Inhibitors of Membrane Receptors Involved with Leukocyte Extravasation

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Abstract: The migration of leukocytes from the blood stream to sites of infection is a key event in cellular immune response, mediated by multiple types of molecules including several adhesion receptors. The inhibition of adhesion receptors holds great promise for novel therapeutical strategies to treat chronic inflammatory disorders or autoimmune diseases. This review reports on recent advances in adhesion-based therapeutics and focuses on structural classification of selectin and integrin inhibitors.

Keywords: Adhesion molecules, autoimmune disease, endothelium, inflammation, integrins, leukocytes, selectins.

1. INTRODUCTION

The human body has an array of defence mechanisms against tissue damages and invasions of pathogenic organisms. Within these mechanisms, the cellular immune response is of key importance. Basis for this defence reaction is the ability of leukocytes to migrate from the blood system to sites of tissue damages or infection, where they attack the invaders with the help of their phagocytotic activity. Lymphocytes display similar activities of monitoring and eliminating pathogenic agents by migrating through lymphoid tissues. As a result of these defence reactions, the body displays typical changes in appearance, such as fever, tumescence, pain; which are also called cardinal inflammatory symptoms: calor, rubor, tumor, dolor and functio laesa. However, dysregulation, i.e. responding to autogenous or non-threatening agents might lead to an uncontrolled excessive infiltration of leukocytes into healthy tissue. This contributes, in part to the pathology of inflammatory and autoimmune reactions, such as rheumatoid arthritis, asthma, multiple sclerosis, Morbus Crohn, psoriasis and many others. Consequently, leukocyte adhesion and emigration appears to be an essential and initial process in pathological inflammations and therefore, a promising target for therapeutical interventions. However, most of the current anti-inflammatory therapeutical approaches, such as NSAD treat downstream consequences of leukocyte defending activities in the tissue. The better insight into the molecular biological processes of leukocyte emigration opens new avenues for novel anti-inflammatory therapeutical strategies.

1.1. Leukocyte Adhesion Cascade in Inflammation

The recruitment of leukocytes from the blood stream is a highly orchestrated process, which proceeds in a cascade-like fashion in postcapillary venules of most organs [1] Fig. **(1)**. Initiated by capturing of flowing leukocytes (tethering), cells begin to roll along the endothelial surface with a systematic decrease in rolling velocity as a functional prerequisite for the subsequent firm adhesion and transmigration. Three families of cell adhesion molecules (CAM) are involved in that process. Tethering and rolling is mediated by selectins, a family of three carbohydrate-binding receptors on both endothelium and leukocytes [2]. A complex array of signaling and binding leads to the activation of several integrins on the rolling leukocytes. Integrins bind their counterreceptors of the immunoglobulin superfamily (IgSF) on the endothelium, and thus mediate firm adhesion and emigration into the tissue through intercellular gaps [3]. In addition to that, contact between endothelial cells is maintained by further adhesion molecules. These junctional adhesion receptors are also important for leukocyte diapedesis, but they should not be considered as therapeutical target in this article.

The regular and overlapping function of all three classes of CAMs is necessary for a sufficient leukocyte emigration, and thus for the immune response. This could be demonstrated in numerous disease models by selective blocking or genetic out knocking of certain receptors. Consequently, the functional blockade of a certain receptor or a receptor family might be sufficient for a therapeutical intervention in pathological inflammations to impair the complete adhesion cascade. In principle, the function of the distinct CAMs can be modulated *via* a number of mechanisms, including competitive inhibition, interfering with expression at the cell surface on a genetic or metabolic pathway, reducing receptor activation (integrins) or increasing the surface cleavage (selectins).

These strategies have been elucidated in some recent reviews of different topics with respect to the progress in clinical trials [4a,b, 5]. This mini review will concentrate on the latest advances in competitive blockers of CAMs. Referring to some well established blockers in advanced clinical trials, special emphasis is laid on structural classification of novel and potential inhibitors, which in most cases are in a preclinical stage.

2. SELECTINS AS THERAPEUTICAL TARGETS

2.1. Selectin Structure and Ligands

As outlined before, selectins mediate the leukocyte tethering and rolling and thus initiate the leukocyte adhesion cascade. Selectins comprise a family of three adhesion

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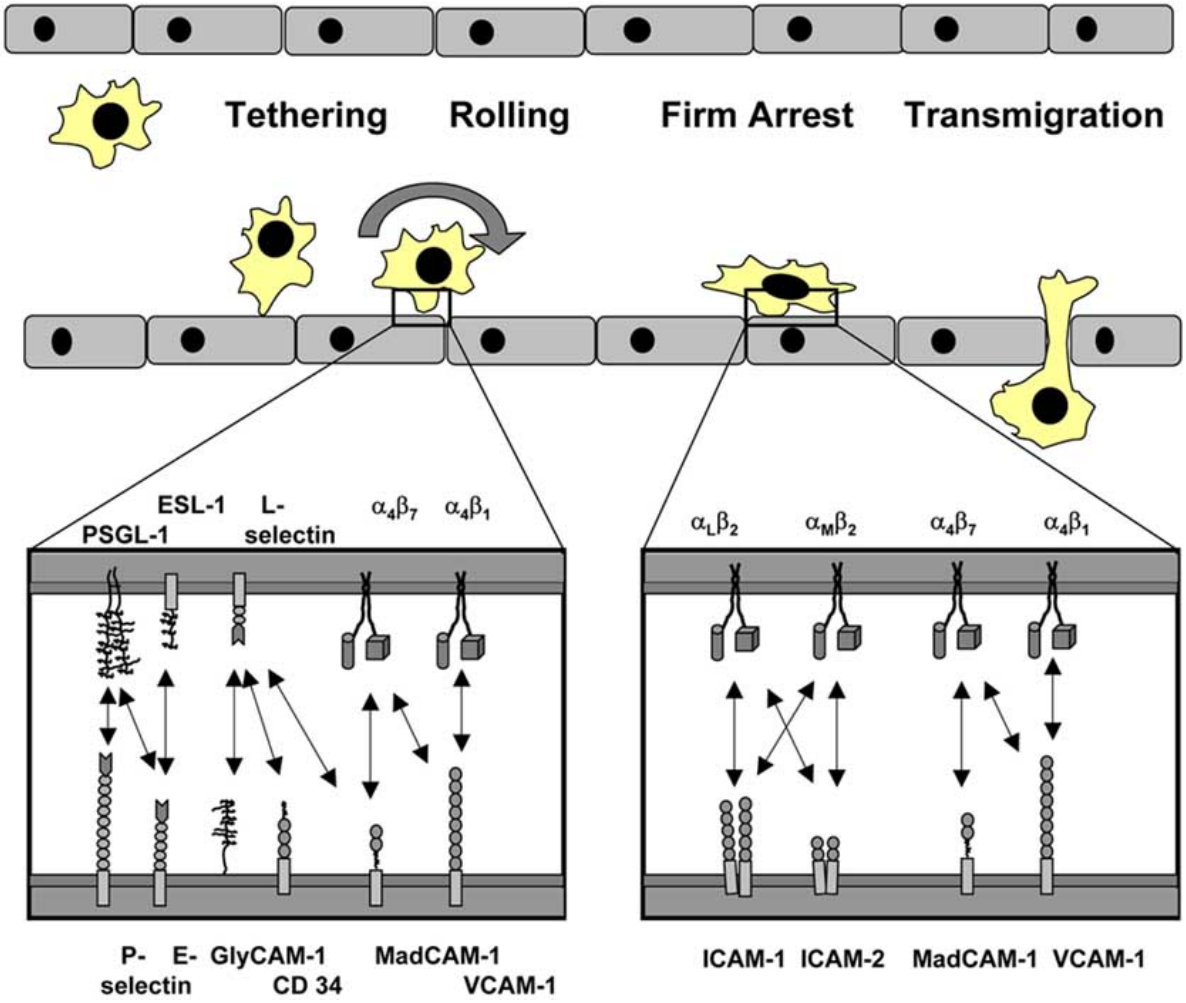


Fig. (1). Schematic presentation of receptors and counter-receptors on endothelial cells and leukocyte surface involved in the molecular mechanisms of leukocyte adhesion and emigration.

Left: Tethering and rolling is mediated by selectins, which bind corresponding mucin-like glycoprotein ligands. α_4 integrins are partly involved in leukocyte rolling.

Right: Firm arrest of leukocyte is mediated by several integrins of the leukocytes, which bind their ligands of the immunoglobulin superfamily.

molecules that bind cell surface carbohydrate ligands. Their nomenclature is based on their localization and original source of identification. E-selectin (endothelium), P-selectin (platelets and endothelium) and L-selectin (leukocytes) display a high degree of structural homology. In general, selectins are glycoproteins and composed of five structural elements. A short cytoplasmatic tail and a membrane spanning component are followed by a variable number of consensus repeats similar to the complement receptor binding region. These complement-like repeats differ in number among the three selectins as well as in similar selectins across different species and cause the elongated shape of these receptors [6]. An epidermal growth factor (EGF) domain and finally the N-terminal (calcium dependent) carbohydrate recognition domain (C-type lectin) realize ligand binding with fast kinetics as prerequisite for leukocyte rolling and subsequent capture and firm adhesion by the integrins.

The three selectins were expressed in a sequential manner correlating with their certain roles in the inflammatory response. P-selectin is stored in secretory vesicles of platelets (alpha-granules) and endothelial cells (Weibel-Palade-bodies) and can be rapidly mobilized to the cell surface following cellular activation by mediators such as histamine or thrombin. E-selectin expression is regulated on a transcriptional level in response to cell activation by cytokines such as TNF-α and interleukin-1 or by lipopolysaccharides. Thus, E-selectin appears on the cell surface with maximal expression 4 – 6 hours after stimulation and it declines by 24 – 48 hours. L-selectin is constitutively expressed by most leukocytes and is rapidly shed after cell activation. The shedding is postulated to be important for the rolling mechanisms as well as in downstream signaling events.

Several cell surface glycoproteins are described to act as specific selectin ligands. L-selectin binds CD 34, mucosal addressin cell adhesion molecule (MadCAM), glycosylation dependent cell adhesion molecule (GlyCAM-1) and some other structures expressed on a variety of tissues. E-selectin binds E-Selectin Ligand-1 (ESL-1). The P-Selectin-

Glycoprotein Ligand (PSGL-1) is the best characterized structure so far, which is also recognized by E- and L-selectin. Most of them are mucin-like glycoproteins, displaying the binding epitopes as carbohydrate side chains in the extended molecules. Multivalency of the epitopes has been regarded as an essential aspect to improve avidity of selectins. The common motifs of binding epitopes for all three selectins are sialylated and fucosylated carbohydrates like the tetrasaccharide Sialyl Lewis^x (sLex) and its related isomer Sialyl Lewis^a. An insight into the structural binding requirements of sLex-selectin interaction could be derived by X-ray data available for E-selectin [7a] as well as for P-selectin bound to its ligand PSGL-1 or to sLex [7b]. These structural informations have been used as guide to create and optimize selectin inhibitors.

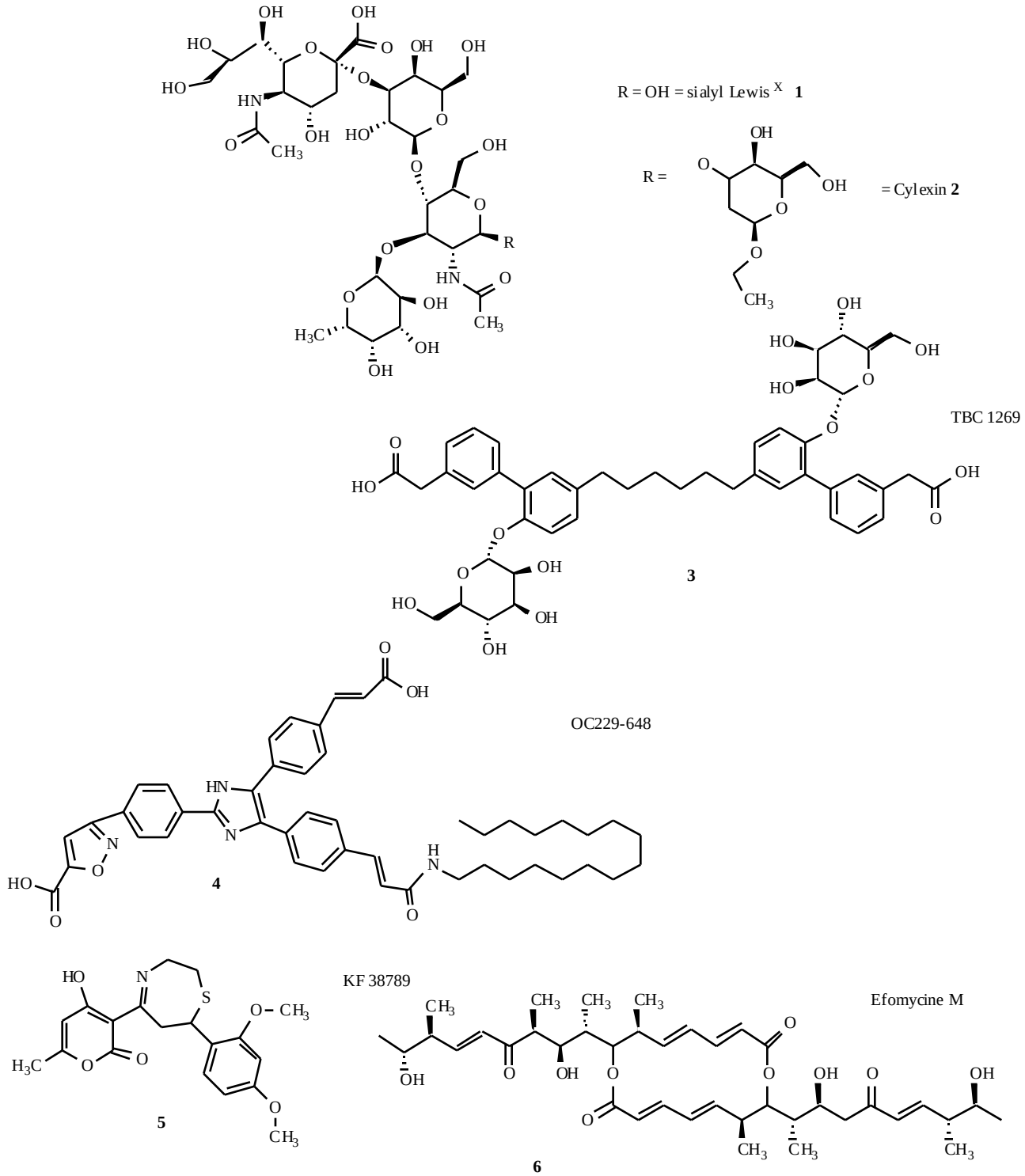


Fig. (2). Structures of the standard selectin binding epitope sialyl Lewis^x and some key inhibitors in preclinical or clinical development.

2.2. Selectin Inhibitors

2.2.1. Rationale

Numerous model experiments using selectin knock-out animals or selectin blocking antibodies clearly prove that all three selectins are implicated in the development of pathological inflammations [8a-d]. Consequently, the blocking of selectins has attracted much attention during the last decade. Antibodies and inhibitors of various chemical structures entered clinical trials focusing on the treatment of different inflammatory disorders. In general, regarding the physiological role of selectins, blocking strategies might also have some important side effects, such as immunosuppression. Those effects can be derived from an extremely rare human syndrome, called leukocyte adhesion deficiency type II (LAD II), which is based on a genetic defect of the fucose metabolism [9]. That results in an insufficient biosynthesis of selectin ligands whereby patients suffer from chronic opportunistic infections. Structurally, the inhibitors used can be classified according to different characteristics. Humanized monoclonal selectin antibodies have been regarded as promising therapeutics. However, preclinical and clinical trials using selectin antibodies have been discontinued, mostly reflecting to an unsatisfying therapeutic efficiency. Since this article focuses on structural parameters of competitive selectin antagonist, antibody strategies should not further be discussed. Consequently, this article refers to a classification into i) small molecule inhibitors derived from the sLex epitope (carbohydrates and non-carbohydrates), ii) protein inhibitors and iii) blocking polymers.

2.2.2. Small Molecule Inhibitors

The insight into the structural requirements of sLex-selectin binding made the sLex tetrasaccharide structure **1,** Fig. **(2)** as an attractive standard to derive potent and simplified carbohydrate-based inhibitors. Various strategies have been applied to reduce the sLex structure to the essential pharmacophoric moieties by replacing carbohydrates in order to get more simple mimetics of higher affinity. These strategies are excellently reviewed in recent papers [10-12].

Cylexin/Cy-1503 (Cytel/Epimmune Inc) **2** was the first selectin inhibitor reaching clinical trials, although it represents a pentasaccharide structure of the non-modified sLex [13a,b]. Cylexin was in a phase II/III trial for reperfusion injury, when the development has been discontinued due to a lacking significant therapeutic benefit over placebo treatment [13c].

Typically, carbohydrate-based mimetics have several restrictions. The binding of selectins to monovalent carbohydrate epitopes is rather weak (K_d~ 1 mM) [14]. Furthermore, carbohydrates suffer from a lack in *in vivo* potency due to enzymatic degradation. The poor and limited oral bioavailability profiles lead to bad pharmacokinetic properties, which enforce a parenteral or topical route of application. Finally, an expensive and difficult synthesis makes those structures bad drug candidates [15a,b].

One exception is Bimosiamose **3** (TBC 1269, Encysive), a rationally designed dimeric mannose-based small molecule (1,6-bis[3-(3-carboxymethylphenyl)-4-(2-α-D-mannopyranosyloxy)-phenyl] hexane), which inhibits all three selectins *in vitro* [16a,b]. The IC_{50} values of 500, 70 and 560 µM against E-, P- and L-selectin in a cell adhesion assay clearly surpass sLex efficiency. This pan-selectin antagonist has been successfully investigated in different preclinical models of inflammatory diseases such as psoriasis, asthma and reperfusion injury [17]. Bimosiamose is at present the most advanced small molecule selectin inhibitor in development [18]. It is in a phase IIa clinical trial for the treatment of allergic asthma, where a single intravenous administration improves several disease parameters such as decreasing eosinophil airway recruitment [18]. Revotar AG continued these studies to develop an inhalative formulation of bimosiamose, which entered clinical phase IIa trials for both single and multiple inhaled doses [19a,b]. Bimosiamose is also in a clinical trial IIa for the topical treatment of psoriasis and atopical dermatitis.

The total replacement of carbohydrate moieties in the search for sLex mimetics should lead to orally applicable agents. The first non-carbohydrate selectin inhibitor was introduced by Ontogen (OC229-648) **4**. Slee *et al*. report on a series of imidazole-based pan–selectin inhibitors, where the lead OC229-648 displays a high *in vitro* efficiency in blocking E- and P-selectin (IC_{50} of 30 µM and 17µM) [20a]. This molecule contains a long hydrophobic alkyl chain, which was postulated to be an important factor to optimize the fitting into the binding pocket of both E- and P-selectin. However, despite excellent *in vitro*-efficiency, preliminary *in vivo* data of OC229-648 in inflammation models are inconsistent [20b] and no further information could be obtained.

Ohta *et al*. reported on a series of non-carbohydrate-based small molecules of 7-phenyl-1,4-thiazepine core structure as potential selectin inhibitors. They could show that the leading structure **5** (KF 38789) is effective in reducing the P-selectin induced cell adhesion with an IC_{50} of about 2 µM, as well as in decreasing leukocyte accumulation in a mice peritonitis model [21]. KF 38789 does not compete with sLex in reducing cell binding, obviously it interferes with the protein interaction between P-selectin and its ligand PSGL-1, which might also explain the influence on signaling functions, such as reduced superoxid production in neutrophils. Consequently, this molecule is a P-selectin specific blocker. With respect to numerous studies that show the pivotal role of both E- and P-selectin for leukocyte rolling as well as their overlapping and mutually compensating functions, the inhibition of only one selectin appears not sufficient for clinical activities [22].

Efomycines are a new class of small molecule selectin inhibitors of macrolidic structure, which were originally isolated from streptomyces fermentation. Partial synthetic modifications resulted in Efomycin M (**6)** as leading structure, which was effective in reducing inflammatory effects in two animal models of psoriasis [23a,b]. New data prove efficiency of Efomycine M in other models of allergic contact dermatitis in current preclinical studies (pers. communic., W.H. Boehncke, Feb. 2004). Molecular modeling data explain the efficiency of Efomycine M by mimicking the essential pharmacophoric groups of sLex.

Two further strategies, which inhibit the cellular biosynthesis of functional selectin ligands are to be shortly

introduced, although they do not represent competitive selectin binding antagonists. The biosynthesis of selectin ligands is related to the activities of several glycosyl transferases, such as fucosyltransferase IV and VII, which mediate terminal sialylfucosylation of for instance PSGL-1 as a prerequisite for P- and E-selectin binding. Using these enzymes as targets, low molecular weight antagonists of fucosyltransferase VII have been investigated as a new tool for reducing leukocyte adhesion. This development is ongoing but has not yet reached clinical trials [24a,b].

Another way to interfere with the synthesis of functional selectin ligands is the use of metabolic inhibitors. Dimitroff *et al*. used a synthetically modified GlcNAc, namely a peracetylated-4-fluorinated-*D*-glucosamine, which is incorporated into the growing poly-*N*-acetyllactosamine chains in the course of leukocyte ligand biosynthesis. The inhibited expression of functional cutaneous ligands was shown to result in a reduced lymphocyte infiltration in different inflammatory skin models in mice [25a,b].

2.2.3. Protein Inhibitors of Selectins

PSGL-1 is the best characterized and understood selectin ligand. PSGL-1 acts as a dimer with a high avidity for P-selectin (K_d~300 nM) [26a], and displays also a certain binding affinity toward L- and E-selectin. Early studies could show that beside the sLex saccharides a moiety of the protein backbone (sulfated tyrosines) is involved in P-selectin binding [26b,c], which explains the much higher affinity of PSGL-1 compared to sLex. This could later be confirmed by the X-ray studies on cocrystallized P-selectin / PSGL-1 by Somers *et al*. [7b]. Consequently, in order to transfer these complex binding conditions to the field of selectin inhibitors, a soluble and truncated recombinant form of PSGL-1 was introduced as protein inhibitor. The rsPSGL-1 has shown promising efficacy in animal models of ischemia-reperfusion injury [27a,b], thrombosis [27c] and transplantation [27d]. rsPSGL-1 (Wyeth) entered a clinical trial phase II for treatment of myocardial infarction [28], but this has recently been discontinued due to discouraging results with respect to therapeutic efficiency (RAPSODY-study, Genetic Institute).

Based on the findings that the extreme N-terminus of PSGL-1 including three sulfated tyrosines is essential for high affinity binding of P-selectin, Leppänen *et al*. synthesized a glycosulfopeptide modeled after the N-terminal region. This peptide GSP-6 binds soluble P-selectin with high affinity (K_d~350 nM) and inhibits PSGL-1 binding to P-selectin [29a]. Using synthetic peptides, potential disadvantages of the expression technology of recombinant PSGL-1, such as cotransfection with fucosyltransferase, could be avoided. Structural modifications gave a better insight into the peptide binding characteristics [29b], however, there is no further information on preclinical studies using this inhibiting peptide.

Molenaar *et al*. used the recombinant phage display technique to search for P-selectin binding peptides [30]. They reported on a series of small peptides that display high affinity for P-selectin in low micromolar range. Although these peptides were not direct structural mimetics of sLex, they are suggested to interact with the sLex binding site of P-selectin. The peptides interfere with P-selectin / PSGL-1 binding in cell adhesion assays in a calcium-dependent manner. Supporting the hypothesis of multivalent selectin bindings for reaching higher affinity, the tetramerization of the peptides dramatically increases the avidity for P-selectin (K_d~10 nM).

2.2.4. Polymers as Selectin Inhibitors

Multivalency of low-affine carbohydrates, such as sLex has been postulated to be the reason for high affinity binding. Based on this, and on lessons learned from PSGL-1, polymers have been established, which display fucosylated oligosaccharides in proximity to multiple sulfate ester groups as potential inhibitors for (P- and L-) selectins. John *et al*. introduced a synthetic polymer of those characteristics with high affinity for P-selectin *in vitro*, which also showed antiinflammatory efficacy in a mice model of allergic airway diseases [31].

Heparin is a naturally occurring sulfated polysaccharide. Some studies reported on a certain heparin binding ability to P- and L-selectin [32]. In order to search for the essential structural requirements of heparin and to optimize the selectin inhibition, Höpfner *et al*. used a series of semi-synthetic heparinoid compounds in an *in vitro* cell rolling assay. The strong affinity for P-selectin could be correlated with molecular weight and sulfation of the heparinoids, although they were ineffective in blocking E-selectin [33].

3. TARGETING INTEGRINS

3.1. Integrin Structure and their Ligands of the Ig-Superfamily

Firm attachment of leukocytes to endothelium and the migration into inflamed tissue is mediated by members of the integrin family. Integrins are heterodimeric cell surface receptors consisting of a non-covalently linked α- and β-subunit. At least 19 α- and 8 β-subunits can associate to form at least 25 heterodimers, which differ in expression pattern and ligand selectivity [3,34]. Integrins are involved in various signaling processes, which also control a rapid transition from a low-affinity to a high-affinity binding state. Integrins mediate cell binding to matrix substrates as well as to cellular ligands.

Several of the integrins expressed on leukocytes are involved in endothelial adhesion, the corresponding ligands on endothelium belong to the Ig-SF.

Two β_2-integrins are important for leukocyte recruitment. $\alpha_L\beta_2$ (LFA-1 = lymphocyte function antigen-1, CD11a/CD18) is expressed on most leukocyte cell types, whereas $\alpha_M\beta_2$ (Mac-1, CD11b/CD18) is expressed primarily on cells of the myeloid lineage. Thus, it is required for the lymphocyte recirculation contributing to the adaptive immune response. Both integrins bind intercellular adhesion molecules (ICAM-1 and 2), two members of the Ig-SF, which are constitutively expressed on the endothelium, although ICAM-1 can be upregulated by proinflammatory cytokines such as TNF-α and IL-1.

The integrin $\alpha_4\beta_1$ (VLA-4; very late antigen-4) is expressed on most types of leukocytes, but preferentially on lymphocytes and monocytes. The related integrin $\alpha_4\beta_7$ is specific for T and B lymphocytes as well as monocytes. In terms of inflammation, both integrins bind to the inducible endothelial counter-receptor vascular cell adhesion molecule-

1 (VCAM-1). Furthermore, $\alpha_4\beta_7$ binds to MadCAM in lymphoid tissues, thus controlling lymphocyte homing.

3.2. Integrin Inhibitors

3.2.1. Rationale

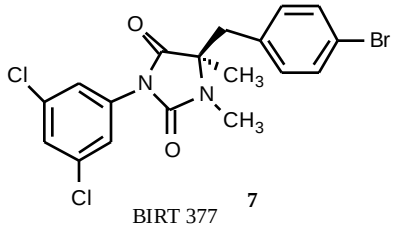
Since the different leukocyte integrins differ in leukocyte subtype specificity, the modulation or inhibition of a certain integrin might be intended either to interfere with acute inflammatory reactions or is rather directed towards autoimmune diseases. Since the roles of the different integrins in leukocyte emigration are partially overlapping, there is no clear classification with therapeutical respect. In general, integrin function can be modulated in differentways; by (i) inhibition of integrin activation by antagonizing chemokines, for example (ii) inhibiting the expression of integrins by blocking transcription factors [35] or the use of anti-sense oligonucleotides [36], (iii) blockade of the integrin binding. The use of monoclonal antibodies is by far the most widely reported way to block integrins [37]. Although many data exist, which demonstrate the therapeutically successful application of mAb's, peptides and small molecule therapeutics might offer several advantages in terms of pharmacokinetics, application, reduced side effects and costs. The two integrins, LFA-1 and VLA-4 are the most important strategic targets for therapeutic approaches, which will be introduced in the following sections.

3.2.2. Inhibiting the LFA-1 / ICAM Interaction

The blockade of the LFA-1 / ICAM- 1 interactions is of broad therapeutic interest. On the one hand, LFA-1 mediates the emigration of neutrophils to acute inflammatory sites as fundamental process in the innate immune response. On the other hand, the LFA-1 / ICAM-1 interaction is essential for T-cell activation and migration, which makes LFA-1 blockade suitable for suppression of autoimmune diseases or immune reactions after organ transplantations [38].

Antibodies

Odulimomab (Antilfa®) is a mAb against CD11a, which showed efficacy in several clinical phase III studies in preventing delayed graft function and transplant rejection [39]. A mAb against ICAM-1 (Enlimomab), which also inhibits the LFA-1-induced leukocyte adhesion by blockade of its ligand, is presently in a clinical phase II trial for burn wound healing [40].



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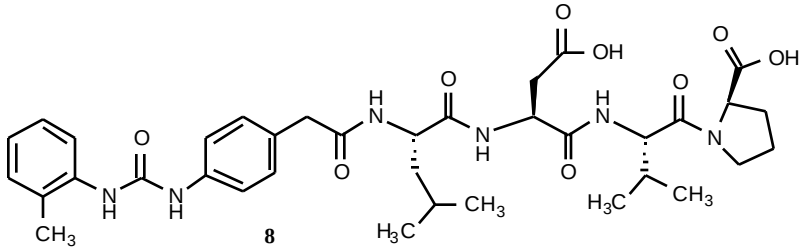

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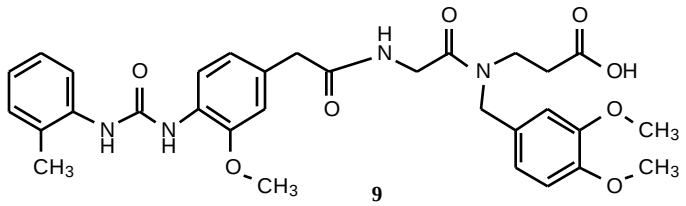

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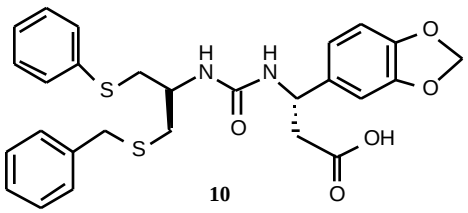

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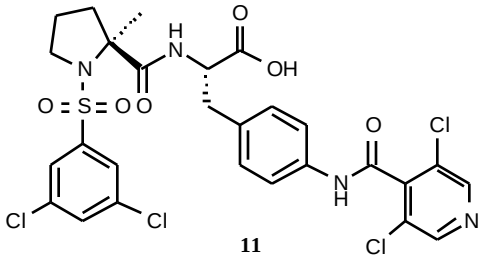

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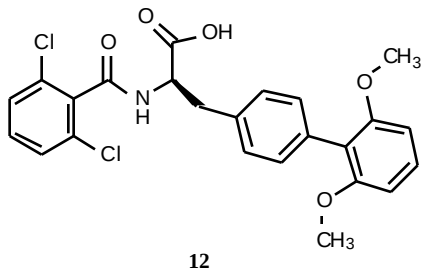

12 TR 14035

Fig. (3). Small molecule inhibitors of the leukocyte integrins. BIRT 377 (**7**) is directed against LFA-1, while the other structures (**8** – **11**) representing a general diphenyl urea moiety are potent blockers of VLA-4. **12** is described to be a dual blocker of both α_4 integrins.

Efalizumab (Raptiva®), a humanized antibody against CD11a, was applied for the treatment of plaque psoriasis. Efalizumab significantly alleviates the symptoms of psoriasis in several phase III trials [41a,b], its approval in USA and Europe is expected in near future.

Peptides

Synthetic peptides or peptidomimetics that mimic the main amino acid motif of the adhesion molecules (cyclic ICAM-1 peptides) could be shown to be able to inhibit the LFA-1-induced T-cell adhesion and function in several *in vitro* models, but have no clinical relevance up to now [42a,b].

Small Molecules

The structural characteristics of the LFA-1 / ICAM-1 interactions as basis for the design of blockers are complex and were described in detail in [5]. A number of (antiinflammatory) drugs indirectly influence LFA-1 activity on the level of activation or expression. This should not be considered in this article, since it does not represent competitive blockings of unique mechanisms, and those applications have no clinical relevance. For instance, an allosteric modulation of LFA-1 was recently described. Statins (inhibitors of HMG CoA reductase) inhibit LFA-1 efficiently in a mice model by binding a novel allosteric site [43].

Boehringer Inc. introduced the first orally available small molecule inhibitor of LFA-1, Fig. **(3)**. BIRT 377 (**7),** (R)-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-1,5-dimethylimidazolidine-2,4-dione blocked the LFA-1-induced cell adhesion under both *in vitro* and *in vivo* situations (K_d ~ 25.8 nM) by a non-covalent interaction with the α-subunit [44a]. Continued studies demonstrated that BIRT 377 acts allosterically by preventing the up-regulation of LFA-1 in its high affinity conformation [44b]. Abbott Lab. identified another series of LFA-1 inhibiting small molecules of a *p*-arylthio-cinnamide structure, which upon structural optimization display activity in a low nanomolar range [45a,b]. However, there is no further information on the present stage of preclinical studies of these compounds.

A recent paper gives an excellent structural insight on how small molecule inhibitors interact with LFA-1 [46]. The authors classify the blockers and finally describe a novel mechanism of interaction with the β_2-subunit.

3.2.3. Blocking the α_4-Integrins

Both therapeutically interesting α_4-integrins are constitutively expressed on a variety of leukocytes and bind to both shared and distinct binding partners, demonstrating their certain roles in the cellular immune response. VLA-4 preferentially binds VCAM-1 on endothelium, whereas the major ligand for $\alpha_4\beta_7$ is MadCAM, expressed on lymphoid tissues. However, since VLA-4 is also implicated in T-cell activation and mast cell function, the blocking of α_4-integrins is in general directed to influence autoimmune inflammatory diseases.

Antibodies

Natalizumab (Elan/Biogen) is a mAb against α_4 integrin, which shows clinical efficiency. In a clinical phase III study for the treatment of multiple sclerosis, a monthly dosis of natalizumab caused a significant decrease in the number of new brain lesions [47]. Positive results could also be generated in a clinical study (III) for the treatment of Crohns disease, where patients display significant higher rates of remission [48].

Peptides

The key motifs for binding of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ to native ligands have been defined. The sequence LDV has been recognized to be critical for VLA-4, LTD-motifs are able to block $\alpha_4\beta_7$ binding to MadCAM [49a,b]. These findings and X-ray crystal structure data of the binding regions were used as guides for the design of peptidic inhibitors [50a,b]. In addition to that, small molecule (petidomimetic) inhibitors were also created based on these findings.

Table 1. Cell Adhesion Antagonists Presently in Clinical Phases

Compound	Target	Clinical indication	Route of administration	State of development	Reference
Bimosiamose	E-, P-, L-selectin	Asthma Psoriasis	inhaled topical	cl. phase II cl. phase II	[18] [19a,b]
Enlimomab (murine mAb)	ICAM-1	Burn wound injury	i.v. s.c.	cl. phase II	[40]
Odulimumab (Antilfa®)	α_L-Integrin CD 11a	Transplant rejection	i.v.	cl. phase III	[39]
Efalizumab (Raptiva®)	α_L-Integrin CD 11a	Psoriasis	i.v.	cl. phase III	[41a,b]
Natalizumab (Antegren®)	α_4-Integrin	Mult. Sclerosis Crohns Disease	i.v.	cl. phase III cl. phase III	[47] [48]
IVL 745	VLA-4	Asthma	inhaled	cl. phase II	
1031	VLA-4	Asthma	inhaled	cl. phase II	
TR 14035	α_4-Integrin	Asthma Inflammations	oral	cl. phase II cl. phase I	[55]
R411	VLA-4	Asthma	oral	cl. phase II	[56a,b]

Small molecule inhibitors

A number of small molecule inhibitors of VLA-4 as well as dual inhibitors of both α_4 integrins have been described in recent years [51]. VLA-4 blockers are basing on a diphenylurea moiety that mimics the structural characteristics of the essential LDV binding motif, Fig. **(3)**. Bio-1211 (collaboration Merck/Biogen) **8** reached clinical phase IIa studies for the treatment of asthma [52]. Trials were discontinued due to disappointing efficacy. Two further compounds by Avensis, IVL-745 (**9**) and 1031 were tested for the same indication after nasal application, the clinical outcome of these phase II trials are not published. Another compound of similar key structure is **10** (TBC 4746, Encysive) on a preclinical stage. Pfizer reported on a series of compounds of similar basic structure and demonstrated high efficiency in mice allergic airway diseases [53a,b]. Merck published animal studies on a compound "A" (**11**), which can interact with both the non-activated and activated VLA-4 structure [54]. It has a 100-fold higher efficiency compared to Bio 1211 in reducing lung inflammatory parameters, when applicated intranasally.

TR 14035 (Tanabe/GlaxoSmithKline) **12** is an orally available dual inhibitor of comparable structure that entered phase II asthma studies and other phase I clinical trials for the treatment of different inflammatory diseases. Binding parameters of those dual inhibitors to both α_4 integrins in activated vs. non-activated stages have recently been analyzed and structurally defined [55].

Roche published a series of experiments on effective phenylalanine-derived VLA-4 blockers [56a,b]. One of these structures (R-411) is currently in clinical phase II studies as an orally available antiasthmatic drug. All antagonists presently in a clinical trial are summarized in Table **1**.

Several other compounds of comparable activity and structure are currently in a late preclinical or clinical stage of development.

4. CONCLUSION

The inhibition of CAMs involved in leukocyte trafficking is a novel and viable approach for the treatment of various inflammatory or autoimmune human diseases. Tremendous efforts have been undertaken in the chemical field to create blocking substrates as promising drug candidates. However, the understanding of binding mechanisms and the efficacy in several preclinical disease models reflect only in part the situation in human that explains several disappointing clinical trials.

Selectins are generally accepted as promising targets for an anti-inflammatory therapy. The carbohydrate nature of selectin ligands, low binding affinity and fast binding kinetics complicate the search for inhibitors and made success in clinical trials elusive. Perhaps, the clinical success of bimosiamose, the most advanced selectin inhibitor will renew the emphasis in this field and might focus the interest on certain promising fields of the anti-inflammatory treatment. Since the chronic inhibition of selectins might provoke unwanted consequences by suppressing the innate immune system, transient, locally controlled treatments such as asthma, skin diseases or reperfusion injury are most promising. Advanced clinical trials will be required to demonstrate the applicability.

The approval and success of different agents blocking β_3-integrins for cardiovascular indications underscore the great potential of integrins as therapeutic targets, which might be conferred to the anti-inflammatory research. The inhibition of certain leukocyte integrins appears very attractive to modulate chronic autoimmune inflammatory disorders. Numerous inhibitors of different structures are in advanced clinical trials. Due to the great number of small molecules in development and the interest of different pharmaceutical companies, significant advances might be expected in the near future.

ABBREVIATIONS

CAM	=	Cell adhesion molecule
ESL-1	=	E-selectin ligand-1
GlyCAM-1	=	Glycosylation dependent cell adhesion molecule
IgSF	=	Immunoglobulin superfamily
IL-1	=	Interleukin-1
ICAM-1	=	Intercellular adhesion molecule-1
LDV	=	Leucin-aspartic acid-valine
LFA-1	=	Lymphocyte function antigen-1
LTD	=	Leucin-threonine-aspartic acid
MadCAM	=	Mucosal addressin cell adhesion molecule,
PSGL-1	=	P-selectin-glycoprotein ligand-1
sLex	=	Sialyl Lewis^x
TNF-α	=	Tumor necrosis factor-α
VCAM-1	=	Vascular cell adhesion molecule-1
VLA-4	=	Very late antigen-4

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