**Title:** E-selectin as a target for drug delivery and molecular imaging

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**Abstract:**

E-selectin, also known as CD62E, is a cell adhesion molecule expressed on endothelial cells activated by cytokines. Like other selectins, it plays an important part in inflammation and in the adhesion of metastatic cancer cells to the endothelium. E-selectin recognizes and binds to sialylated carbohydrates present on the surface proteins of certain leukocytes. E-selectin has been chosen as a target for several therapeutic and medical imaging applications, based on its expression in the vicinity of inflammation, infection or cancer. These systems for drug delivery and molecular imaging include immunoconjugates, liposomes, nanoparticles, and microparticles prepared from a wide range of starting materials including lipids, synthetic polymers, polypeptides and organo-metallic structures.

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1. Introduction

The selectin family is represented by three receptors composed of calcium-dependent type I transmembrane glycoproteins with an extracellular lectin-like domain. They are classified by their site of expression into E-selectin (activated endothelium [1, 2]), P-selectin (platelets [3, 4] and endothelial cells [5, 6]), and L-selectin (lymphocytes [7]). Selectins allow adhesion between leukocytes and platelets in contact with the vascular endothelium during inflammation or tissue damage [8]. At a molecular level they are able to recognize sialylated, fucosylated and sulfated glycans found on glycoproteins, glycolipids or proteoglycans [9] to mediate the initial attachment or “tethering” of free-flowing leukocytes to the vessel wall through reversible adhesion that permits the cells to roll in the direction of flow [10, 11]. The physiological expression of selectins is strictly controlled in order to limit inflammatory reactions, and it is modified in inflammation and cancer metastasis to allow adherence of leukocytes or cancer cells, respectively, on endothelial cells.

Advances in molecular and cellular biology have elucidated the role played by each of these receptors in a range of pathological disorders involving aberrant trafficking of immune cells. P-selectin is involved in inflammatory disorders such as acute lung injury [12], psoriasis [13], and rheumatoid arthritis [14]. It plays a role in hemo-stasis [15, 16] and hematogenous spread of tumor cells [17, 18]. For detailed information about the pathological roles and therapeutic targeting of P-selectin, the reader is directed to the review published by Ludwig and co-workers [19]. It is also well documented today that E-selectin is deeply implicated in many disorders including inflammatory diseases, cardiovascular disorders, cancer and metastasis. On the other hand, there is little evidence for the direct involvement of L-selectin in pathology, although some studies have reported variations in levels of the receptor and/or its soluble form (sl-selectin) in serum in some patients with HIV-infection [20], insulin-dependent diabetes mellites [21], meningeval leukemia [22], multiple sclerosis [23] and sepsis [20]. However, its soluble form, sl-selectin, was found to be decreased in patients with Kawasaki Syndrome and in patients with risk factors for acute ischemic stroke even in the absence of disease [24].

In the light of these observations, interest in this family of receptors has been growing during the last 2 decades; in particular in the possibility of using them as a pharmacological target for the treatment of the diseases mentioned above. In fact, different selectin-based therapeutic strategies have been proposed, including the inhibition of their expression in pathological situations, targeting their ligands, or using them as molecular targets for delivery of therapeutic and diagnostic agents.

In this present review, a summary of physiological and pathological roles of E-selectin will be given, followed by a detailed presentation of systems which have been proposed to target it in inflammation, cancer, cardiovascular disorders, for drug delivery, gene transfer and medical molecular imaging. A general discussion summarizes the key points in the development of such systems.

2. E-selectin: an interesting target for drug delivery

2.1. Physiology of E-selectin

E-selectin (64 kDa) also known as CD62E, ELAM-1 and LECAM-2, is expressed specifically by endothelial cells. Relative molecular weight values of 100 and 115 kDa have been detected for different glycosylated forms [25]. The primary structure of E-selectin contains several domains: an amino terminal lectin-like domain, followed by an epidermal growth factor (EGF)-like domain and six repeated motifs (about 60 amino acids each) similar to those found in some complement-binding proteins [2]. The lectin-like domain, and (EGF)-like domain mediate interaction with leukocytes, while the role of the complement binding-like regions is not yet well defined, but they probably serve, at least in part, as spacers to hold the other domains away from the cell surface [26].

E-selectin is not constitutively expressed by endothelial cells [1, 2]; its expression is stimulated by inflammatory molecules such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1) and bacterial lipopolysaccharide (LPS) [2, 27, 28]. However, some recent studies have detected its expression in non stimulated endothelial cells in vitro [29]. Its expression is maximal 4 h after cytokine stimulation and decreases rapidly thereafter. E-selectin expressed on the surface of the cell is gradually internalized by endocytosis [30] and degraded in lysosomes [31] so that it is no longer detectable after 24 h [2]. It was recently demonstrated that E-selectin clusters in both clathrin-coated pits and lipid rafts of endothelial cells but is internalized only by clathrin-coated pits. The distribution of E-selectin into two different domains on the cell membrane markedly enhances its ability to mediate leukocyte rolling in flow conditions [32].

A soluble form of E-selectin (sE-selectin) is generated by enzymatic cleavage or when activated endothelial cells shed damaged parts of their surface. It seems that the concentration of sE-selectin is directly correlated with cell surface expression [33]. The exact role of SE-selectin is not known, however, as some studies have found that it exerts a chemoattractant signal towards neutrophils, it may trigger the migration of these cells [34]. Other reports suggest that it may limit E-selectin-mediated rolling of activated leukocytes by competing for binding sites on the cell surface, thus “downregulating” the inflammatory response [35]. Whatever the case, the circulating concentration of circulating E-selectin can be used as a marker of activation of the endothelium and is therefore useful as a clinical tool for diagnosis of tumors and acute inflammatory processes.

E-selectin recognizes several structures carried by glycoproteins. Among the ligands that have attracted the most attention are: E-selectin ligand-1 (ESL-1) [36] which binds specifically to E-selectin but not to P- and L-selectin, P-selectin glycoprotein ligand-1 (PSGL-1) [37], L-selectin [38], CD43 [39], CD44 [40], β2 integrins [41] and death receptor-3 (DR3) [42].

Selectins have been found to recognize particular carbohydrate motifs: Sialyl lewis x (SLEX) and Sialyl lewis a (SLEA) found at the terminus of the structure of glycoproteins and glycolipids on the surface of leukocytes and tumor cells [43, 44]. The molecular bases of E-selectin-SLEA interaction are well documented [45, 46]. Many groups have reported the synthesis of SLEA analogs able to recognize and bind to E-selectin [47, 48].

Interaction of E-selectin with its ligand leads to the rolling of leukocytes on inflamed endothelial cells [49] which is the first step of the firm adhesion and transmigration to the surrounding tissue. More information about selectins, their ligands, role in physiology and pathology as well as their targeting are available in several published reviews [8, 19, 50–52].

2.2. Pathological implication of E-selectin

As mentioned above, although E-selectin has an important role in the physiological mechanisms regulating inflammation reactions, it is well documented today that it is also involved in many diseases. Since the pathological implications of E-selectin have also been previously reviewed only a brief summary will be provided here.

2.2.1. E-selectin and inflammation

The expression of E-selectin on endothelium during acute or chronic inflammatory reactions has been extensively studied, focusing on its involvement in the recruitment of immune cells [9, 50] through interaction with its ligands located on their surface. E-selectin has been detected on vascular endothelium in liver sections from patients with primary biliary cirrhosis, acute allograft rejection, alcoholic liver disease, but not on normal liver endothelium.
Vascular endothelium in retroocular connective tissues of patients with Graves' ophthalmopathy is strongly positive for E-selectin [55]. E-selectin has been found to be expressed at low level in non inflamed tissue, including the synovium [33], but its expression is up-regulated during rheumatoid arthritis (RA) in the inflamed synovial endothelium [56–58]. It is also expressed in the rheumatoid nodules in patients with PA [56] and is implicated in the recruitment of inflammatory cells into the tissue [59]. This expression is reduced after TNF-α blocking therapy [60].

High levels of the soluble form (sE-selectin) have also been reported in patients with inflammatory diseases: bronchial asthma [61], eczema [62], Graves disease [63], Guillain–Barre syndrome [64], Kawasaki disease [65], atopic dermatitis [66], psoriasis [67] and inflammatory bowel disease [68].

These observations in patients have been complemented by some information from animal models. In a murine model of anti-glomerular basement membrane glomerulonephritis, 2 h after disease induction 75% of the glomeruli were positive for E-selectin while it was undetectable in untreated mice [69]. In mice with experimental autoimmune uveoretinitis, the vessels of the inner retina showed strong staining for E-selectin, in contrast to the eyes of normal mice [70]. Still in mice, corneal vascular expression of E-selectin is involved in the pathogenesis of ischemic-reperfused zones [71].

2.2.2. E-selectin and cancer vascularisation

The pathological role of E-selectin is not restricted to inflammatory disorders. It has also been detected in some cancer tissues, indicating a potential involvement in the pathogenesis of this disease [72].

E-selectin has been detected on the endothelial cells of small vessels adjacent to cancer nests in patients of human colorectal cancer. This expression appears to be induced by some stimuli originating from the cancer cells, since the degree of expression is inversely correlated to the distance of the blood vessels from the cancer nests [73]. In primary gastric cancer, de novo expression of E-selectin on blood vessels was observed, particularly in highly vascularized tumor areas [74]. Furthermore, the expression of E-selectin was found to be significantly higher in malignancies than in benign tumors in head and neck [75] and breast tumors [76].

In vitro, it was observed that conditioned medium from Ehrlich ascites tumor (EAT) cells was able to induce expression of E-selectin in human umbilical vein endothelial cells (HUVECs) suggesting that tumor cells secrete cytokines or other factors stimulating expression of this receptor [77].

2.2.3. E-selectin and metastasis

Adhesion of tumor cells on vascular endothelium of the target organ is a key step in the cascade of metastasis. It is now well established that the couple SLEx/E-selectin is an important mediator in the adhesion of cancer cells on the endothelium in metastasis formation [78] by a mechanism similar to that of the adhesion of immune cells (Scheme 1).

Takada et al. have determined that the adhesion of 12 cultured human epithelial cancer cell lines of colon, pancreas lung and liver origin on the endothelium was achieved in an E-selectin-dependent way [79]. Also E-selectin is an intermediary in the adhesion of H-59 murine carcinoma and two highly metastatic human colorectal carcinoma lines to the liver endothelium [80] and of breast cancer cells to endothelial cell monolayers [81, 82]. When injected into the spleens of nude mice, human colon carcinoma cell lines with high levels of surface SLEx colonized to the liver more efficiently than low SLEx cells. This adhesion was partially inhibited by blocking antibodies specific for E-selectin [83].

E-selectin is involved in hepatic metastases of breast [84] and colorectal cancer [73]. Other data indicate a role for the SLEx/E-selectin interaction in bone metastases of prostate cancer [85] and liver metastases of gastric cancer [86].

3. E-selectin as a target

As a result of this demonstrated expression of E-selectin in the vicinity of inflammation, infection or cancer, it has become a natural target for therapeutic intervention. Different strategies to exploit and modulate E-selectin-mediated binding include blocking its interaction with its ligands, blocking its ligands and inhibiting the glycosyl transferases associated with biosynthesis of selectin carbohydrate binding determinants [51]. These strategies could inhibit immune and cancer cell adhesion in inflammation and metastasis, respectively.

An important strategy is the use of E-selectin itself as a receptor for the delivery of anti-inflammatory or anti-cancer drugs and for medical imaging systems containing an imaging agent (Scheme 2). This last approach will be described in this review.

3.1. General targeting — proof of concept

The different studies that have demonstrated the efficiency of targeting E-selectin with a view to developing drug delivery systems are listed in Table 1. They are classified by the recognition element employed; i.e. antibody-based systems and small molecular ligands and analogs.

3.1.1. Antibody-based targeting

Both liposomes and microspheres have been used as supports to couple anti-selectin antibodies.

3.1.1.1. Liposomes. The group of Dr. Bendas has targeted E-selectin by means of liposomes decorated with monoclonal anti-E-selectin antibodies (mAb) coupled directly to the surface. It could be shown that these immunoliposomes selectively bound to selectin-expressing HUVECs under either static or simulated blood flow conditions. The lipid/antibody ratio was found to be an essential factor determining the efficacy of the system [87].

In a second study, they chose to use polyethylene glycol (PEG) stabilized liposomes which would have a longer circulation time in the organism. When anti E-selectin mAb was directly connected to the surface of the liposomes (inserted among PEG chains), a higher PEG concentration reduced the E-selectin-mediated binding with E-selectin–transfected Chinese Hamster Ovarian cells, as a result of either reduced antibody accessibility or reduced efficiency of antibody coupling or both. When the mAb was attached to the distal ends of PEG, higher concentrations of PEG led to higher cell binding [88].

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A proportion of the liposomes bound to the cells was subsequently internalized, mainly by an endocytotic mechanism involving, E-selectin-mediated endocytosis to accumulate in the endosomes [89].

Spragg et al. prepared liposomes of various types conjugated with the anti-E-selectin H18/7 mAb. The best binding to activated HUVECs was obtained when the mAb was attached to the surface without steric stabilization, while PEG-stabilized liposomes showed lower cell association because the PEG reduced the amount of antibody that could be coupled to the surface and also limited the accessibility of the antibody to the receptor. Among the different formulations tested, cationic immunoliposomes showed a relatively high level of nonspecific binding due to interactions with the anionic cell surface [90].

In an attempt to enhance binding by using the synergy between two ligands, Gunawan and Auguste [91] prepared immunoliposomes bearing two distinct antibodies directed against intracellular cell adhesion molecule 1 (aICAM) and E-selectin (aELAM). The presence of the two Abs increased the cellular uptake of immunoliposomes by IL-1α-activated HUVECs compared with the uptake of liposomes bearing only aICAM or aELAM antibodies. They reported greater
binding and internalization of DOPC immunoliposomes in which the lipids are in a fluid state at temperatures above −20 °C than DPPC immunoliposomes in which the lipids are in a gel state at physiological temperature [91]. This influence of lipid mobility the aICAM:aE-selectin ratio on cell binding only occurred in conditions under which lipid rafts were present. When the formation of lipid rafts was prevented by deleting the cell membrane of cholesterol, binding of both DOPC and DPPC immunoliposomes was reduced to the non-specific binding level, suggesting that the presence of lipid rafts in ECs is critical for targeted drug delivery [92].

3.1.1.2. Targeting with E-selectin ligands and analogs

Minaguchi et al. have used liposomes on which SLEE® moieties were bound via human serum albumin, encapsulating a fluorescent marker. After intravenous injection in mouse model of arthritis induced by an anti-collagen-antibody, fluorescent imaging showed a signal confined to the inflammatory site in an inflammation-dependent manner and with colocalization with the vascular endothelial cell marker (CD31) and E-selectin [97].

Banquy et al. prepared nanoparticles from poly(lactic acid) co-polyethylene glycol (PLA-PEG) microparticles conjugated to with anti-human E-selectin mAb (68-5H11) via a biotin-neutravidin bridge with high densities (>200,000 mAbs per particle) demonstrated significant selective adhesion (up to 15-fold more) to inflamed endothelium compared with noninflamed endothelium, under physiologically relevant in vitro flow conditions (1.5 dyn/cm²) [95], which is strikingly more avid than was achieved previously with passive adsorption of mAbs onto biodegradable particles [99]. In an in vivo model, the intrascrotal injection of TNF-α which elicits E-selectin expression [96], followed by injection of particles 2 h later showed a significant (6-fold higher) selective adhesion for cytokine-inflamed endothelium than for non-cytokine-treated endothelium [95].

All these findings have demonstrated the effectiveness of specific antibodies to target E-selectin using liposomes and polymeric particles. The key factors that determine the efficiency of such systems is the position of the antibody on the surface of the vector and the way with which it is conjugated to the lipidic or polymeric backbone. More studies are needed to evaluate the in vivo toxicity of these systems, including the immunogenicity after antibody administration especially with long term treatment.

3.1.2. Targeting with E-selectin ligands and analogs

E. Jubeli et al. prepared nanoparticles from poly(lactic acid) cova-

lently linked to a mannose based analog of SLEx. In vitro adhesion tests showed strong adhesion of nanoparticles mediated specifically by E- and P-selectin because the pretreatment of activated cells with free ligand inhibited the adhesion of the particles [29].

Our group has prepared nanoparticles from an amphiphilic block co-
polymer composed of poly(lactic acid), PEG methacrylate (PEGMA) and
functionality at the distal end of the PEG chains with the mannose-based analog mentioned above. These nanoparticles allowed efficient targeting of TNF-α-stimulated E-selectin-expressing HUVECs with subsequent internalization (unpublished data). In these experiments, approximately two-thirds of the observed cell association was inhibited by pretreatment with anti-E-selectin antibody, suggesting that nanoparticles entered cells mainly in an E-selectin-mediated way, the remaining internalization may be mediated by interaction with P-selectin which was not blocked by the specific E-selectin antibody.

Peptide-quinic acid derivatives have been developed as non carbohydrate analogs of SLE. These constructions antagonize the adhesion of SLE-expressing HL-60 cells to both E-selectin and P-selectin-coated plates. When these analogs were conjugated to Hydroxypropyl methacrylamide (HPMA) copolymers, they showed about 3 orders of magnitude higher affinity than the monomolecular ligand and were internalized by E-selectin expressing endothelial cells mainly by endocytosis [98].

In order to design a targeted ultrasound diagnostic tool, lipidic microparticles based on phosphatidylcholine were functionalized with thiolated peptide ligands for E-selectin. These particles showed effective binding to human bladder carcinoma cells expressing E-selectin under flow conditions. Reduced binding in the presence of free ligand demonstrated the specificity of the binding; however, this was inhibited by serum proteins. Increasing the phospholipid chain length from 18 carbons to 22 carbons improved the stability of the particles during ultrasound exposure, without compromising their acoustic properties [99].

In conclusion, small molecule ligand-based targeting systems seem to be safer than antibodies based ones and probably much cheaper. Although they have given very good results in both in vitro and in vivo models, the clinical trials are still missing compared with system targeting other receptors by small ligands such as folic acid based carriers targeting folic acid receptor.

3.2. Targeting therapeutic agents

3.2.1. Targeting inflammation

Dexamethasone (DXM) is widely used to treat inflammatory and autoimmune diseases due to its broad spectrum of pharmacological effects. The various adverse effects of corticotherapy, especially during long-term treatment, suggest that targeting by E-selectin could be advantageous in dexamethasone therapy. This drug has often been used as a model in studies of anti-inflammatory drug targeting to activated endothelium.

In the work of Everts et al., DXM was covalently attached to H18/7 anti-E-selectin mAb. This conjugate selectively bound to TNF-α-stimulated endothelial cells, was internalized and routed to lysosomes, allowing DXM to be released and to down-regulate the expression of the proinflammatory IL-8 gene [100]. It is noteworthy that the antibody carrying an average of two conjugated dexamethasone molecules per antibody molecule, still recognized its target antigen. However, higher dexamethasone loading ratios (up to 10 dexamethasone molecules per Ab molecule) reduced the solubility of the conjugate and diminished its recognition of E-selectin [100]. Furthermore, the internalization and lysosomal degradation of an iodine-125 (125I-radiolabeled) dexamethasone-anti-E-selectin immunoconjugate in activated endothelial cells have been reported. The binding, internalization and degradation of the conjugate followed the kinetics of E-selectin expression and internalization. In contrast, free DXM entered both resting and activated endothelial cells by passive diffusion [101].

Immunoliposomes encapsulating DXM and bearing anti-E-selectin Ab were selectively internalized by activated endothelial cells, but their binding to E-selectin was saturated at a slightly lower concentration than the molecular immunconjugate described above, probably because of the presence of multiple Ab molecules on the surface of one liposome [102]. In an in vivo model of murine delayed-type hypersensitivity known to induce E-selectin expression in the affected skin, both immunonjugates and immunoliposomes accumulated in activated endothelial cells in the inflamed area. However, the liposomes were also detected in control skin, although to a lesser extent, and in macrophages of the liver and the spleen. The authors argued that this behavior could be explained by the coupling of intact antibody molecules in a random orientation to the liposomes, allowing recognition by the Fc receptors on macrophages because of clustering of Fc portions of the antibody on the liposomal membrane [102]. This important issue of specificity should be treated with special attention for any further clinical trials.

Similar liposomes conjugated with MES-1 anti-E-selectin Ab were internalized by activated mouse endothelial cells in vitro through E-selectin-mediated endocytosis. After intravenous administration in mice afflicted with anti-glomerular basement membrane
glomerulonephritis, accumulation of targeted liposomes in the kidney was 3.6 times higher than that of nontargeted IgG liposomes, whereas the accumulation of both types of liposomes in the liver, spleen, heart and lungs was comparable. In glomeruli, targeted delivery of DMX reduced expression of P-selectin, E-selectin, and vascular cell adhesion molecule-1 on the glomerular endothelial by 60–70% [103]. These liposomes reduced renal injury, improved glomerular function, strongly inhibited the formation of crescents in glomeruli and did not affect blood glucose levels, in contrast to free dexamethasone [103].

Instead of antibodies, SLE³ itself has also been used as a targeting ligand for DXM-loaded liposomes. In a model of murine experimental autoimmune uveoretinitis, intravenous injection of these conjugated liposomes led to their accumulation in the retina of EAU mice. Pre-treatment with anti-E-selectin antibody inhibited the accumulation. Again, liposomes were detected in the liver as well as in inflamed tissue. Two-fold higher dexamethasone concentration was achieved in the inflamed eyes after administration of 2 μg of encapsulated drug than that obtained with 1 mg of free dexamethasone [70].

3.2.2. Cancer targeting

Two strategies based on E-selectin targeting have been developed: inhibition of cancer cell adhesion by competition with ligand-bearing particles, and delivery of anti-cancer drugs to tumor cells via neighboring activated endothelial cells expressing E-selectin (Table 2).

As mentioned above, E-selectin is involved in the first step of the adhesion cascade taking place on the endothelial cell surface metastatic process [9, 82, 104] (Scheme 1). Therefore, occupation of this receptor by targeted structures could prevent this adhesion and thereby inhibit metastasis.

Zeising et al. reported the preparation of several formulations of liposomes functionalized with SLE³ which inhibited in vitro adhesion of HT29 colon and Lewis lung carcinoma LL cancer cells expressing SLEx and/or SLE³ to the endothelium [105]. Similarly multivalent sialyl Lewis x-peptide ligands and SLE³-bearing liposomes were used to block E-selectin-mediated binding of HepG2 hepatoma cells. Their ability to inhibit tumor cell binding to activated HUVECs was as follows: \( \text{SLE}^3 = \text{SLE}^2 > \text{SLE}^1 \) (20-fold) and \( \text{SLE}^3 > \text{SLE}^2 > \text{SLE}^1 \) (2000-fold). In the case of glycopeptides, if the first bond between one SLEx residue and a receptor has been formed, the free mobility of the other residues is strongly restricted, while the mobility of glycolipids in the lipidosome bilayer renders them almost independent of one another, making them very active inhibitors [106].

A trisaccharide analog of SLE³ known as GSC-150 inhibited the adhesion of a human colon carcinoma-derived cell line (KH12-HX) over-expressing SLE³ to HUVECs activated with TNF-α. Coadministration of GSC-150 with these cells into the spleens of BALB/c nude mice reduced the number of cells distributed to the liver and the number of cancer nodules in the liver even in mice injected with lipopolysaccharide (LPS), which is known to induce high E-selectin expression [107].

Mann et al. have identified a thioaptamer ligand (ESTA-1) that selectively recognizes E-selectin, thus allowing it to bind specifically to the inflamed tumor-associated vasculature of human carcinomas derived from breast, ovarian, and skin, but not to normal organs. It is able to block the adhesion (over 75% inhibition) of SLE³-positive HL-60 selectin on endothelial cells. Intravenously injected E-selectin-1 was detected binding to the tumor vasculature in a breast cancer xenograft model [108]. The advantage of this thioaptamer compared with SLE³ is its nanomolar binding affinity (KD = 47 nM) combined with minimal cross-reactivity with P- and L-selectin making it a selective ligand compared with many others including SLE³ itself that may interact with other selectins.

Inhibition of the E-selectin-mediated adhesion of metastatic cells could be used to prevent or at least to reduce metastatic tumor spread under certain conditions of high risk of dissemination such as during tumor surgery.

Carriers targeting E-selectin have also been used to deliver antitumor drugs. Liposomes conjugated with the anti-E-selectin H18/7 mAb and loaded with the cytotoxic agent doxorubicin caused a marked decrease of survival of activated cells, whereas nonactivated HUVECs were unaffected by this treatment [90].

Hydroxypropyl methacrylamide (HPMA) functionalized with a high-affinity E-selectin binding peptide (ESBP) bound to E-selectin on the surface of vascular endothelial cells with an affinity in the low nano-molar range, 10-fold higher than that of non-targeted copolymers. Once bound, E-selectin facilitated rapid internalization and lysosomal trafficking of the copolymers. When these immunopolymers were conjugated to doxorubicin they had 150-fold higher cytotoxicity than non targeted HPMA–DOX conjugates [109].

Two studies have been based on the fact that E-selectin vascular expression is significantly increased in malignant breast cancer. Mice bearing transplanted mammary tumors showed a significant increase in E-selectin expression on endothelial cells after tumor irradiation [110]. Immunoliposomes conjugated with anti-E-selectin mAb (10E9.6), loaded with the angiogenesis inhibitor combretastatin disodium phosphate and injected after a single dose of radiation, inhibited the growth of blood vessels, which led to an inhibition of tumor growth. Fractionated radiation plus fractionated doses of immunoliposomes resulted in further tumor growth delay [110].

In another study, mice bearing spontaneous mammary adenocarcinoma were treated with SLE³-targeted liposomes loaded with the anticancer agent merphanal prodrug. The treated mice showed higher average time before palpable tumors could be detected and higher average survival time than mice treated with non-targeted merphanal-loaded liposomes. As well as the essential influence of the glycoconjugate itself, the authors highlight the role of its hydrophilic nature in the stabilization of liposomes in the circulation [111].

SLE³-targeted liposomes loaded with cisplatin (CDDP) were injected in mice transplanted with Ehrlich ascites tumors in which the endothelial cells in the vicinity of the tumor expressed E-selectin. Treated mice showed a survival rate of 75% at 14 days (compared with 0% in mice treated with free CDDP). Accumulation of CDDP-SLE³-liposomes in the tumor was about 6 times more than that of non targeted liposomes. In a mouse xenograft model of A549 lung carcinoma cells, CDDP liposomes with or without SLE³ induced a suppression of tumor growth due to the enhanced permeability and retention effect (EPR), with slightly better suppression for targeted liposomes through the binding of SLE³ to the tumor-associated vascular endothelial cells [112].

3.2.3. Targeting cardiovascular system

Targeting endothelial cells is a potential strategy for treating cardiovascular anomalies. An example of drug delivery by targeted carriers is the treatment of restenosis, which remains a serious complication after angioplasty. In accord with the observation that E-selectin is expressed on the endothelium of atherosclerotic lesions [113, 114], Tsuruta et al. detected E-selectin in the carotid artery after balloon angioplasty in rats. When this local inflammation was significantly larger than those in all other groups, demonstrating that such a system could effectively prevent restenosis after angioplasty [115].

Thrombin is a mediator in vascular pathologies involving activation of the endothelium. In order to deliver hirudin, a potent and specific inhibitor of thrombin, it was covalently cross-linked to the H18/7 mAb. This immunoconjugate selectively bound to IL-1-activated but not to non activated HUVECs. During the process of cell activation with IL-1, immunoconjugate binding increased in parallel with cell-surface procoagulant activity. When bound to activated cells, the immunoconjugate significantly inhibited endogenous thrombin activity, and down regulated cellular responses that are mediated via the thrombin receptor [116].
3.3. Targeting E-selectin for gene transfer

Endothelial cells are an attractive target for gene therapy because they are intimately involved in disease processes associated with inflammation and angiogenesis and because they are readily accessible to gene therapy vectors via the circulation. Successful gene delivery requires good cellular targeting, internalization, endosomal escape and translocation to the nucleus. Targeted delivery systems can help to increase the specificity through promoting entry and intracellular trafficking of the genetic material.

One of the first reports using E-selectin as a targeting ligand for gene transfer was the work of Wickham et al. [117] in which an adenovirus was coupled to a bispecific antibody (bsAb) (recognizing both an epitope of adenovirus and an anti-E-selectin antibody). This bsAb was able to direct binding and entry of adenovirus into TNF-α-activated endothelial cells increasing then β-galactosidase transduction, while non activated cells were not efficiently transduced. The targeting was E-selectin specific, since the majority of transduction was blocked by preincubating the HUVECs with the anti-E-selectin mAb [117].

In further work with the adenovirus vector, the anti-E-selectin mAb (1.2B6) was coupled directly to the virus. Gene transduction of cultured endothelial cells was increased 20-fold compared with a non-specific control Ab coupled to the virus. The vector transduced around 30% of the intimal endothelial cells in segments of pig aorta cultured with cytokines ex vivo, compared with less than 0.1% of transduced cells with the control [118].

In an attempt to improve the specificity of viral gene carriers, Ogawara et al. carried out PEGylation of the adenovirus to inhibit its interaction with the coxsackie-adenovirus receptor and conjugated an E-selectin Ab to the ends of the PEG chains. This system showed longer persistence in the circulation with plasma concentrations 12-fold higher than those of unmodified virus, and selectively localized in inflamed skin in mice exhibiting a delayed-type hypersensitivity inflammation, resulting in local expression of the reporter transgene luciferase [119].

Another strategy to prolong the circulation time of viral vectors was proposed by Bachtarzi et al. who coated adenovirus with an amino-reactive polymer based on poly N-(2-hydroxypropyl) methacrylamide. H18/7 mAb was conjugated to these particles via protein G to provide optimal antibody orientation. This construction enhanced the transduction of TNF-α-activated endothelium in vitro and in a human umbilical vein cord model ex vivo. When the virus was targeted using a chimeric P-selectin Glycoprotein Ligand-1-Fc fusion (PSGL-1) protein (that recognized both P- and E-selectin), it showed significant uptake into HepG2 xenografts following systemic administration in mice, with 36-fold more genome copies than non-modified virus [120].

Although viral vectors are efficient at transfecting cells in vitro and in vivo, there are still some safety issues to be resolved. Therefore, much research has been devoted to non viral vectors, based on lipids or on polymers which are safer than viral vectors, relatively non immunogenic, may not be pro-inflammatory and generally easy to make. Targeting strategies towards E-selectin have also been applied to these systems.

George et al. proposed a method of coupling anti-E-selectin 1.286 Ab to liposomes already complexed with DNA, using mild heat treatment to aggregate the immunoglobulin G (IgG). The transfection rate was increased in CHO cells transfected to express E-selectin, TNF-α-activated endothelial cells, and TNF-α-stimulated human saphenous veins ex vivo. Transfection was inhibited by an excess of free antibody, demonstrating the specificity of targeting [121].

Amido amine dendrimers (PAMAM) carry a large number of positively charged terminal amines and hence bind DNA electrostatically, condensing and protecting it from various cellular, extracellular and bacterial nucleases [122] and thus represent an alternative to liposomes for non viral gene delivery. When the anti-selectin 1.286 Ab was conjugated to PAMAM by means of biotin/avidin interaction, the transfection efficacy of complexed DNA increased 4–7 fold in CHO cells expressing E-selectin, in primary human saphenous vein endothelial cells activated with TNF-α and in human saphenous vein segments compared to plain dendrimers [122].

These in vitro trials of E-selectin-mediated targeting of viral and non-viral gene delivery seem to be promising. However, no data are available from in vivo studies of such systems. Future development will have to deal with general issues of gene delivery in vivo such as gene activation, integrating the gene into the cellular genome and avoiding harmful side effects.

Another aspect of nucleic acid delivery is the use of smaller sequences (antisense oligonucleotides and siRNA) to silence genes. One example of this strategy in the treatment of inflammation was the use of SLEβ-bearing liposomes loaded with antisense oligonucleotides (AS-ODNs) directed against the adhesion molecule ICAM-1 to activated vascular endothelial cells [123]. Two different AS-ODNs were tested and both were able to reach their target in the cytoplasm and inhibit protein expression. The AS-ODN concentrations needed to inhibit ICAM-1 protein expression were ten-fold lower than those required when lipofectin was used as the vector for ODN transport.

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Table 2: Summary of delivery systems directed towards E-selectin for cancer therapy.

<table>
<thead>
<tr>
<th>System</th>
<th>Type of cancer/cancer cells</th>
<th>Recognition element</th>
<th>Target</th>
<th>Evaluation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes (several formulations)</td>
<td>HT29 colon and Lewis lung carcinoma LL cancer cells</td>
<td>SLEβ</td>
<td>Inhibition of cancer cells adhesion to endothelium</td>
<td>In vitro</td>
<td>[106]</td>
</tr>
<tr>
<td>Multivalent peptide ligands and liposomes</td>
<td>HepG2 hepatoma cells</td>
<td>SLEβ</td>
<td>Inhibition of cancer cells adhesion to endothelium</td>
<td>In vitro</td>
<td>[106]</td>
</tr>
<tr>
<td>Free ligand</td>
<td>Human colon carcinoma-derived cell line (KHT2-HX)</td>
<td>SLEβ trisaccharide analog</td>
<td>Inhibition of cancer cells adhesion to endothelium</td>
<td>In vitro</td>
<td>[107]</td>
</tr>
<tr>
<td>Free ligand</td>
<td>1) HL-60 2) Breast, ovarian, and skin carcinomas</td>
<td>Thioaptamer ligand</td>
<td>1) Inhibition of cancer cells adhesion to endothelium</td>
<td>In vitro</td>
<td>[108]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>–</td>
<td>Anti-E-selectin H18/7 mAb</td>
<td>Targeting endothelial cells with doxorubicin</td>
<td>In vitro</td>
<td>[90]</td>
</tr>
<tr>
<td>HPMA polymer</td>
<td>–</td>
<td>E-sel binding peptide</td>
<td>Targeting endothelial cells with doxorubicin</td>
<td>In vitro</td>
<td>[109]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Transplanted mammary tumors</td>
<td>Anti-E-selectin mAb (10E9.6)</td>
<td>Inhibition of angiogenesis with combretastatin disodium phosphate</td>
<td>In vitro</td>
<td>[110]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Mammary adenocarcinoma</td>
<td>SLEβ</td>
<td>Treat cancer and increase survival time with Merphalan</td>
<td>In vivo</td>
<td>[111]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Ehrlich’s ascites tumors</td>
<td>SLEβ</td>
<td>Treat cancer and increase survival time with cisplatin</td>
<td>In vivo</td>
<td>[112]</td>
</tr>
</tbody>
</table>
A similar approach to silence the gene for a cellular adhesion molecule was the addition of siRNA targeting the gene for VE-cadherin to the cationic amphiphilic lipid known as “SAINT” which has been conjugated to the H18/7 anti-selectin mouse Ab. These lipoplexes delivered significantly more siRNA to activated endothelial cells in vitro and in human kidney tissue slices subjected to inflammatory conditions ex vivo. The enhanced uptake of siRNA was combined with improved silencing of both gene and protein expression of VE-cadherin in activated HUVECs, indicating that SAINT delivered functionally active siRNA. The association of the lipid complex with cells was correlated with the expression pattern of E-selectin [124]. This is an important result because endothelial cells are notoriously difficult to transfect.

3.4. Applications for imaging

E-selectin has been used to improve the specificity of various types of imaging agents including radioactive (Table 3), fluorescent and MRI (Table 4) markers. We present below some published data in this domain, including in vitro and in vivo approaches and clinical trials.

3.4.1. Radioactive agents

Early pioneering work was done by Peters, Haskard and co-workers using Indium (111In)-radiolabeled E-selectin mAb 1.2B6 or its F(Ab)2 fragment (111In-F(Ab)2 1.2B6) for the imaging of vascular endothelial activation [125–127]. After intravenous injection of these imaging agents, they could be clearly detected 20–24 h later associated with the inflamed tissues in models of phytohaemagglutinin (PHA)-induced arthritis [125] and urate-crystal-induced arthritis [126] in pigs. The in vivo accumulation was many fold higher than controls and was correlated with E-selectin expression in the inflamed tissue.

Pig skin sites injected with IL-1, TNF-α, phytohaemagglutinin, or phosphoryl-mystate acetate showed specific accumulation of technetium-99m(Tc)-labeled anti-selectin 1.2B6 mAb after intravenous injection [128]. This approach allowed changes in vascular luminal expression of E-selectin to be quantified and leukocyte traffic and other signs of inflammatory responses to be detected in vivo [128].

An E-selectin-binding peptide (ESbp) conjugated with 99mTc had high affinity for E-selectin in vitro. In vivo imaging in a rat adjuvant-induced arthritis model showed specific binding of 99mTc-ESbp to the rat ankle joint which preceded clinical manifestations of inflammation [129].

Garood et al. described imaging of human synovial tissue transplanted into SCID mice using a radiolabeled 1.2B6 mAb and nano single photon emission computed tomography/computed tomography. The tissue vasculature was stimulated with TNF-α by an intra-graft injection 5 h prior to intravenous injection of 111In-labeled anti E-selectin. A significant difference in graft radioactivity was observed at 4 h and 24 h with a significantly higher uptake of 111In-anti-E-selectin compared with control antibody of the same isotype [130].

In a clinical study carried out with patients suffering from rheumatoid arthritis, prominent and well defined uptake of 111In-labeled F(ab′)2 fragments of 1.2B6 anti-E-selectin mAb was clearly visible in inflamed joints of all patients as compared with nonspecific immunoglobulin which was not specifically localized. Targeted 111In-1.2B6 scintigraphy is a sensitive method which yielded more intense and specific imaging than technetium-99m-labeled human immunoglobulin (99mTc-Hlg). However, the optimum time for imaging 111In-1.2B6 was 24 h after injection, whereas good results are obtained with 99mTc-Hlg as soon as 4 h post injection. Furthermore, Tc is a more suitable isotope for scintigraphy than In [131].

Based on the fact that E-selectin is expressed in venules of actively inflamed mucosa in inflammatory bowel disease (IBD), scintigraphy using 111In-labeled F(ab′)2 mAb was carried out. This technique yielded comparable results to the standard technique with Tc-99m-labeled leucocytes in 14 patients out of 17, with those positive for both (10/17) showing similar disease localization and extent [68].

A similar approach was applied to rheumatoid arthritis. When a fragment of 1.2B6 anti-E-selectin was labeled with 99mTc, image contrast in RA patients was slightly better at 4 h than 1.2B6-labeled 111In-1.2B6 F(ab′)2, showing a clear advantage of the 99mTc-F(ab′) as it could be used in a one-day protocol. Plasma clearance of 99mTc-Fab was faster and its diagnostic accuracy was better than that of 111In-F(ab′)2 and the commonly used tracer 99mTc-oxidronate (99mTc-HDP) [132]. It should be mentioned that 99mTc-Fab appeared somewhat unstable in vivo, as shown by radioactivity in the thyroid gland and bowel [132].

3.4.2. Fluorescent agents

Recently, fluorescence has become to replace radioactivity as a means of imaging pathological tissues. Near-infrared labels are necessary for in vivo studies to avoid excessive absorption of the emitted fluorescence by the tissues.

Graft copolymers of poly-(L-lysine) and methyl-PEG were covalently conjugated with a mAb fragment, F(AB′)2 of the H18/7 Ab and labeled with near-infrared indocyanine fluorophores (Cy5.5). The system was employed for in vitro imaging of E-selectin expression on human endothelial cells activated with IL-1, it showed high binding specificity (20–30 fold more than non-specific uptake) [133].

Small E-selectin-binding ligands have also been used to target fluorescent markers. The E-selectin-binding peptide (ESBP) was conjugated to crosslinked dextran-coated iron oxide (CLO) nanoparticles labeled with (Cy5.5). Internalization by activated HUVECs was rapid and mediated by E-selectin. In vivo, the fluorescent targeted particles bound both to Lewis lung carcinoma cells and endothelial cells, although the cancer cells had a much lower expression of E-selectin. This suggests that the system was a very sensitive detector of E-selectin expression [134]. Wittmann et al. have reported chemoenzymatic synthesis of a fluorescently labeled mono and bivalent SLE® conjugates. The staining of CHO and HUVEC cell lines expressing E-selectin was comparable to that for fluorescent anti E-selectin mAb [135].

### Table 3

Summary of radioactive imaging agents directed towards E-selectin.

<table>
<thead>
<tr>
<th>System</th>
<th>Imaging agent</th>
<th>Recognition element</th>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocongjugate</td>
<td>111In</td>
<td>E-se1 mAb 1.286</td>
<td>In vivo: phytohaemagglutinin induced arthritis [125]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>111In</td>
<td>E-se1 F(Ab)2 fragment (1.286)</td>
<td>In vivo: urate-crystal-induced arthritis [128]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>99mTc</td>
<td>E-se1 mAb 1.286</td>
<td>In vivo: skin sites injected with IL-1, TNF-α, phytohaema-glutinin, or phosphoryl mystate acetate [128]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>99mTc</td>
<td>E-se1 binding peptide</td>
<td>In vivo: tissue vasculature stimulated with TNF-α [130]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>111In</td>
<td>E-se1 mAb 1.286</td>
<td>Clinical study: rheumatoid arthritis [131]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>111In</td>
<td>E-se1 F(Ab)2 fragment (1.286)</td>
<td>Clinical study: inflammatory bowel disease [68]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>99mTc</td>
<td>E-se1 mAb 1.286</td>
<td>Clinical study: rheumatoid arthritis [132]</td>
<td></td>
</tr>
<tr>
<td>Immunocongjugate</td>
<td>111In</td>
<td>E-se1 F(Ab)2 fragment (1.286)</td>
<td>Clinical study: rheumatoid arthritis [132]</td>
<td></td>
</tr>
</tbody>
</table>
Liposomes have been used as a carrier for fluorescent imaging with anti-E-selectin Ab. A Cy5.5 fluorescent marker was encapsulated. These liposomes efficiently recognized HUVECs after cytokine activation in vitro, and were able to localize in an Ehrlich ascites tumor in mice [77]. The same formulation also localized in arthritic joints in a mouse model [136].

### 3.4.3. Magnetic resonance imaging

MRI can give much useful information about the structure and function of soft tissues in vivo by recording changes in the state of water protons in a magnetic field. However, since water is ubiquitous, this technique has limited sensitivity and specificity unless appropriate contrast agents are used to highlight the tissue of interest. Direct targeting of MR contrast agents to receptors within the pathological region could provide greater specificity than “passive” targeting approaches. Therefore, a number of systems utilizing E-selectin targeting have been developed for MRI. The contrast agents that have been used most frequently are diethylene triamine pentaacetic acid (DTPA) coupled with gadolinium (Gd) and superparamagnetic iron oxide particles.

Mulder et al. prepared pegylated liposomes labeled with H18/7 mAb covalently coupled to the distal end of the PEG-chains incorporating 20–30 mol% of Gd-DTPA – bis (stearyl amide). The encapsulation of the contrast agent did not affect the stability of the liposomes. A rhodamine derivative was also incorporated in order to follow the liposomes by fluorescence microscopy. Both MRI and fluorescence microscopy revealed the specific association of the solonar MR contrast agent with TNF-α-stimulated HUVECs [137]. The same group synthesized a molecular conjugate consisting of an analog of SLE<sup>α</sup> coupled to DTPA in order to target sites of inflammation. They complexed this ligand with gadolinium to prepare a new contrast agent known as Gd-DTPA-B (SLEx) [138]. When this agent was injected intravenously to Con A-treated mice, they induced a significantly lower attenuation of liver signal intensity than USPIO-SLE<sup>α</sup> injected into healthy mice, or USPIO injected to Con A-treated mice, suggesting that the specific contrast media is retained extracellularly as a result of interaction with E-selectin overexpressed on the vascular endothelium [142].

Cross-linked iron oxide nanoparticles (CLIO) have been decorated with anti-human E-selectin F(Ab)2 fragments. These particles showed 100–200 times higher binding to HUVECs treated with IL-1β than to control cells, and as a result the HUVECs became intensely superparamagnetic [145]. This system was evaluated in vivo using a model of adoptive human endothelial cell (HUVEC) implanted in athymic mice in a Matrigel scaffold. E-selectin was induced by IL-1β treatment. The MR high-resolution images recorded after injection of the nanoparticles revealed a three-fold higher accumulation and six-fold stronger contrast effect than with unmodified CLIO [146].

In another study, Reynolds et al. used USPIO nanoparticles conjugated to the anti-murine E-selectin F(Ab)2 fragment bound to CHO cells expressing mouse E-selectin in vitro [147]. In an in vivo mouse model of collagen hypersensitivity to oxazolone in the ear, the injection of functionalized nanoparticles resulted in their accumulation in the affected ear but not in the noninflamed ear. Accumulation of antibody-directed nanoparticles in the liver and spleen was also observed [147].

Nanoparticles with an iron oxide core coated with more complex polysaccharides were prepared by van Kasteren et al. [148]. These particles each contained 10<sup>7</sup> to 10<sup>9</sup> sugars per particle. In fact, only nanoparticles carrying the tetra saccharide SLE<sup>α</sup> showed strong and selective binding to an E-selectin-F<sub>c</sub> fragment. These particles were shown to bind to CHO cells expressing mouse E-selectin in vitro [147]. In an in vivo mouse model of collagen hypersensitivity to oxazolone in the ear, the injection of functionalized nanoparticles resulted in their accumulation in the affected ear but not in the noninflamed ear. Accumulation of antibody-directed nanoparticles in the liver and spleen was also observed. Therefore, this type of particle could be used in early, preclinical detection of MS and other...
neuropathologies including multi-infarct dementia, HIV-associated encephalitis, or Parkinson’s disease [148].

4. Discussion

Targeting therapeutic agents by means of specific molecular recognition is a very promising strategy for the treatment of complex diseases such as cancer, inflammation, autoimmune disorders, and neuropathologies. These systems could be expected to be more effective and to reduce side effects as well as allowing more accurate and earlier diagnosis by the development of more specific imaging agents.

The body of work described above shows how E-selectin has become one of the molecular targets for this type of specific therapy because of its expression during different disease states.

One of the most important features of targeted delivery systems is their surface properties. Several factors have emerged as important: steric stabilization using PEG [88, 105, 119], dextran [134, 142], or methacrylamide [149] chains; the manner in which the antibody or ligand is attached to the surface (it is clear that covalent linking is more efficient than physical adsorption [93, 94]), ligand accessibility and flexibility, where the positioning of the ligand/antibody at the end of the chain of PEG stabilizing chain has often been observed to be more effective than direct connection to the surface of the carrier [88, 105] and antibody attached to mobile lipids rather than gel-forming lipids aids effective binding [91, 92]; the number of drug molecules attached to each antibody molecule in the particular case of immun conjugates that may affect their solubility [100] and the density of the antibody or ligand on the surface in the case of particulate system [95, 124, 136].

The multivalency or “cluster” effect due to the presence of several copies of antibody or ligand carried by the conjugate/vector has been found to be an important factor that improves the adhesion with E-selectin [148]. This multivalency enhanced the binding of immun conjugates compared with free antibody fragments [133], polymers carrying 7–8 copies of E-selectin-binding peptide compared with free peptide [109], immunoliposomes compared with immunonjugate [90, 102], and nanoparticles carrying antibody fragments compared with free antibody fragments [145]. In all these examples, cooperative binding occurs when multiple weak bonds result in overall strong adhesion, mimicking the adhesion of leukocytes to the endothelium.

Negative charge has been claimed to be an advantageous property for injectable systems because it leads to electrostatic repulsion with cells such as vascular endothelial cells, erythrocytes and leukocytes which are also negatively charged. This repulsion is expected to limit non specific interaction with these cells [70, 77, 112, 136].

Different systems have been used for targeting, including simple conjugates, functionalized polymers, liposomes, micro and nanoparticles. Each platform has its own advantages and limits.

Conjugates are small molecules that can be prepared with relatively few steps of preparation and a smaller amount of adjuvant material administered, but their solubility could be an issue [100] and in most cases they have only one recognition element by molecule, so there is no multivalency effect. Liposomes and nanoparticles can carry a wide variety of both hydrophilic and hydrophobic therapeutic or diagnostic agents, provide a larger drug payload per particle, protect encapsulated agents from metabolic processes, and allow a high degree of cooperative binding to target cell antigens. In addition, the lipid composition of liposomes or the polymer composition of the nanoparticles can be modified to obtain other desirable properties such as prolonged circulation in the blood by adding PEG or polysaccharides to their surface.

Liposomes have a phospholipid bilayer structure that mimics the cell membrane and renders them biocompatible and non toxic, but suffer from poor stability on storage (complicated freeze drying) and after administration (interactions with serum proteins and lipoproteins). Progress in polymer chemistry over the last few decades has allowed polymer-based systems with very large spectrum of physico-chemical and biological properties to be synthesized. Polymeric nanoparticles are usually more stable than liposomes but their biocompatibility is an important issue that should be always well evaluated in the development of therapeutic or diagnostic agents.

It is clear that the choice of recognition element that will guide the delivery system is as important as the target itself. Monoclonal antibodies are still the most specific tool for molecular recognition, but their immunogenicity and the large size of the final system have prompted an evolution towards antibody fragments (i.e. F[ab]₂ and Fab fragments) and even antigen-binding domain fragments. These fragments can be excreted rapidly, and minimize the risk of an undesirable immunological reaction. Another disadvantage of antibodies is that some of them may have restricted species specificity, i.e. they are active in animal models but does not cross-react with the human epitope. In all cases, the complex purification and high cost of antibody production remains a practical problem.

Peptides are easily prepared in large quantity and high purity by solid-phase synthesis, and the sequence can be modified to change their biological half-life; however, very few studies of targeting E-selectin using binding peptides have been reported [98, 99, 109].

Recent progress in glycobiology and the determination of the mechanism by which E-selectin binds to its biological ligands have rendered the use of saccharide ligands to be an attractive alternative to overcome the problems mentioned above. The use of a proven, small molecule ligand such as SLE⁵ and its analogs has the advantage of transferable cross-species activity. However, it should be mentioned that SLE⁵ has a weak affinity for E-selectin, and the system will be in completion with leukocytes carrying SLE⁵.

Synthetic analogs of SLE⁵ could be an advantageous solution, especially because some of these molecules have been reported to have higher affinity than the natural ligands [47, 48]; hence they can bind E-selectin more effectively than leukocytes. Moreover, the hydrophilization of the carrier surface due to the presence of sugar derivatives can participate, to a limited but non negligible extent, to the protection of the delivery system against the adsorption of opsonin proteins in blood plasma and phagocytosis by macrophages [109, 111, 136]. On the other hand, it should be kept in mind that the synthesis of these saccharide molecules is complex but not very expensive.

Most in vitro tests under static conditions were carried out using HUVECs or Chinese hamster ovary cells. A number of dynamic methods have been developed and used to simulate circulation conditions; these systems are reviewed elsewhere [50]. Although the in-vitro flow models provide useful information and allow some phenomena to be studied in a more relevant way than in static adhesion assays, they represent an approximation and do not completely simulate in vivo conditions where the presence of other blood components such as erythrocytes and blood proteins in circulation [150], as well as the modification of vasculature and flow profile within different disease settings [151], may affect interactions with endothelial cells.

The most important concern for the development of clinically acceptable system is to tackle problems of specificity and selectivity of targeting. Although most systems tested in-vitro have showed very good results, in vivo data often shows a lack of specificity since the targeted conjugate/carrier could be detected in sites other than those to which is was targeted, especially in the liver [70, 102, 132, 147], spleen [102, 139, 147], heart and lungs [103] and sometimes the skin [102]. This could be due to formulation issues such as the use of ligands that can recognize other structures in addition to selectins [142], or the use of excess antibody or ligand in the formulation that was not firmly attached to the conjugate or carrier and could serve to mask the target receptor [122].
Future development will depend mainly on how such systems can improve the therapeutic index in the treatment of serious inflammatory disease and cancer compared with conventional therapies.

5. Conclusion

Although many clinical trials have been carried out in the field of medical imaging, targeted systems for drug delivery to E-selectin are still mostly at the level of laboratory research with animal models. The question of large-scale production for clinical use is now needed to screen these ligands to confirm their specificity before clinical studies. The question of large-scale production for such innovative molecular drug delivery systems also needs to be addressed.

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