Treat Respir Med 2005; 4 (2): 85-94
1176-3450/05/0002-0085/$34.95/0

Selectin Antagonists

Therapeutic Potential in Asthma and COPD

Suzanne J. Romano

Novasite Pharmaceuticals Inc., San Diego, California, USA

Abstract

Asthma and COPD are chronic inflammatory conditions that affect hundreds of millions of patients worldwide. New therapeutics are desperately needed, especially those that target the underlying causes and prevent disease progression. Although asthma and COPD have distinct etiologies, both are associated with reduced airflow caused by excess infiltration of inflammatory cells into healthy lung tissues. As selectin-mediated adhesion of leukocytes to the vascular endothelium is a key early event in the initiation of the inflammatory response, selectin inhibition is thought to be a good target for therapeutic intervention.

Three known selectins are expressed in distinct subsets of cells: P-selectin is presented on the surface of activated platelets and endothelial cells, L-selectin is constitutively expressed on leukocytes, and E-selectin synthesis is upregulated in activated endothelial cells. They mediate cell-cell adhesion in the shear flow of the bloodstream via specialized interactions with clusters of oligosaccharides presented on cell surface glycopeptide ligands. The role of selectin-ligand interactions in the inflammatory response has been demonstrated in various animal models, prompting considerable attention from the pharmaceutical industry.

Drug discovery efforts have yielded many different classes of selectin inhibitors, including soluble protein ligands, antibodies, oligosaccharides and small molecules. Although many selectin inhibitors have shown activity in preclinical models, clinical progress of selectin-directed therapies has been slow. Early approaches employed carbohydrate-based inhibitors to mimic the natural ligand sialyl Lewis X; however, these compounds proved challenging to develop. Cytel's CY 1503, a complex oligosaccharide, progressed to phase II/III trials for reperfusion injury, but further development was halted when it failed to demonstrate clinical efficacy. Two protein-based selectin inhibitors have reached phase II development. These included Wyeth's recombinant soluble P-selectin ligand, TSI (PSGL-1), which was discontinued after disappointing results in myocardial infarction trials and Protein Design Labs' humanized anti-L-selectin monoclonal antibody, which is currently in development for trauma. Bimosiamose, discovered by Encysive Pharmaceutical and presently being developed by Revotar Biopharmaceuticals, is an 863 g/mol molecular weight dimer with minimal carbohydrate content and is, to date, the leading selectin inhibitor in clinical development. This compound has shown promise in a phase IIa 'proof of concept' trial in patients with asthma, reducing airway recruitment of eosinophils after intravenous administration. Further clinical development of an inhaled formulation is underway.

Despite a significant need for new therapeutics, selectin inhibitors have not yet been explored for the treatment of COPD. Bimosiamose represents an important proof of principle, and hopefully continued success will spark renewed interest in selectin-directed therapeutics for respiratory diseases.

Asthma and COPD have an enormous impact on the world population. An estimated 600 million people have COPD, and an additional 155 million have asthma.[1] These two conditions accounted for nearly $US11 billion in drug treatment costs worldwide in the year 2000.[2] The prevalence of both respiratory diseases is increasing, and new therapeutic options are desperately needed – especially those that address the underlying causes rather than merely treating symptoms.

Asthma and COPD are chronic respiratory diseases that are characterized by excessive and uncontrolled infiltration of inflammatory cells into healthy lung tissues. Patients experience restricted airflow and disabling and often irreversible damage to their

lungs. While asthma and COPD have distinct etiologies and different inflammatory mediators are responsible for pathogenesis of the two diseases, it is thought that intervening at the initial stages of the inflammatory process may provide the best opportunity for halting the progress of both diseases.[3] However, most available treatments address the downstream consequences of excess leukocyte infiltration into pulmonary tissue (figure 1). For example, bronchodilators relax smooth muscle in the airways and alleviate bronchoconstriction, while leukotriene inhibitors counteract the release of pro-inflammatory mediators from invading inflammatory cells. Corticosteroids have broader anti-inflammatory activity, but cause a number of adverse effects that limit long-term use.

To provide safer and more effective therapeutic agents for chronic respiratory diseases, pharmaceutical and biotechnology companies are making concerted efforts to discover drugs that specifically target the upstream triggers and mediators of these diseases.[4] One such strategy is to interfere with the 'leukocyte adhesion cascade', a key early process in inflammation during which a series of chemoattractants, cytokines, and cell adhesion molecules (CAMs) work in a programmed, sequential manner to direct inflammatory cells to inflamed tissue (figure 2).[5] By inhibiting this process it may be possible to reduce tissue damage caused by excess cellular infiltration and stop the feedback loop that amplifies the inflammatory response.

Three known families of CAMs participate in the leukocyte adhesion cascade: selectins, integrins, and members of the immunoglobulin superfamily. The initial event of this cascade, the tethering and rolling of leukocytes (including lymphocytes) along the blood vessel wall, is mediated by the selectins. Selectin-ligand interactions act like 'Velcro' reducing the flow of inflammatory cells through the blood vessels and slowing them sufficiently to allow firm adhesion and extravasation via the integrin and immunoglobulin family members. Selectins may also enhance inflammation by activating second messenger pathways, including members of the mitogen-activated protein (MAP) kinase cascade.[7] Because of their critical role in the earliest stages of the immune response, the selectins are potential targets for novel therapeutics to treat inflammatory conditions such as asthma and COPD.[3,8]

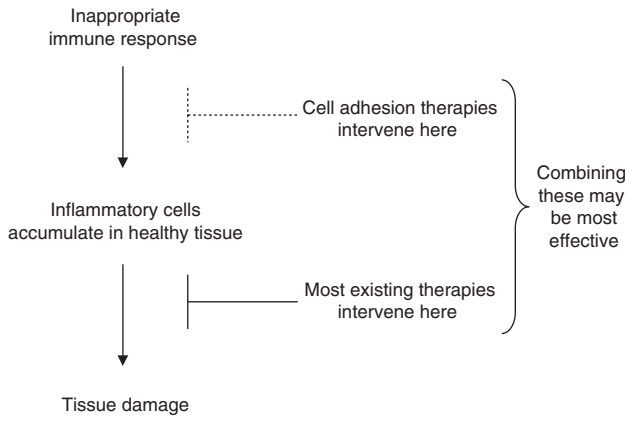


Fig. 1. Strategies for inhibiting the inflammatory response in obstructive pulmonary disease.

1. The Structure and Function of Selectins

The selectins are a small family of transmembrane glycoproteins that mediate cell-cell adhesion by binding to cell surface carbohydrate ligands.[9-11] To date, three members have been identified, each expressed in a distinct subset of cells: P-selectin (platelets and endothelial cells), L-selectin (leukocytes), and E-selectin (endothelial cells). They are also known by their cluster of differentiation (CD) antigen designations CD62P, CD62E and CD62L. The three selectin proteins share similar structural features, including a calcium-dependent (C-type) lectin binding domain, an epidermal growth factor (EGF)-like domain, a series of short consensus 'complement regulatory (CR) protein' repeat sequences, and a transmembrane anchor segment. The number of CR repeats and the size of their cytoplasmic domains vary.

The ligands for the selectins are cell surface glycoproteins that present clusters of specialized oligosaccharides.[7,12] Key motifs recognized by all three selectins include sialylated and fucosylated tetrasaccharide sialyl Lewis X (sLe^X; figure 3) and the related sialyl Lewis a (sLe^a);[12-14] however, there is considerable variability in the ligands' glycoprotein scaffolds and how they present their carbohydrate moieties. L-selectin is known to bind GlyCAM-1, CD34 and MAdCAM-1; each is expressed on different tissues and each presents many L-selectin binding sites distributed over the length of the scaffold. In comparison, TSI, the P-selectin ligand, is a dimer that presents a single binding site at the tip of each subunit. (P-selectin also binds CD24, the smallest of the ligands; this interaction may be involved in migration of cancer cells.[15]) The functional E-selectin ligands have not yet been clearly identified, but candidates include the E-selectin-specific ESL-1 and the shared ligand, TSI.

The various selectin-ligand pairs interact with distinct binding affinities and kinetics, allowing the selectins to mediate tethering, rolling and adhesion between various cell types in a wide range of *in vivo* settings. Specialized interactions allow them to perform their functions in the shear flow forces of the bloodstream, including rapid association/dissociation (on/off) rates, ligand clustering and dimerization, and multivalent oligosaccharide binding sites.[16] Remarkably, selectin-mediated adhesion is actually enhanced by shear stress.[17]

The unique properties of the selectin-ligand interaction have made it difficult to measure selectin activity using *in vitro* assays,

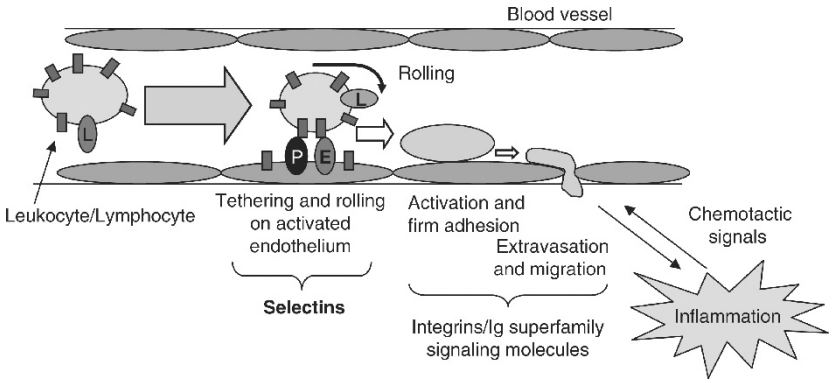


Fig. 2. Schematic presentation of the role of selectins in the leukocyte adhesion cascade, leading to excess infiltration of inflammatory cells into the target tissue (reproduced from Romano and Slee.,[6] with permission from PharmaPress Ltd). **E** = E-selectin; **L** = L-selectin; **P** = P-selectin.

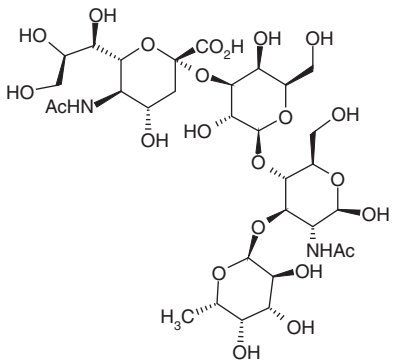
and may have hindered progress in the field. The need for shear flow, complex ligands and multivalent interactions do not lend themselves to the high throughput methods currently used in most drug discovery programs. Some groups have successfully established binding assays and identified inhibitors using synthetic ligands and recombinant selectin proteins,[18,19] for example using the system developed by Foxall et al.[20] or modified versions using cultured cell lines.[21] However, static adhesion assays often give inaccurate estimations of the activity of a compound and make it difficult to obtain an accurate measurement of the ability of a compound to block selectin-mediated rolling in shear flow.[8,22,23] To address this concern, mechanistic assays that measure cell-cell adhesion and rolling under flow conditions *in vitro* and *in vivo* have been used to determine the intrinsic selectin-inhibiting potential of test compounds.[22,24-26] These mechanistic assays can serve as important proof of principle to demonstrate mechanism of action and support further testing using *in vivo* models of inflammation.

2. The Role of Selectins in the Inflammatory Response

The three selectins are expressed in unique temporal and spatial patterns, allowing significant flexibility in the modulation of the inflammatory response.[7,27] Furthermore, the expression of each selectin is regulated via a different mechanism, providing several opportunities for blockade. P-Selectin is stored in secretory vesicles of endothelial cells (Weibel-Palade bodies) and platelets (α-granules), and is rapidly transported to the cell surface upon activation by mediators such as histamine or thrombin. E-Selectin is regulated at the transcriptional level, and thus appears on the surface more slowly. It is expressed on endothelial cells in response to pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α or interleukin (IL)-1β. L-Selectin is constitutively expressed on all leukocytes, and upon cell activation it can be down-regulated from the surface by protease-mediated cleavage known as 'shedding'.

The role of selectins in the inflammatory response has been well established. Mouse knockouts of the selectins alone and in various combinations have been useful tools to elucidate their roles *in vivo*.[28] It has been demonstrated that P-selectin mediates rolling in the basal state, while L-selectin is critical for lymphocyte homing to peripheral lymph nodes. All three selectins mediate leukocyte rolling during inflammation, with P-selectin appearing early in the inflammatory cascade and E- and L-selectin involved later. Importantly, the knockout data also suggest that it may be necessary to inhibit more than one of the selectins to have a significant impact on the inflammatory response.

Inhibitors such as antibodies, soluble protein ligands, and various sLe^X mimetics have been used to further confirm that blocking the selectins can reduce the inflammatory response in a variety of animal models (see reviews by Lowe and Ward[8] and Rosen and Bertozzi[12]). Proof of concept in humans comes from the study of a very rare genetic disease termed type II leukocyte adhesion deficiency (LAD II), which has been described in only a few



sialyl Lewis X

Fig. 3. The structure of the oligosaccharide sialyl Lewis X, the primary native selectin ligand.

patients to date.[29,30] The LAD II mutation causes a defect in fucose metabolism, resulting in a complete lack of the selectin ligand sLe^X. Because of this, selectin-mediated events such as leukocyte rolling and neutrophil chemotaxis are greatly reduced in these patients.[31,32] Clinically, LAD II patients experience chronic, severe periodontitis as well as mental and growth retardation; however, their developmental abnormalities are likely attributed to the general defect in fucose metabolism rather than a defect in leukocyte adhesion.[30]

In addition to acting at sites of inflammation, selectins are important components of the normal immune response. The body's surveillance system relies on selectins for directing blood leukocytes to sites of tissue damage or infection, and for trafficking of circulating lymphocytes through lymphoid tissues. One concern is that blocking selectins would make already weakened patients more susceptible to infection. This theory was supported by observations in E-, P-, and E/P-selectin double-knockout mice, which had a higher frequency of opportunistic bacterial infection and higher morbidity when challenged with *Streptococcus pneumoniae*.[33,34] However, clinical experience suggests that selectin blockade may not create significant problems in human patients. Adults with LAD II are perhaps the best approximation of a patient treated long-term with a highly effective selectin inhibitor. Aside from chronic periodontitis, LAD II patients do not show greater incidence of systemic infection, nor do they require prophylactic antibacterials.[30] Mice deficient for α-1,3-fucosyltransferase Fuc-TVII, a mutation that closely mimics human LAD II, were also resistant to opportunistic infections.[35] This is a less severe phenotype than what has been observed in the knockout mice, as described above. The apparent differences in susceptibility may be attributed to the fact that the knockouts completely lack selectin proteins, while the fucose metabolism mutants may retain some selectin binding function due to non-fucosylated ligands.[30] It may also reflect differences in laboratory settings and mouse strains.

2.1 The Role of Selectins in Airway Inflammation

Data from the LAD II patients, knockout mice, and other animal models strongly suggest that inhibiting selectins can safely reduce an inappropriate immune response by attenuating cellular infiltration. Thus, it seems reasonable to postulate that selectin inhibitors could effectively treat airway diseases such as asthma and COPD, where accumulation of inflammatory cells in the lung tissue causes reduced lung function.[36] Data from animal models of airway inflammation have been promising. Ward and colleagues have demonstrated the role of selectins in several models of acute lung injury using blocking antibodies, soluble ligands and carbohydrate inhibitors.[37,38] Murine models of antigen-induced airway inflammation have also provided useful data. Mouse knockouts suggest that both P-selectin and L-selectin are important. Antigen-challenged P-selectin-deficient mice had reduced airway hyperresponsiveness (AHR) and decreased pulmonary eosinophilia in comparison with wild-type controls.[39,40] In L-selectin knockout mice, cellular infiltration was not significantly reduced but the population of invading cells was slightly different and AHR was remarkably reduced.[41,42] While the murine models do not reproduce human asthma or COPD with complete accuracy, they demonstrate that selectins participate in the recruitment of leukocytes to the lung and suggest that each of the selectins is responsible for a different aspect of the inflammatory response.

Perhaps the most compelling preclinical data come from sheep and monkey studies, where anti-selectin monoclonal antibodies (mAbs) have shown efficacy. These models are attractive because they most closely approximate the two key manifestations of the human asthmatic response: bronchoconstriction, which comprises an acute early airway response (EAR), a late airway response (LAR), and prolonged AHR. In fact, the sheep model has become a 'gold standard' for preclinical efficacy studies, and nearly every new asthma drug that has progressed to clinical trials has been tested in this system. An L-selectin mAb reduces all phases of the airway response in antigen-challenged sheep.[43] In the monkey model, an E-selectin antibody reduced neutrophil influx and attenuated the LAR.[44]

3. Selectin Inhibitors

Because of their role in inflammatory disease, selectins have been the target of drug discovery programs at many pharmaceutical and biotechnology companies over the past several years. As described above, expression of the selectins is regulated via several different mechanisms, providing a number of potential therapeutic opportunities.[45,46] For example, cell-surface expression of selectins can be blocked by small molecules[47] or antisense nucleotides.[48] Alternatively, it may be possible to identify compounds that induce cleavage or shedding of the selectins from the cell surface.[49] This mechanism may already be the target of existing treatments: some NSAIDs, including indometacin, ketoprofen and aspirin (acetylsalicylic acid), induce the release of L-selectin from neutrophils.[50]

The most common and straightforward approach for blocking selectin action is to inhibit the selectin-ligand interaction directly. Over the past decade, more than 20 pharmaceutical companies have reported selectin inhibitors and a number of these have been validated in animal models (see recent reviews[45,46,51,52]); unfortu-

Table I. Selectin antagonists in clinical development for asthma and other indications

Developing Ccmpany (originating company)	Compound	Type of inhibitor	Selectin/ selectins targeted	Development Status	Indication
Revotar Biopharmaceuticals (Encysive Pharmaceuticals)	Bimosiamose (TBC 1269)[21]	Mannose-containing small molecule dimer	P, E, L	Phase II Phase I	Asthma, psoriasis, COPD, ARDS
Protein Design Labs Scil Technology	Anti-L-selectin monoclonal antibodies[53]	Antibody	L	No development reported	Trauma
Wyeth	TS-1 (PSGL-1)[54]	Glycoprotein	P	Phase II (discontinued)	Myocardial infarction
Cytel	Cylexin® (CY 1503)[55,56]	Oligosaccharide	P, E	Phase III (discontinued)	Reperfusion injury

ARDS = acute respiratory distress syndrome; **E** = E-selectin; **L** = L-selectin; **P** = P-selectin.

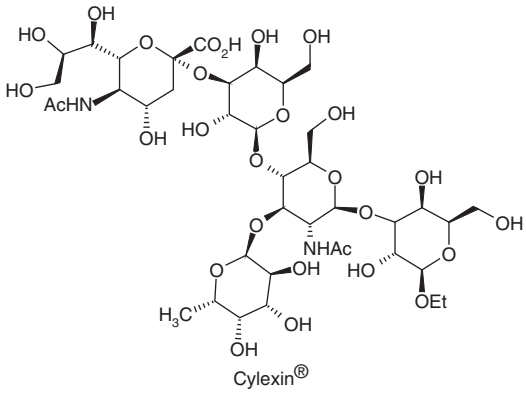
nately, only a few of these have been tested in patients, and clinical success has been elusive (table I).

The first-generation selectin inhibitors, represented by Cytel's (now Epimmune) CY 1503 (Cylexin® [1]; figure 4),[55,56] were complex oligosaccharides that competed with ligand binding by mimicking the natural ligand, sLe^X (figure 3). Of the selectin inhibitors that have been nominated for clinical development, Cylexin® has progressed furthest. Unfortunately, it showed no benefit in placebo-controlled phase II/III trials for reperfusion injury, and development was discontinued in early 1999.[57,58] This result can likely be attributed to the carbohydrate nature of the drug. Pharmaceutical development of oligosaccharide-based compounds has proven difficult for several reasons, and clinical success has been limited.[57,59] These compounds often have poor potency and are metabolically unstable *in vivo*; they also tend to be technically challenging and expensive to synthesize. Furthermore, because they require parenteral administration, the therapeutic applications of such compounds are limited.

Although they share some of the same problems, protein drugs have, in general, met with better clinical success. Unfortunately, this has not been the case for protein-based selectin inhibitors. Protein Design Labs licensed its humanized anti-L-selectin mAb, aselizumab, to Scil Technology for development in Europe, and planned to use Scil's trial data to aid internal clinical efforts. Recently, however, published results reveal that aselizumab failed to demonstrate statistically significant efficacy in a phase II evaluation in trauma pateients, and as a result the future of this drug is uncertain.[60] Wyeth's recombinant soluble P-selectin ligand, TSI,[54] was discontinued after disappointing phase II results in myocardial infarction, but may be examined for other indications. Like cytexin, the glycoprotein and antibody drugs are difficult to manufacture and require parenteral administration. Despite these concerns, protein-based anti-selectin approaches should not be summarily dismissed. The recent success of the anti-TNFα protein drugs etanercept and infliximab suggest that this may be a viable approach for the treatment of some chronic inflammatory disease[61].

For asthma and COPD, however, patients and clinicians are in need of new drugs that can be administered orally or, if necessary, via inhalation. Thus, the search continues for potent, small molecule selectin inhibitors with minimal carbohydrate content. A greater understanding of the selectin structures[62,63] and the key interactions between selectins and their ligands[7,13,64] has aided the search. Several groups have reported non-oligosaccharide inhibitors with better properties,[65-67] but few have succeeded in identifying compounds with potent *in vivo* activity.

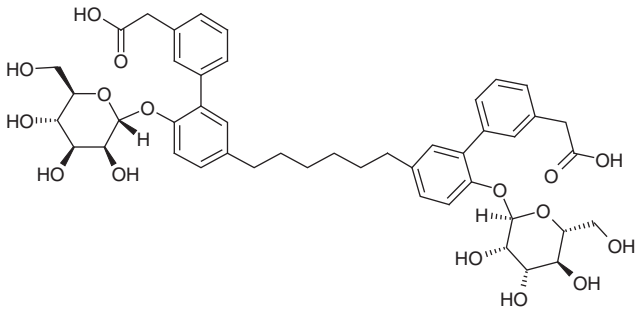
The leading 'second-generation' selectin inhibitor is bimosiamose, which was discovered by Encysive Pharmaceuticals and is currently being developed by Revotar Biopharmaceuticals. Bimosiamose, shown in figure 5, is an 863 g/mol molecular weight dimer with minimal carbohydrate content. This approach minimizes carbohydrate content of the inhibitor while maintaining the



Cylexin®

Fig. 4. The chemical structure of a first-generation oligosaccharide inhibitor, Cylexin® CY 1503.[55,56] Clinical development of this compound was discontinued due to poor efficacy in phase III clinical trials.

1 The use of trade names is for product identification purposes only and does not imply endorsement.



Bimosiamose

Fig. 5. The chemical structure of bimosiamose[21] (TBC-1269), the most promising selectin inhibitor undergoing clinical development for asthma and other indications.

multivalent presentation of sugar moieties thought to be required for potency. Bimosiamose has been reported to have IC_{50} values (concentration producing 50% inhibition) of 500, 70 and 560 μmol/L against E-, P-, and L-selectin, respectively, in cell adhesion assays and 105, 17 and 87 μmol/L against E-, P-, and L-selectin, respectively, in ELISA.[21,68] It has efficacy in several animal models of inflammation[68] and has shown promise in a phase IIa clinical trial for allergic asthma[52,69] (see section 4.1).

There have been few reports of small molecule selectin inhibitors that completely lack carbohydrate content, yet maintain potency and *in vivo* activity. One such molecule at the preclinical stage is Ontogen Corporation's OC 229648 (figure 6). The lead compound in a series of potent, pan-selectin inhibitors, OC 229648 has IC_{50} values of 300 nmol/L and 3 μmol/L in P- and L-selectin ELISAs, respectively, and 10–50 μmol/L IC_{50}s in P-, L- and E-selectin cell adhesion assays; this compound reduces selectin-mediated leukocyte rolling in inflamed vessels, and exhibits activity in animal models of cutaneous and peritoneal inflammation.[18,70,71] It remains to be seen whether such 'third-generation' selectin inhibitors will be successful in the clinic.

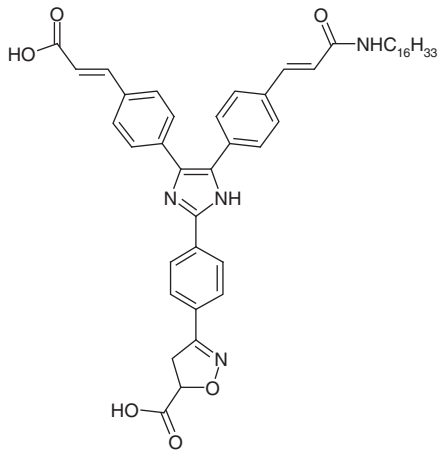
4. Selectin Therapeutics for the Treatment of Respiratory Diseases

As described above, many different selectin inhibitors have been identified and a number of these have been validated in animal models. These compounds span a broad range of physical properties, selectivity and potencies. However, with one notable exception (bimosiamose), these early-stage successes have not translated to the clinic.[36] Carbohydrate-based and protein inhibitors have proved technically challenging to develop for respiratory diseases, where inhaled administration is common and an oral formulation is preferred.

4.1 Targeting Asthma

Asthma is a well studied condition, and much is known about its immunology and cell biology. Reversible airway obstruction and hyperreactivity are the hallmarks of this chronic inflammatory disease. It is generally thought that asthma is an innate, IgE-mediated allergic response triggered by airborne allergens. However, a closer examination of the clinical data suggests that there may be multiple phenotypes of asthma or 'wheezing illness', each with distinct causes and mediators.[72] This variability may explain some of the clinical failures seen in the past few years – most recently, the anti-IL-5 monoclonal antibodies.[73] These new findings also raise important questions about future diagnosis and treatment of patients with asthma, and highlight the need for new, targeted therapeutics that are directed at specific mediators. Many such drugs are in clinical trials as pharmaceutical companies seek alternatives to the current standard of care consisting of the broadly acting corticosteroids.[74-77]

One of those new drug candidates is the pan-selectin inhibitor bimosiamose (figure 4), currently in phase II clinical trials for asthma.[21,68] In preclinical testing, the compound reduced the EAR, LAR and AHR in a sheep model after intravenous and inhaled administration.[43] Some may question why a selectin inhibitor, which targets a vascular target, would be effective via the inhaled route; however, inhaled formulations of both selectin and integrin antagonists have proven efficacious in animal models of asthma.[78] The simplest explanation for these results is that the drug is absorbed into the systemic circulation through the lung epithelium, but it is also possible that the compounds work on resident cell-cell interactions directly in the lung tissue. Encysive Pharmaceuticals circumvented potential bioavailability issues in



OC 229648

Fig. 6. The chemical structure of OC 229648,[18] a third-generation selectin inhibitor that lacks sugar moieties. This compound is still in the preclinical stage of development for asthma and COPD.

their phase IIa 'proof of concept' trial by intravenous dosing. In the first clinical success of a cell adhesion inhibitor, intravenous bimosiamose 30 mg/kg reduced airway recruitment of eosinophils in asthma patients.[36] Toxicity data indicate that bimosiamose is safe and suggest that this compound may have an advantage over corticosteroids.[68,79]

The clinical development of bimosiamose continues with Revotar Biopharmaceuticals. Two phase I, double-blind trials of the inhaled formulation were completed in early 2002.[52] In an escalating single-dose study, the drug was well tolerated and no serious adverse events were observed. Plasma levels were achieved at the higher doses, demonstrating that the drug can, in fact, enter the bloodstream via the inhaled route.[52] The second trial examined the effects of various dose levels given twice per day over a 7-day period. Again, the drug was well tolerated. Based on these trials, the maximum tolerated dose and the dose required to achieve systemic drug levels were established. Revotar Biopharmaceuticals has completed a double-blind, placebo-controlled, randomized, cross-over study to evaluate the effects of inhaled bimosiamose on LAR following inhaled allergen challenge in patients with mild allergic asthma. The results of this proof of concept study demonstrated positive clinical results of the pan-selectin antagonist, bimosiamose, where pre-treatment with nebulized bimosiamose decreased the allergen challenge-induced LAR in patients by 50% compared to placebo.[69] Bimosiamose is also in clinical devleopment for psoriasis, COPD, and ARDS. The continued success of bimosiamose represents an important proof of principle and suggests that a selectin inhibitor will have use in the treatment of asthma and other inflammatory diseases.

Next-generation selectin inhibitors – smaller molecules that lack carbohydrate moieties and are amenable to oral administration – are under investigation by several groups, but are likely years away. The structures of the three key selectin inhibitors shown in figure 4, figure 5 and figure 6 illustrate the progression towards smaller compounds with minimal carbohydrate content. Ontogen's OC 229648 (figure 4), which has no sugar moieties, was active in mouse models of inflammation but did not show efficacy in preliminary sheep airway studies.[46] This result is probably not due to insufficient potency, since OC 229648 is more active than bimosiamose in *in vitro* P-, E- and L-selectin assays. It is possible that bimosiamose has multiple functions *in vivo* which enhance its efficacy in asthma. In addition to reducing leukocyte trafficking to the lung, it may inhibit selectin-mediated signaling between resident leukocytes in the lung.[36] Indeed, in the sheep model, it reduced mast cell histamine release during the EAR.[43] Taking these characteristics into account, future drug discovery efforts may lead to selectin inhibitors with improved *in vivo* properties.

4.2 Targeting Chronic Obstructive Pulmonary Disease

While COPD is often mentioned together with asthma, the two are very different diseases. COPD includes chronic bronchitis and emphysema, and is characterized by obstructed airflow without the airway hyperreactivity seen in asthma. Approximately 80–90% of COPD cases are caused by smoking, and 15% of all cigarette smokers develop COPD. This chronic respiratory condition can also be due to exposure to other lung irritants. In rare cases (<5%), hereditary, early onset COPD is caused by a deficiency in α1-antitrypsin, a protective protease inhibitor.

COPD is six times more prevalent than asthma and is responsible for more than 100 000 deaths annually in the US; it was the fourth leading cause of death in 1999.[80] Despite the large patient population and the severe unmet medical need, COPD drugs comprise only 17% of the respiratory disease market and new treatments are desperately needed.[2] The lack of a predictive animal model that incorporates all of the key aspects of COPD may have slowed progress towards new therapies.[81] Currently, the most effective way to manage COPD is smoking cessation therapy that may include nicotine replacement or treatment with buproprion. Other available therapies include corticosteroids and bronchodilators. In addition, antibacterials and vaccines against the influenza virus are used to reduce the threat of respiratory infection in patients with COPD. Lung transplant and lung reduction surgery are more invasive options. Unfortunately, these treatments only attempt to treat the symptoms, and none affect the progression of the disease; even corticosteroids are largely ineffective.[82] In fact, most of the drugs currently used to treat COPD were originally developed for asthma.[83] Given the mechanistic differences between the two diseases, their lack of efficacy is not surprising.

While asthma and COPD are both associated with an excessive influx of inflammatory cells and airway obstruction, the etiology of the two conditions is quite different.[84] COPD is associated with the infiltration of CD8-positive lymphocytes, neutrophils and macrophages into lung tissues; in contrast, the invading cells in the lungs of asthmatic patients are primarily CD4+ T cells and eosinophils. Selectins are key molecules that direct leukocytes to the lung,[43,55,85,86] and there is evidence that the expression of selectins and their ligands are elevated in the lung tissue and on the peripheral leukocytes of COPD patients.[87,88] Thus, it would seem reasonable to target selectins for the treatment of COPD. Selectin antagonists have not yet been pursued for the treatment of COPD in the clinic, but the successful use of bimosiamose in asthma may well generate greater interest in this area. In fact, preclinical studies with bimosiamose suggest that it has preferential effects on

neutrophils,[89] so it may be a good candidate for reducing cellular infiltration in the lung tissue of COPD patients.

5. Conclusions

Selectin-mediated tethering of leukocytes to the vascular endothelium is a key, early event in the pathogenesis of acute inflammation, and many agree that it is a reasonable target for therapeutic intervention. Animal data strongly suggest that blocking selectins can prevent excessive infiltration of inflammatory cells associated with airway disease, and early results with the pan-selectin inhibitor bimosiamose have been promising. However, the poor showing of selectin inhibitors and other carbohydrate-based drugs in clinical trials has caused the pharmaceutical industry to remain cautious.[57]

Development of selectin inhibitors to treat respiratory disease is a difficult undertaking. Asthma and COPD are complex, pleiotropic diseases, and redundancy in the pathways that lead to respiratory disease may make it difficult to identify single agents that are effective for all patients.[77] Pairing a selectin inhibitor with an agent that works further downstream, for example a leukotriene inhibitor, may result in enhanced efficacy. The combination approach is often used in the treatment of asthma and COPD (e.g. pairing corticosteroids and bronchodilators), but this has not yet been examined with selectin inhibitors. Many new drugs targeting various aspects of the signaling pathways are in development for respiratory disease;[4,74,75,77,82,83] therefore, new tools for such studies should be available in the coming years.

In addition to being the target of blockade, selectins are receiving increased attention in the area of targeted drug delivery. Because they are expressed in distinct spatial and temporal patterns during inflammation, selectins may be useful for targeting drugs directly to the site of inflammation. Everts and colleagues have developed a novel approach to link dexamethasone to an E-selectin antibody, in order to target the drug to inflamed tissue.[90] This method could be used to minimize the dose and avoid some of the unwanted adverse effects of nonspecific drugs like corticosteroids, commonly used for the treatment of respiratory disease. It may also have the added benefit of selectin blockade, making it a targeted, combination treatment.

A greater understanding of the underlying mechanisms responsible for disease initiation and progression will hopefully lead to more effective, and eventually more personalized, treatments. For example, the recent findings that suggest there are multiple forms of asthma[72] offer a potential mechanism for targeting treatments to the appropriate patient population. The fact that bimosiamose appears to have preferential effects on neutrophils[89] suggests that it may have a higher probability of efficacy in 'non-eosinophilic' asthma, rather than the classic form. If information such as this can be built into clinical trials, the chances of success may be greater – and clinical success will be necessary to revive pharmaceutical company interest in selectin blockade as a therapeutic approach. New treatments for asthma, and especially COPD, are desperately needed. Hopefully, selectin inhibitors will someday be a part of the pulmonary physician's arsenal.

Acknowledgments

The author wishes to thank Drs Gerhard Wolff, Rainer Zahlten and colleagues at Revotar Biopharmaceuticals AG (Henningsdorf, Germany), as well as Kurt Berens for reviewing the manuscript and providing insightful comments. No grant or other support has been used in the preparation of this review. The author was employed at Ontogen and participated in the discovery and preclinical development of OC 229648

References

1. World Health Organization. World Health Report 1998. Life in the 21st century: a vision for all. Geneva: World Health Organization, 1998
2. News and commentary: fighting for breathing space in the respiratory market [news article]. Curr Drug Disc 2002 Jul:10-1
3. Schleimer RP, Bochner BS. The role of adhesion molecules in allergic inflammation and their suitability as targets of antiallergic therapy. Clin Exp Allergy 1998; 28 Suppl. 3: 15-23
4. Vanderslice P, Biediger RJ, Woodside DG, et al. Development of cell adhesion molecule antabonists as therapeutics for asthma and COPD. Pulm Pharmacol Ther 2004; 17 (1): 1-10
5. Albelda SM, Smith CW, Ward PA. Adhesion molecules and inflammatory injury. FASEB J 1994; 8: 504-12
6. Romano SJ, Slee DH. Targeting selectins for the treatment of respiratory diseases. Curr Opin Investig Drugs 2001; 2 (7): 907-13
7. Vestweber D, Blanks J. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999; 79 (1): 181-213
8. Lowe JB, Ward PA. Therapeutic inhibition of carbohydrate-protein interactions in vivo. J Clin Invest 1997; 99 (5): 822-6
9. McEver R. Selectins. Curr Opin Immunol 1994; 6 (1): 75-84
10. McEver RP, Moore KL, Cummings RD. Leukocyte trafficking mediated by selectin-carbohydrate interactions. J Biol Chem 1995; 270 (19): 11025-8
11. Tedder TF, Steeber DA, Chen A, et al. The selectins: vascular adhesion molecules. FASEB J 1995; 9 (10): 866-73
12. Rosen SD, Bertozzi CR. The selectins and their ligands. Curr Opin Cell Biol 1994; 6 (5): 663-73
13. Lasky L, Presta L, Erbe D. Structure-function aspects of selectin-carbohydrate interactions. In: Metcalf B, Dalton B, Poste G, editors. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994: 37-53
14. Bertozzi C. Cracking the carbohydrate code for selectin recognition. Chem Biol 1995; 2 (11): 703-8
15. Aigner S, Sthoeger Z, Fogel M, et al. CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. Blood 1997; 89 (9): 3385-95
16. Lawrence M. Selectin-carbohydrate interactions in shear flow. Curr Opin Chem Biol 1999; 3 (6): 659-64
17. Lawrence MB, Kansas GS, Kunkel EJ, et al. Threshold levels of fluid shear promote leukocyte adhesion through selectins (CD62L,P,E). J Cell Biol 1997; 136 (3): 717-27
18. Slee D, Romano S, Yu J, et al. The development of potent non-carbohydrate imidazole-based small molecule selectin inhibitors with anti-inflammatory activity. J Med Chem 2001; 44 (13): 2094-107
19. Ohmoto H, Nakamura K, Inoue T, et al. Studies on selectin blocker 1. structure-activity relationships of sialyl Lewis X analogs. J Med Chem 1996; 39 (6): 1339-43

20. Foxall C, Watson SR, Dowbenko D, et al. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis X oligosaccharide. J Cell Biol 1992; 117 (4): 895-902
21. Kogan TP, Dupre B, Bui H, et al. Novel synthetic inhibitors of selectin-mediated cell adhesion: synthesis of 1,6-bis[3- (3-carboxymethylphenyl)-4- (2-α-D-mannopyranosyloxy)phenyl]hexane (TBC1269). J Med Chem 1998; 41: 1099-111
22. Jenison R, Jennings S, Walker D, et al. Oligonucleotide inhibitors of P-selectin-dependent neutrophil-platelet adhesion. Antisense Nucleic Acid Drug Dev 1998; 8 (4): 265-79
23. Sanders W, Gordon E, Dwir O, et al. Inhibition of L-selectin-mediated leukocyte rolling by synthetic glycoprotein mimics. J Biol Chem 1999; 274 (9): 5271-8
24. Reinhardt P, Kubes P. Differential leukocyte recruitment from whole blood via endothelial adhesion molecules under shear conditions. Blood 1998; 92 (12): 4691-9
25. Sriramarao P, Anderson W, Wolitzky B, et al. Mouse bone marrow-derived mast cells roll on P-selectin under conditions of flow in vivo. Lab Invest 1996; 74 (3): 634-43
26. Lawrence M, Springer T. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell 1991; 65 (5): 859-73
27. Whelan J. Selectin synthesis and inflammation. Trends Biochem Sci 1996; 21 (2): 65-9
28. Frenette P, Wagner D. Insights into selectin function from knockout mice. Thromb Haemost 1997; 78 (1): 60-4
29. Etzioni A, Tonetti M. Leukocyte adhesion deficiency II-from A to almost Z. Immunol Rev 2000; 178: 138-47
30. Etzioni A, Doerschuk C, Harlan J. Of man and mouse: leukocyte and endothelial adhesion molecule deficiencies. Blood 1999; 94 (10): 3281-8
31. von-Andrian U, Berger E, Ramezani L, et al. In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. J Clin Invest 1993; 91 (6): 2893-7
32. Price T, Ochs H, Gershoni-Baruch R, et al. In vivo neutrophil and lymphocyte function studies in a patient with leukocyte adhesion deficiency type II. Blood 1994; 84 (5): 1635-9
33. Frenette P, Mayadas T, Rayburn H, et al. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. Cell 1996; 84 (4): 563-74
34. Munoz F, Hawkins E, Bullard D, et al. Host defense against systemic infection with Streptococcus pneumoniae is impaired in E-, P-, and E/P-selectin deficient mice. J Clin Invest 1997; 100 (8): 2099-106
35. Maly P, Thall A, Petryniak B, et al. The α (1,3)fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. Cell 1996; 86: 643-53
36. Berens K, Vanderslice P, Dupre B, et al. Selectin antagonists: therapeutics for airway inflammation. In: Hansel T, Barnes P, editors. New drugs for asthma, allergy and COPD. Vol. 31. Basel: Karger, 2001: 306-9
37. Ward P, Mulligan M, Vaporciyan A, et al. Adhesion molecules in experimental lung inflammatory injury. In: Ward P, Fantone J, editors. Adhesion molecules and the lung. Vol. 89. New York: Dekker, 1996: 159-76
38. Ward P, Mulligan M. Adhesion molecules in inflammatory lung injury. In: Paul L, Issekutz T, editors. Adhesion molecules in health and disease. New York: Dekker, 1997: 523-37
39. De Sanctis GT, Wolyniec WW, Green FHY, et al. Reduction of allergic airway responses in P-selectin-deficient mice. J Appl Physiol 1997; 83 (3): 681-7
40. Broide D, Sullivan S, Gifford T, et al. Inhibition of pulmonary eosinophilia in P-selectin and ICAM-1-deficient mice. Am J Respir Cell Mol Biol 1998; 18 (2): 218-25
41. Fiscus L, Herpen JV, Steeber D, et al. L-Selectin is required for the development of airway hyperresponsiveness but not airway inflammation in a murine model of asthma. J Allergy Clin Immunol 2001; 107 (6): 1019-24
42. Tang M, Fiscus L. Important roles for L-selectin and ICAM-1 in the development of allergic airway inflammation in asthma. Pulm Pharmacol Ther 2001; 14 (3): 203-10
43. Abraham W, Ahmed A, Sabater J, et al. Selectin blockade prevents antigen-induced late bronchial responses and airway hyperresponsiveness in allergic sheep. Am J Respir Crit Care Med 1999; 159 (4): 1205-14
44. Gundel RH, Wegner CD, Torcellini CA, et al. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase obstruction in monkeys. J Clin Invest 1991; 88 (4): 1407-11
45. Dasgupta F. Selectin antagonists. In: Kahn M, editor. High throughput screening for novel anti-inflammatories. Basel: Birkhauser Verlag, 2000: 123-44
46. Romano S, Slee D. Targeting selectins for the treatment of respiratory diseases. Curr Opin Investig Drugs 2001; 2 (7): 907-13
47. Stewart A, Bhatia P, McCarty C, et al. Discovery of inhibitors of cell adhesion molecule expression in human endothelial cells: 1. Selective inhibition of ICAM-1 and E-selectin expression. J Med Chem 2001; 44 (6): 988-1002
48. Bennett F, Condon T, Grimm S, et al. Inhibition of endothelial cell adhesion molecule expression with antisense oligonucleotides. J Immunol 1994; 152 (7): 3530-40
49. Hafezi-Moghadam A, Thomas K, Prorock A, et al. L-Selectin shedding regulates leukocyte recruitment. J Exp Med 2001; 193 (7): 863-72
50. Diaz-Gonzales F, Gonzales-Alvaro I, Campanero MR, et al. Prevention of in vitro neutrophil-endothelial attachment through shedding of L-selectin by non-steroidal antiinflammatory drugs. J Clin Invest 1995; 95 (4): 1756-65
51. Tilton R, Berens K. Functional role for selectins in the pathogenesis of cerebral ischemia. Drug News Perspect 2002; 15 (6): 351-7
52. Aydt E, Wolff G. Development of synthetic pan-selectin antagonists: a new treatment strategy for chronic inflammation in asthma. Pathobiol 2003; 70: 297-301
53. Schlag G, Redl H, Till G, et al. Anti-L-selectin antibody treatment of hemorrhagic-traumatic shock in baboons. Crit Care Med 1999; 27 (9): 1900-7
54. Kumar A, Villani M, Patel U, et al. Recombinant soluble form of PSGL-1 accelerates thrombolysis and prevents reocclusion in a porcine model. Circulation 1999; 99 (10): 1363-9
55. Ridings P, Holloway S, Bloomfield G, et al. Protective role of synthetic sialylated oligosaccharide in sepsis-induced acute lung injury. J Appl Physiol 1997; 82 (2): 644-51
56. Park I, Lee D, Song M, et al. Cylexin: a P-selectin inhibitor prolongs heart allograft survival in hypersensitized rat recipients. Transplant Proc 1998; 30 (7): 2927-8
57. Alper J. Searching for medicine's sweet spot. Science 2001; 291: 2338-43
58. Cytel Corporation halts clinical trial of Cylexin [company press release]. San Diego, 1999
59. Service R. After the fall. Science 2001; 291: 2340-1
60. Seekamp A, van Griensven M, Dhondt E, et al. The effect of anti-L-selectin (aseulizumab) in multiple traumatized patients: results of a phase II clinical trial. Crit Care Med 2004; 32 (10): 2021-8
61. Lorenz HM, Kalden JR. Perspectives for TNF-a-targeting therapies. Arthritis Res 2002; 4 Suppl. 3: S17-24
62. Graves BJ, Crowther RL, Chandran C, et al. Insight into E-selectin/ligand interaction from the crystal structure and mutagenesis of the Lec/EGF domains. Nature 1994; 367 (6463): 532-8
63. Somers W, Tang J, Shaw G, et al. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to sLe^X and PSGL-1. Cell 2000; 103 (3): 467-79
64. Poppe L, Brown G, Philo J, et al. Conformation of sLe^X tetrasaccharide, free in solution and bound to E-, P-, and L-selectin. J Am Chem Soc 1997; 119: 1727-36
65. Norman K, Anderson G, Kolb HC, et al. Sialyl Lewis X (sLe^X) and an sLe^X mimetic, CGP69669A, disrupt E-selectin-dependent leukocyte rolling in vivo. Blood 1998; 91 (2): 475-83
66. Todderud G, Nair X, Lee D, et al. BMS-190394, a selectin inhibitor, prevents rat cutaneous inflammatory reactions. J Pharmacol Exp Ther 1997; 282 (3): 1298-304
67. Tsukida T, Moriyama H, Kurokawa K, et al. Studies on selectin blockers. 7. Structure-activity relationships of sialyl Lewis X mimetics based on modified Ser-Glu dipeptides. J Med Chem 1998; 41 (22): 4279-87
68. Pradella L. TBC-1269: Texas Biotechnology Group. Curr Opin Anti-Inflamm Immunomod Invest Drugs 1999; 1 (1): 56-60
69. Beeh K-M, Beier J, Buhl R, et al. Influence of inhaled bimosiamose (TBC 1269) a synthetic pan-selectin antagonist, on the allergen-induced late asthmatic response (LAR) in patients with mild allergic asthma [abstract]. Am J Resp Crit Care Med 2004; 167 (7): A321

70. Slee D, Romano S, Yu J, et al. Development of potent non-carbohydrate small molecule selectin inhibitors. American Chemical Society 221st National Meeting; 2001 Apr 1-5; San Diego

71. Romano SJ, Slee DH, John JK, et al. OC 229-648, A novel, non-carbohydrate small molecule selectin inhibitor with anti-inflammatory activity [abstract]. Inflamm Res 2000; 49 Suppl. 2: S90

72. Douwes J, Gibson P, Pekkanen J, et al. Non-eosinophilic asthma: importance and possible mechanisms. Thorax 2002; 57 (7): 643-8

73. Giembycz M. Are eosinophils out of asthma? Trends Pharmacol Sci 2001; 22 (2): 61-2

74. Rogers D, Giembycz M. Asthma therapy for the 21st century. Trends Pharmacol Sci 1998; 19 (5): 160-4

75. Adcock I, Matthews J. New drugs for asthma. Drug Discov Today 1998; 3 (9): 395-9

76. Liebermann P. 57th AAAAI: novel drugs. IDrugs 2001; 4 (6): 639-42

77. Paterson D. Asthma: new drug targets and innovative therapeutics. SMi Conference. IDrugs 2001; 4 (6): 646-9

78. Henderson WJ, Chi E, Albert R, et al. Blockade of CD49d (α4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. J Clin Invest 1997; 100 (12): 3083-92

79. Texas Biotechnology Corp. Annual report. Houston (TX): Texas Biotechnology Corp., 1999

80. Hoyert D, Arias E, Smith B, et al. Deaths: final data for 1999. Natl Vital Stat Rep 2001; 49 (8): 1-114

81. Vyas B. Severe asthma and COPD: the good, the bad and the ugly. IDrugs 2001; 4 (9): 1002-4

82. Chavannes N, Schayck CV. Developments in the treatment of chronic obstructive pulmonary disease: the clinical picture. Curr Opin Investig Drugs 2000; 1 (1): 75-8

83. Barnes P. New treatments for COPD. Nat Rev Drug Discov 2002; 1 (6): 437-46

84. Barnes P. Mechanisms in COPD: differences from asthma. Chest 2000; 117 (2 Suppl.): 10S-4S

85. Mulligan M, Paulson J, DeFrees S, et al. Protective effects of oligosaccharides in P-selectin-dependent lung injury. Nature 1993; 364 (6433): 149-51

86. Mulligan M, Watson S, Fennie C, et al. Protective effects of selectin chimeras in neutrophil-mediated lung injury. J Immunol 1993; 151 (11): 6410-7

87. DiStefano A, Maestrelli P, Roggeri A, et al. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. Am J Respir Crit Care Med 1994; 149 (3 Pt 1): 803-10

88. Witt C, Schumacher A, Liebers U, et al. Comparative analysis of P-selectin glycoprotein ligand-1 (PSGL-1) expression on leukocytes from patients with allergic asthma, COPD, and smokers [abstract]. Am J Resp Crit Care Med 2004; 169 (7): A840

89. Davenpeck K, Berens K, Dixon R, et al. Inhibition of adhesion of human neutrophils and eosinophils to P-selectin by the sialyl Lewis antagonist TBC1269: preferential activity against neutrophil adhesion in vitro. J Allergy Clin Immunol 2000; 105 (4): 769-75

90. Everts M, Kok R, Asgeirsdottir S, et al. Selective intracellular delivery of dexamethasone into activated endothelial cells using an E-selectin-directed immunoconjugate. J Immunol 2002; 168 (2): 883-9

Correspondence and offprints: Dr *Suzanne J. Romano*, Novasite Pharmaceuticals Inc., 11095 Flinkote Avenue, San Diego, CA 92121, USA. E-mail: Sromano@Novasite.com