organization of the enzyme domains. The catalytic domain and* src*-homology 2 (SH2) and SH3 domains are coloured green, blue and white, respectively. The enzyme is shown in its inactive conformation. However, for clarity, ATP (red) has been docked into the catalytic site of the model. Courtesy of Keith Menear, based on the original structure by Michael J. Eck [*Nature *(1997) 385, 595–602].*

presented. Paul Ponath (LeukoSite, Cambridge, MA, USA) provided a list of options for development of chemokine receptor (CCR) antagonists, many of which were endorsed by Noel Snell (Bayer, Slough, UK) for development of IL-4 receptor antagonists:

- Chemokines modified to bind receptors without signalling – for example monocyte chemotactic protein 1 (MCP-$1^{9-76}$), which reverses arthritis in an experimental model;
- Viral encoded proteins that look like chemokines – for example vascular macrophage inflammatory protein 1α (V-MIP-1α);
- Small molecules – which both Ponath and Snell considered currently to be the least likely clinical candidates;
- Certain chemokines themselves – for example eotaxin, which will block CCR2, and MCP-1 for CCR3;
- Monoclonal blocking antibodies – LeukoSite's main thrust, for example 7B11 and 2A8, which are CCR3 selective, and 2D4, which is CCR1 selective.

Binding of the circulating cytokine, using soluble receptors or monoclonal antibodies, will also lead to inhibition of activity. Phase II trials have shown good anti-inflammatory activity for nebulized recombinant human IL-4 receptor (rhIL-4R). A soluble form of the receptor for granulocyte-macrophage colony stimulating factor has been developed by coupling it to the Fc portion of murine IgG 2a (sGM-CSFRα-Fc) (William Williams, SmithKline Beecham, USA). The resultant heterotrimer specifically binds GM-CSF. Clinical studies are awaited. Since last year's meeting, SCH55700, a humanized neutralizing antibody to IL-5 developed from the animal antibody using CDR (complementarity determining region) grafting techniques, has entered Phase I clinical trials (Bob Egan, Schering-Plough, Kenilworth, NJ, USA). At a dose of 0.3 mg $kg^{-1}$ intravenously it had a $t_{1/2}$ of 25 days, which indicates that quarterly dosing is an option. Phase II studies are under way.

Another approach to inhibition of cytokine activity is to block cytokine synthesis. Greg Pahel (Glaxo Wellcome, Research Triangle Park, NC, USA) described the discovery of tumour necrosis factor α (TNF-α) converting enzyme (TACE) which cleaves and releases TNF-α from cells. TACE inhibitors are now in development. For example, 'Compound Y' is highly effective in preclinical *in vitro* and *in vivo* models, and clinical studies are planned for early next year, initially for inflammatory bowel disease and rheumatoid arthritis.

## Protein kinase inhibition

Kinases propagate and amplify membrane receptor signals through protein phosphorylation of tyrosine or serine/threonine residues to produce a cellular response. Inhibitors of these molecules are an attractive therapeutic option for several pharmaceutical companies (Keith Menear, Novartis, Horsham, UK; Don Griswold, SmithKline Beecham, USA; Tony Manning, Signal Pharmaceuticals, San Diego, CA, USA). However, with >2400 kinases in the human genome (700 of which can be accessed on public databases), and ~200 kinases per cell, development of kinase-selective inhibitors is a considerable challenge. Nevertheless, compounds are being developed, with many using selectivity for the ATP-binding site as a design rationale (Fig. 2).

Inhibition of T cell-specific kinases is an attractive area for development of anti-asthma drugs, with inhibition of lck, Janus kinase 3 (JAK3) and, in particular in Menear's view, ZAP-70 mimicking 'immuno-modulation'. Blockade

of intracellular signalling pathways from cytokine receptors [for example with inhibitors of NF-κB-inducing kinase (NIK) or FCεR1 receptors (with inhibitors of Syk or lyn) may have 'anti-inflammatory' effects. With its involvement in TNF-α and IL-1 signalling, p38 mitogen-activated protein (MAP) kinase is of particular interest. p38 MAP kinase was found to be the target of a group of SmithKline Beecham molecules, termed cytokine-suppressive anti-inflammatory drugs (CSAIDs), which although exhibiting anti-inflammatory activity, were not 'classical' anti-inflammatory drugs. Several molecules have been evaluated preclinically, including SB203580, VK19577 and L167307, but are associated with limiting side-effects, in particular hepatotoxicity. However, this does not seem to be a class effect and 'follow-up' compounds, some with potencies in the picomolar range, have been selected for potential development. Interestingly, airways remodelling may be targeted by inhibitors of receptor protein kinases for epidermal growth factor (EGF), for example CGP59326 (currently in Phase I trial; Fig. 1), and platelet-derived growth factor (PDGF), for example CGP53716 (Fig. 1).

**Summary**

By the end of the meeting, the impression was that remarkable progress had been made in just a year: therapeutic targets were being meticulously characterized, drug molecules were being designed specifically for those targets, and some new drugs were now not only in clinical trial but were also giving encouraging results. Although too early to tell, and with experience of many 'false dawns' in drug discovery, it is distinctly possible that there will be several new drugs aimed at conquering airway inflammation on the market early into the new millennium.

For the 1999 meeting, see http://www.nhli.ac.uk/mtgs.htm#dg

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# Hunting for PPAR ligands

A crystal clear view of the peroxisome proliferator-activated receptor-γ (PPAR-γ) has been seen by US researchers, which could reveal clues to designing novel drugs for several diseases including atherosclerosis, cancer and diabetes.

The receptors for oestrogen, thyroid hormone, retinoic acid and retinoid X act as intermediaries between hormones and DNA. Drugs that act on these receptors are keenly sought. One class member, the PPAR, is crucial for normal development and gene regulation but is an 'orphan' receptor having no known ligand.

PPAR-γ is a transcription factor found in the nucleus of adipocytes and in macrophages. Although no bona fide ligand has yet been recognized, there are several compounds known to activate it. For instance, in its role in lipid regulation, several fatty acids, oxidized lipids and prostaglandin J derivatives can interact with the receptor.

Activation of PPAR-γ by a ligand causes it to bind to specific DNA sequences in the nucleus of adipocytes. It also controls differentiation of muscle cells to fat cells. Having more fat cells to respond to insulin and metabolize glucose enables better control of diabetes, so understanding the activation could lead to new drugs for this disease.

**Dietary fats and cancer**

Recent research on a mouse genetic model, reported in *Nature Medicine* at the beginning of September by Enrique Saez of the Salk Institute (La Jolla, CA, USA) and colleagues also suggested that PPAR-γ could be the genetic switch that allows fats to trigger colorectal cancer – at least in the mouse model [(1998) *Nat. Med.* 4, 1058–1061]. However, other laboratories [Spiegelman (Harvard) and Koeffler (UCLA)] have shown that PPAR-γ ligands halt or slow the growth of human colon tumour cells and tumour cells from other diet-related cancers such as prostate and breast cancer.

A better understanding of the site of ligand binding in PPAR-γ and discovering its natural ligands could provide clues to developing novel drugs for treating several diseases, including cancer, diabetes and atherosclerosis.

**Crystal clear pocket**

Now, a collaboration between Michael Milburn and his colleagues at Glaxo Wellcome (Research Triangle Park, NC, USA) and Christopher Glass and Michael Rosenfeld at UCSD Howard Hughes Medical Institute (La Jolla, CA, USA) has focused on the ligand-binding centre of the protein and built a high-resolution X-ray crystal structure to examine its interactions with a coactivator required for PPAR-γ to alter gene expression [Nolte R.T. *et al.* (1998) *Nature* 395, 137–143].

The crystal structure of the 'empty' PPAR-γ revealed a large binding pocket. This, says Rosenfeld, may explain the diversity of ligands for PPAR-γ. The team also examined the pocket when occupied. The ternary complex containing the PPAR-γ ligand-binding domain (LBD), the antidiabetic ligand rosiglitazone, and a stretch of human steroid receptor coactivating factor-1 (SRC-1) – a coactivator in the transcriptional

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