

Received: 2004.12.06
Accepted: 2005.02.04
Published: 2005.08.15

Peptide-based approaches to treat asthma, arthritis, other autoimmune diseases and pathologies of the central nervous system

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Source of support: by grants from the MHRC, MMSF, and DFG (Lo 823/3-1).
M. Los is supported by CRC “New Cancer Therapy Development” program.

Summary

In this review we focus on peptide- and peptidomimetic-based approaches that target autoimmune diseases and some pathologies of the central nervous system. Special attention is given to asthma, allergic rhinitis, osteoarthritis, and Alzheimer’s disease, but other related pathologies are also reviewed, although to a lesser degree. Among others, drugs like Diacerhein and its active form Rhein, Pralnacasan, Anakinra (Kineret), Omalizumab, an antibody “BION-1”, directed against the common β -chain of cytokine receptors, are described below as well as attempts to target β -amyloid peptide aggregation. Parts of the review are also dedicated to targeting of pathologic conditions in the brain and in other tissues with peptides as well as methods to deliver larger molecules through the “blood-brain barrier” by exploring receptor-mediated transport, or elsewhere in the body by using peptides as carriers through cellular membranes. In addition to highlighting current developments in the field, we also propose, for future drug targets, the components of the inflammasome protein complex, which is believed to initiate the activation of caspase-1 dependent signaling events, as well as other pathways that signal inflammation. Thus we discuss the possibility of targeting inflammasome components for negative or positive modulation of an inflammatory response.

Key words: Anakinra • BION-1 • β -amyloid • Diacerhein • Kineret • Omalizumab • osteoarthritis • Pralnacasan • Rhein • secretase • Zafirlukast

Abbreviations: AD – Alzheimer’s disease, BBB – blood-brain barrier, CARD – caspase recruitment domain, CNS – central nerve system, CPP – cell penetrating peptides, ECM – extracellular matrix, HIV – human immunodeficiency virus, IL – interleukin, IL-1ra – IL-1 receptor antagonist, IL-1R – IL-1 receptor, ICE – IL-1 β -converting enzyme, NGF – nerve growth factor, NSAIDs – non-steroidal anti-inflammatory drugs, OA – osteoarthritis, RA – rheumatoid arthritis, RMT – receptor mediated transport, Syk – spleen tyrosine kinase, Th1 – T helper type 1, VIP – vasoactive intestinal peptide.

Full-text PDF: http://www.aite-online/pdf/vol_53/no_4/7740.pdf

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INTRODUCTION

Peptides derived from critical interaction or cleavage sites are the preferred leads (starting points) for the development of new drugs. In one of the recent issue we discussed in great detail recently developed peptide-derived anticancer drugs as well as attempts to selectively target cancer cells using peptide-based approaches⁸⁹. In this review we summarize the progress in three other areas that strongly rely on peptide-based pharmacologic innovations, namely: 1) asthma and autoimmune diseases, 2) pathologies of the central nerve system (CNS), and finally, 3) novel peptide-based strategies to deliver larger molecules into mammalian cells. These areas of pharmacologic development, although very different, all frequently use peptides as starting points for the development of pharmaco-active substances.

In the following sections we will discuss recent developments that target adverse inflammatory responses using peptide-derived molecules or antibodies directed against caspase-1 or other elements of the inflammatory process. This will be followed by a discussion of peptide-based approaches to treat pathologies of the central nervous system. Finally, we will reveal recent developments of peptides that have the capacity to transport much larger molecules into mammalian cells.

CASPASES AND THE INFLAMMASOME

Several peptide-based inhibitors of inflammatory caspases have been developed in recent years in attempts to modulate the inflammatory response^{61, 79}. The group of inflammatory caspases includes human caspase-1/interleukin-1 β -converting enzyme (ICE), caspase-4, and capsase-5. Inflammatory caspases have so far only been found in vertebrates. All mammalian caspases have so-called CARD (caspase recruitment domain) interaction motifs at their N-terminus, followed by the enzymatic domains^{13, 66, 84}. Although inflammatory caspases may be involved, to some degree, in programmed cell death⁷⁸, they are primarily activated by inflammatory stimuli. The inflammatory response, like apoptosis^{21, 107}, is intended to keep a healthy organism in a delicate balance. Many viruses, however, have developed mechanisms to evade inflammatory caspases and components of apoptotic pathways in order to disarm the inflammatory response against them²⁰. Learning more about how viruses accomplish this task could lead to interesting future targets for the development of peptidomimetics.

The key components and events that govern the activation of inflammatory caspases have recently been

described (reviewed in⁸⁴). According to the model, yet to be defined intracellular signals triggered by inflammatory stimuli bring about the formation of a multi-protein complex called the “inflammasome” (Fig. 1). The core components of the complex are caspase-1 and a NALP family member. Depending on the function and composition, the inflammasome may signal either inflammation or apoptosis or both events simultaneously. Other components that are found in some inflammasomes are ASC, CARDINAL, Ipaf, and caspase-5⁸⁴. The inflammasome provides multiple potential targets for modulation that may lead to future therapies for some of the many diseases resulting from improper regulation of inflammation.

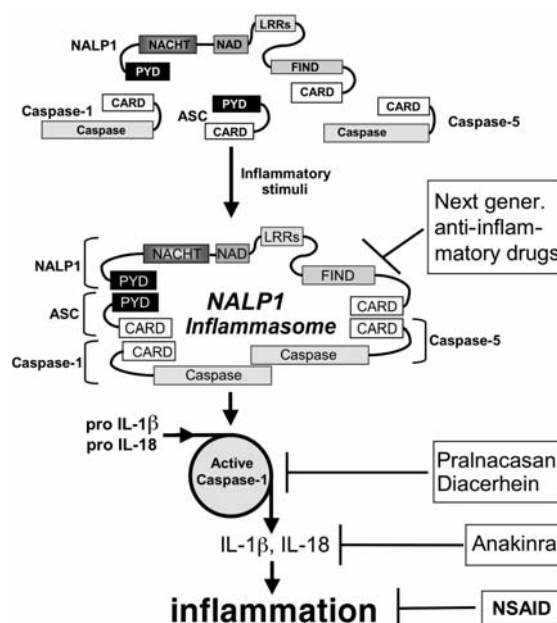


Figure 1. Inflammasome, the intracellular sensor of inflammatory stimuli that leads to the proteolytic activation of IL-1 β and IL-18. Yet to be identified bacterial or viral components trigger the activation of the inflammasome. The NOD-LRR family member NALP1 forms the backbone of this type of inflammasome. The other components include ASC, a small adaptor protein with CARD domain, and two pro-caspases. Pro-caspase-5 and NALP1 interact directly through the CARD domains, whereas pro-caspase-1 interacts directly with ASC (CARD-CARD interaction). The PYD (Pyrin domain) of ASC connects with the other end of NALP1. These interactions bring both caspase domains into a proximity that probably leads to proteolytic cross-activation of both⁸⁴. To the right side of the scheme we indicate some of the new anti-inflammatory drugs that are discussed in greater detail in the text.

INHIBITION OF CYTOKINE PROTEOLYTIC MATURATION, A PROMISING NEW APPROACH FOR THE TREATMENT OF OSTEOARTHRITIS AND OTHER INFLAMMATORY DISEASES

Osteoarthritis (OA) is a common multifactorial arthropathy that involves the slow progressive

destruction of articular cartilage in the joints. An imbalance between synthetic and destructive processes in the cartilage leads to degeneration of the articular cartilage, affecting in a large part the extracellular matrix (ECM). Since the ECM is what gives the cartilage its biomechanical properties, an alteration in the ECM of OA patients leads to mechanical joint failure.

Cytokines play an important role in the pathophysiology of osteoarthritis⁵². Increased levels of the pro-inflammatory cytokine interleukin (IL)-1 β have been shown to play an important role in the destructive process of osteoarthritis^{3, 92, 96, 97}. The increased production of IL-1 β is associated with increased levels of matrix metalloproteinases (MMPs). These enzymes degrade collagen and proteoglycan, which makes up the cartilage matrix¹³⁴. IL-18 is also known to produce similar effects⁸⁷ to those of IL-1 β . Cytosolic IL-1 β and IL-18 are inactive and require proteolytic activation by ICE/caspase-1^{17, 28, 78, 80}. The processing and release of IL-1 β necessitates activation of the IL-1 receptor (IL-1R)⁶³. The interleukins, together with other inflammatory mediators, are also responsible for the induction of clinical symptoms of inflammation such as hyper-perfusion of the inflamed site, fatigue, and pain⁵⁵. Several studies indicate that inhibition of ICE, with the use of an irreversible ICE inhibitor, significantly suppresses mature IL-1 β and IL-18 levels^{31, 92, 109, 120, 125}.

TREATMENTS AIMED AT DECREASING PRO-INFLAMMATORY CYTOKINE LEVELS IN OSTEOARTHRITIS AND OTHER INFLAMMATORY DISEASES

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is a common treatment for arthritis. However, these drugs treat only the symptoms of inflammation and pain without intervening in cartilage degradation⁹³. The development of disease-modifying drugs is therefore an important step. Diacerhein/Rhein, Pralnacasan, and Anakinra represent new disease-modifying strategies for the treatment of arthropathies and other inflammatory diseases. Mechanisms involve inhibiting ICE/caspase-1 and interfering with the activation of the IL-1R to prevent proteolytic maturation of IL-1 β and IL-18⁶². The primary goal is to alleviate cartilage destruction and regain joint function.

Diacerhein and Rhein

Diacerhein, an ICE-inhibitor, is used in the treatment of osteoarthritis. Diacerhein is converted to Rhein, its active metabolite, which then inhibits IL-1 production and activity¹⁴². Another study focused on

ICE levels in human osteoarthritic cartilage to determine whether decreased ICE levels correlated to a decrease in IL-1 β and IL-18 levels. Indeed, suppression of ICE resulted in decreased levels of these two molecules⁹². A potential side effect of Diacerhein is increased prostaglandin synthesis due to the increase of cyclooxygenase-2¹⁰⁴. Questions arose in a study done with an accelerated canine model of osteoarthritis¹² as to whether or not Diacerhein is in fact an effective disease-modifying drug, perhaps due to the counterbalancing effects of prostaglandin. A recent study bringing new evidence on the effect of Diacerhein contradicts this previous study. It reported decreased metalloproteinase levels and also suggested that Diacerhein had a positive effect on matrix synthesis⁸³. These results again give credence to the use of Diacerhein as a disease-modifying drug.

Pralnacasan

Pralnacasan is a specific inhibitor of ICE/caspase-1. It is an orally administered pro-drug recently approved for the treatment of rheumatoid arthritis (RA). Phase I/II trials showed Pralnacasan actively inhibited human ICE, and *ex vivo* assays demonstrated inhibition of mature IL-1 β production¹¹². ICE is an important enzyme in intestinal inflammation, utilizing IL-1 β and IL-18 as its biological effectors¹²⁵. Pralnacasan is currently being considered for treatment of osteoarthritis, inflammatory bowel disease, and possibly other autoimmune diseases. It was shown to significantly improve disease activity in dextran sulfate sodium-induced colitis in mice⁶. A reduction in joint damage was shown in collagenase-induced OA and spontaneously induced murine models of OA. This was confirmed by histopathological examination of the joints¹¹⁸. Pralnacasan is the first potent orally available drug to specifically target ICE/caspase-1. In laboratory studies and clinical trials Pralnacasan has shown promise as a future treatment for inflammatory and autoimmune disease.

Anakinra (Kineret)

Anakinra is a human recombinant IL-1 receptor antagonist (IL-1ra) that exhibits identical activity to its naturally occurring counterpart, IL-1ra¹¹⁵. Lower than normal levels of IL-1ra in the OA synovium have been observed, possibly due to the effect of increased nitric oxide seen in OA^{29, 123}.

Anakinra is categorized as a biological response modifier. It is the first biological modifier to demonstrate improvement in cartilage erosion when used in monotherapy or in combination with methotrexate, an antimetabolite¹. Combination treatment with

etanercept, a tumor necrosis factor α inhibitor, showed an increase in the number of opportunistic infections in patients with RA³⁷. This establishes the significance to study the effects of Anakinra in combination with other disease-modifying antirheumatic drugs. The most pronounced side effect observed upon the administration of Anakinra and ceftriaxone, a cephalosporin antibiotic, has been mild injection-site reactions⁹⁵.

ALLERGIC RHINITIS AND ASTHMA: PEPTIDE-BASED THERAPEUTIC INTERVENTIONS

Allergies and asthma are an ever increasing problem, particularly in industrialized nations¹²⁷. This results in thousands of dollars in lost wages, decreased productivity, missed school days, and reduced concentration at work or school. Individual allergic responses range from the minor annoyance of itching, watery eyes, or sneezing to life-threatening airway swelling and anaphylaxis⁵⁸. Sensitization to an antigen occurs by an unknown mechanism¹³³ involving antigen presentation to the immune system under conditions that favor an inflammatory response. This leads to the production of antigen-specific IgE antibodies. IgE binds its high-affinity receptor, Fc ϵ RI, on basophils and mast cells. Subsequent exposure to the antigen causes aggregation of the receptors via the IgE-antigen complexes, leading to mast cell degranulation. Within 10–15 min, stored chemical mediators, such as histamine, are released⁸. The cascade of events that follows increases the release of many T helper type 2 (Th2) cytokines and chemokines (i.e. IL-3, IL-4, IL-5, GM-CSF). This ultimately pushes the balance of inflammation away from the Th1 phenotype and favors inflammation. The result is increased IgE production and recruitment of inflammatory cells, such as eosinophils, that serve to induce vasodilation, mucus secretion, and bronchoconstriction and mediate a late phase response by stimulating further production of cytokines and pro-inflammatory mediators, including arachidonic acid metabolites, such as leukotrienes¹⁸. Widespread dissemination of these mediators can even result in anaphylaxis, where decreased blood pressure and swelling of the airway becomes a life-threatening condition. Many of the same mechanisms involved in allergic rhinitis are also responsible for the heterogenic disease known as asthma²⁶. Airway hyperresponsiveness in response to multiple causative agents leads to bronchoconstriction and increased mucous secretion which prevent the expulsion of air from the alveoli, inhibiting meaningful gas exchange, and can quickly lead to death in asthma¹¹¹. Long-term increases in lung inflammation can even result in alterations of the airway physiology that decreases lung function indefinitely¹¹¹.

Current standard therapy for asthma and allergy involves avoidance of the triggering allergen, which is not always possible or convenient. Another option is long-term administration of glucocorticoids to limit the inflammatory response. However, the significant side effects of glucocorticoid therapy disqualifies them from long-term use, especially in children and adolescent patients. As we expand our knowledge of the etiology of allergic rhinitis and asthma, we uncover an ever-increasing number of potential targets for their treatment.

Antigen immunotherapy

One of the oldest forms of peptide therapy for allergies, known as allergen immunotherapy, has been in use since the 1920s³². The antigen is injected at a high dose and forces the immune system into tolerance. The mechanism of anergy induction is unclear, but may involve T cell regulation or the lack of co-stimulation^{2, 133}. This method is associated with side effects, such as anaphylaxis, and may require life-long maintenance. It is also specific to the antigen used for treatment⁶⁷. However, sublingual formulations¹⁰³ and standardized dosing are being developed, so allergen immunotherapy may become more plausible in the future.

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP) is a natural peptide that potently causes smooth muscle relaxation in combination with nitric oxide⁴³ and has been a target for the development of peptidomimetics. Unfortunately, early attempts to deliver synthetic VIPs to the lungs were discouraging due to their rapid inactivation by peptidases⁴³. More recent analogues showed more promise in their effects *in vitro*⁹⁹ and in animal models⁹⁸. However, it was noted in one clinical trial that the results were not as satisfactory when compared with current therapy⁷³.

Monoclonal antibodies as a therapeutic strategy

A new trend in immunotherapy has been the development of monoclonal antibodies (Table 1). Antibody therapies have progressed largely due to the development of strategies for humanizing the proteins, which minimize the potential for patient reactions while maintaining the convenience of using non-human recombination systems to design the antibodies. Of course, the potential for reactions to the antibodies as foreign antigen, regardless of species, still exists. The reaction could degrade the protein available for therapy or, under the worst circumstances, exacerbate the allergic condition. An anti-IgE protein, Omalizumab, has recently been

Table 1. Representative approaches to the modulation of various components of the aberrant immune system

Mode of action	Target	Ligand model	Type of interaction	Reference
Adhesion molecule antagonist	ICAM-1 intracellular domain	T lymphocyte adhesion	competitive inhibition of T lymphocyte migration across the vascular endothelium	42
	lymphocyte function antigen-1, CD11a	humanized monoclonal antibody to CD11a, Efalizumab	blocks T lymphocyte adhesion to vascular endothelium	36
Cytokine/chemokine agonist (Th1)	IFN- γ	gene transfer or recombinant IFN- γ	enhances the Th1 response	53, 71
	IL-12	recombinant IL-12	enhances the Th1 response	15
Cytokine/chemokine antagonist (Th2)	IL-4	soluble IL-4 receptor	sequesters IL-4 inhibiting Th2 differentiation and isotype switching to IgE	11
	IL-5	monoclonal antibody to IL-5, TRFK-5	sequesters IL-5 and decreases eosinophil levels	126
Intracellular signal antagonists	Zap-70 protein tyrosine kinase, i.e. Syk	tandem SH2 domain	competes with T cell receptor for binding	47
		tandem SH2 domain	competes for binding of Syk with high affinity IgE receptor, Fc ϵ RI	119
Receptor agonist	Vasoactive intestinal peptide (VIP)	IK312532	relaxes tracheal smooth muscle constriction with longer duration than VIP	73, 98
Receptor antagonist	low affinity receptor (Fc ϵ RII), CD23 β -chain (β c) subunit common to the GM-CSF, IL-3, and IL-5 receptors	primatized monoclonal antibody to CD23	inhibits IgE synthesis	116
	GM-CSF receptor	monoclonal antibody to β c of receptor, BION-1	blocks binding of GM-CSF, IL-3, and IL-5, inhibiting eosinophil function and lifespan	88
		GM-CSF analogue E21R (Glu21Arg)	not fully understood	88
	CD4	IL-16 mimetic	blocks ligand binding without agonizing receptor	23, 74
	chemokine receptors (CCR3)	N-terminal-truncated CCL14	binds CCR3 and causes internalization, desensitization, and a lack of eosinophil chemotaxis	33
	leukotriene receptor	LTD4 mimetic, Zafirlukast	selective receptor antagonist, stabilizes mast cells	60
Antibody to IgE	serum IgE	humanized monoclonal antibody to IgE, Omalizumab	competes with the high-affinity IgE receptor, Fc ϵ RI, for binding of the CH3 domain	19, 69
Protease inhibitor	tryptase	β II tryptase structure	inhibits serine protease activity associated with mast cell degranulation	30
Antigen mimetic	peptide immuno-therapy	various specific allergens including: BeeVenom-PLA2, Cat allergen-Fel d I	desensitizes immune system against specific antigens	2, 53

approved for use in severe allergic asthma by the United States Food and Drug Administration. Clinical studies are currently under way to expand Omalizumab's use to include prevention of anaphylaxis⁶⁹ (Table 1). Omalizumab serves to bind and reduce the levels of free IgE in the serum⁵⁶, reducing the severity of symptoms¹⁹, and increasing tolerance to antigens⁶⁹. Although it is well tolerated overall⁴⁵, a few serious effects have been reported, as have long-term side effects due to chronic inhibition of the body's IgE levels.

Monoclonal antibodies are also being investigated for their ability to bind receptors without activating them, as is the case with primatized anti-CD23. The

antibody recognizes the low-affinity IgE receptor, Fc ϵ RII, which seems to have an influence on IgE synthesis. The anti-CD23 antibody showed a dose-dependent decrease in IgE in human trials, and although the trial showed no change in lung function, the anti-CD23 was well-tolerated compared with placebo and shows promise for the future¹¹⁶.

The major Th2 cytokines act to increase inflammation by binding receptors on their target cells. These receptors are made up of subunits, including the β -chain (β c), which is common to the receptors for IL-3, IL-5, and GM-CSF. Another antibody approach under investigation by McClure et al.⁸⁸ is

BION-1, an anti- β c antibody. BION-1 acts to antagonize the subunit common to several Th2 cytokine receptors and blocks activation by the cytokines, as shown in animal models¹¹⁶. Future studies will be required to show whether the BION-1 antibody will continue to show promise.

Targeting intracellular signaling

Binding of the β c leads to recruitment of downstream kinases, including Lyn, Lck, Fgr, and Jak³⁹. Modulation of these signaling cascades represents a more comprehensive approach to immunomodulation as the signals converge on these cascades. However, this brings with it its own set of problems with regard to cellular entry and functional blockade of events involved in far greater processes. One target currently under investigation is the src homology-2 tandem repeat domain of spleen tyrosine kinase (Syk), a cytosolic protein tyrosine kinase. Development of peptides that can competitively inhibit Fc ϵ RI binding to Syk would block IgE signal transduction, therefore limiting the allergic response to antigen¹¹⁹. A more thorough review of the potential intracellular targets has recently been published^{39, 82}.

Zafirlukast: a leukotriene mimetic

An alternative to antibody-based therapies is to design proteins that mimic the natural ligand, as is the case with Zafirlukast, a leukotriene D₄ mimetic. Zafirlukast competitively antagonizes the leukotriene receptors, stabilizing the mast cells and preventing the release of pro-inflammatory cytokines. Zafirlukast is generally well tolerated at the recommended dose and patients show improvement in asthma control as well as overall quality of life^{26, 59}.

Cytokine mimicry

Many of the therapeutic approaches targeting cytokines have shown disappointing results due to the toxicity associated with their delivery or simply

because they showed far less effectiveness than anticipated⁹. Intravenous IL-12 promised to augment the Th1-type response via suppression of IgE and eosinophil migration. Murine studies showed a reduction in IgE production and airway eosinophilia and an increase in IL-12 production; however, human trials showed significant toxicity¹⁵. This certainly precludes its use in human treatment in its current form; nevertheless, an alteration in the formulation may decrease toxicity. Conversely, the soluble receptor used to sequester the Th2 cytokine IL-4 was well tolerated in human trials; unfortunately, conflicting evidence with regard to its efficacy exists⁹. IL-4 is considered to be involved in isotype switching to IgE and Th2 cell differentiation. A study using nebulized IL-4R showed a reduction in dependence on steroids; however, a larger study, by Borish et al.¹¹, showed no significant difference from placebo⁹. These less than satisfying results are likely due to the complexity of allergy as a disease, the choice of endpoint measures, and the lack of correlation between human disease and existing animal models³⁵. With such a wide range of mediators involved in developing and maintaining allergy, it is unlikely that blocking any single mediator will resolve the disease state.

One of the major problems with the immunomodulatory therapies to date has been that most require intravenous or subcutaneous delivery. These methods will likely limit the therapies to very severe cases due to poor patient compliance and the great cost associated with this method of delivery. However, the use of peptidomimetics is clearly an interesting approach to the treatment of a potentially life-threatening disease. Further investigation into combination therapies, alternative deliveries, and novel target sites are warranted.

CNS: PEPTIDES AND PEPTIDOMIMETICS AS PROMISING THERAPEUTICS

In recent years there have been a number of studies attempting to apply small peptides to treat diseases in the CNS (Table 2). Peptides and peptidomimetics

Table 2. Examples of central nervous system diseases and peptidomimetics

Peptidomimetic	Mechanism of action	Disease
Prosapide [™]	peptidomimetic of prosaposition, the precursor to saposins A, B, C, and D, and a neurotrophic factor	Parkinson's disease, stroke, pain ^{75, 81, 90, 141}
2,3-Benzodiazepin-1,4-diones	peptidomimetic inhibitor of γ -secretase	Alzheimer's disease ^{86, 110, 138}
Saquinavir, Ritonavir, Indinavir and Amprenavir	HIV-1 and HIV-2 protease inhibitors, thus decrease viral assembly and budding	HIV ^{113, 124}
D3	peptidomimetic of TRkA-selective NGF	Alzheimer's disease, cognitive impairment associated with age ⁷⁵
Multiple	opioid receptor agonists	pain ⁵¹

are being developed that one day will successfully target chronic pain, Parkinson's disease, Alzheimer's disease (AD), ischemia, and CNS-changes induced by human immunodeficiency virus (HIV). Attempts are even being made to target mild cognitive impairment using neurotrophin-based peptidomimetics¹⁴. Currently the only approved peptidomimetics-based therapies are proteasome inhibitors, such as saquinavir, used in the treatment of HIV-induced pathologies of the CNS.

Transport problems of peptidomimetics to the CNS

The vasculature of the CNS has evolved to keep the brain and spinal cord protected/separated from many natural metabolites. This barrier, called the blood-brain barrier (BBB), prevents essential molecules such as neurotransmitters from leaving the CNS, maintains ionic homeostasis, and keeps unwanted and potentially toxic substances, as well as many drugs that are used for targeting signaling pathways in the periphery, from entering the CNS.

The blood vessels supplying the brain are composed of a single layer of endothelial cells, astrocyte foot processes, and pericytes. The extremely tight junctions between the endothelial cells of the brain blood vessels are an important and characteristic component of BBB. The tightness of these junctions are assured by an elaborate connection of claudins, occludins, and other junctional adhesion molecules⁴. Due to these tight junctions, para-cellular transport or diffusion is negligible, and in order for molecules to enter the brain, they must be small (<400–500 Da)¹⁰¹, lipophilic for diffusion through the cells, or actively transported¹⁰². This rule applies for all areas of the brain except circumventricular organs, which are important for the release of circulating hormones^{4, 50, 100}. The BBB is disrupted following pathological events such as ischemia, although weakening of the BBB is delayed by 4–6 h⁷. Therefore, even therapeutics engineered to prevent ischemia-associated cell death have to cross the BBB since they are most effective within the first 3 h after the occurrence of the ischemic pathology^{62, 100, 101}.

Since direct injection of treatments into the CNS, such as intrathecal administration, are invasive and difficult, strategies have been used to increase the transport of molecules across the BBB. These include increasing the osmolarity of the blood, often with mannitol, to open up the BBB tight junctions; increasing the lipophilicity of the treatment; and finally conjugating the peptide treatments to take advantage of receptor-mediated uptake through the BBB^{101, 102, 128}.

Receptor-mediated transport of molecules into the brain

The most important challenge during the development of peptidomimetics for the treatment of CNS pathologies is to design molecules that cross the BBB. Besides passive diffusion, researchers aim to take advantage of the BBB's own receptor-mediated transport in order to deliver larger molecules. Please also consult the next section for an overview of approaches that aim to assist the transport of large molecules into cells. Receptor-mediated transport (RMT) is the most promising mechanism to circumvent the BBB and obtain access for therapies into the brain. RMT can be used to transport large-molecule therapeutics into the CNS that cannot normally enter otherwise¹⁰². RMT takes advantage of naturally occurring transport proteins such as the transferrin receptor, the insulin receptor, or the immunoglobulin Fc receptor. For example, brain-derived neurotrophic factor has been fused to an anti-transferrin receptor monoclonal antibody. The transferrin receptor monoclonal antibody binds to the transferrin receptor in the endothelium cells and is transported into the brain where it can bind to neurotrophin tyrosine-kinase receptor B, leading to the protection of hippocampal neurons following ischemia in rats^{139, 140}. However, it must be noted that the intracellular pathways that govern the RMT require further study¹⁰¹.

Peptidomimetics-based AD therapy approaches

AD is a neurodegenerative disorder and a leading cause of dementia. It is manifested by memory loss, impaired thinking, and personality changes. Most frequently it is found in elderly people. AD currently affects around 4.5 million people in the United States, 12 million worldwide²². This is expected to increase 3-fold in the next 50 years⁴⁹, mainly due to changes in global population demographics. Among the hypotheses that attempt to explain the mechanism of the observed pathologic changes in AD, the "amyloid hypothesis" appears to be the most widely accepted. According to this hypothesis, the accumulation of amyloid- β 42 (A β 42, the 42-residue isoform of the amyloid- β peptide) leads to aggregate formation, neuronal cell death, and AD-typical dementia manifestation.

The treatment of choice has been cholinesterase inhibitors such as donepezil, galantamine, or rivastigmine to retard depletion of acetylcholine levels in the brain. However, these are only mildly beneficial¹²². Recently, the N-methyl-D-aspartate receptor antagonist memantine has received approval for treatment of severe AD. However, the currently available therapies only treat the symptoms of AD and do not prevent or even delay the progression of the disease.

One of the main hurdles of therapies for AD to prevent progression of the disease is the fact that a definitive diagnosis can only be made at autopsy. The amyloid deposits occur years prior to the onset of cognitive decline, and therefore early diagnosis is critical¹⁰². Saito et al.¹²¹ were able to show that conjugating amyloid- β 1-40 (composed of the first 40 amino acids of amyloid- β) with a monoclonal OX26 antibody facilitated uptake of the “amyloid- β 1-40” peptide into the CNS through the transferrin receptor in rat. This peptide complex was radio-labeled with [¹²⁵I] iodine. It is believed that one day this radio-labeled peptide can be used in conjunction with the SPECT-detection system to diagnose AD patients early^{65, 68, 121}.

Multiple peptidomimetics are under development to treat AD using different pathways. One of the main goals is to disrupt amyloid- β aggregation. This approach has entered phase III trials with a glycosaminoglycan mimetic that is supposed to disrupt aggregates²². Eighteen patients showed improved cognition over controls over 16 months in the Neurochem company phase II clinical trial.

A second approach for treating AD is to modulate the production of highly amyloidogenic A β 42 peptide produced from amyloid- β protein by secretases. However, A β 42 is not the only product of amyloid precursor protein (APP). There are 3 secretases that process APP, α -secretase, β -secretase, and γ -secretases. Most of the research in this area has focused on peptidomimetics that inhibit γ -secretases, as inhibition of this protease decreases the levels of all the A β isoforms²². The work of Wolfe et al.¹³⁸ has focused on this pathway. They have identified the hydroxyethyl moiety that mimics the transition state of the aspartyl protease catalysis¹³⁸. This approach is similar to one applied for the development of HIV protease inhibitors. There are limitations regarding inhibiting γ -secretases, as APP is not the only protein it cleaves. γ -Secretase substrates include NOTCH1 receptor and NOTCH ligands. Thus potential toxic side effects may be related to this inhibitory activity²². One method around this problem is the development of helical peptidomimetics that take the advantage of γ -secretase cleaving the transmembrane portion of APP directly in the lipid bilayer to produce A β 42¹³⁸. Although many groups are actively pursuing the development of γ -secretase inhibitors, the current literature remains limited. Some pharmaceutical companies have focused on developing β -secretase inhibitors instead, although these efforts are in a rather early stage as well²². Some existing drugs already possess such an activity. Weggen et al.¹³⁶ have shown that common NSAIDs, such as ibuprofen,

indomethacin and sulindac sulfide, were able to reduce A β 42 production through inhibition of γ -secretase.

Another, indirect approach aims to use neurotrophic factors such as nerve growth factor (NGF) to rescue the cholinergic neurons that undergo cell death in AD. This approach has no effect on the production or accumulation of A β 42, but as a trophic factor it prevents neuronal cell death. NGF, however, is a large polar molecule that does not cross the BBB if administered systemically¹³¹. Phase II clinical trials were conducted using intrathecal injections of NGF which had to be stopped due to back pain, weight loss, and the impracticality of intrathecal injection²². Small neurotrophin mimetics have been proposed to overcome these side effects as well as the short half life of neurotrophins¹⁰⁸. The NGF-directed approach is further complicated by the fact that cholinergic neurons lose expression of the NGF receptor TrkA in AD. Recently, Bruno et al.¹⁴ developed a peptidomimetic to activate this pathway. Although this treatment improved cognition in aged mice, it has yet to prove its benefit in AD treatment. Thus, large-scale clinical trials are necessary in order to determine if any of the newly discovered molecules actually alter the course of the disease.

PEPTIDES AS CARRIERS/TRANSPORTERS OF LARGE MOLECULES INTO THE CELL

The delivery of large biomolecules into cells for therapeutic applications has proven to be difficult due to the physiological nature of the cytoplasmic cell membrane. Composed of a lipid bilayer, the cell membrane selectively excludes large polar compounds while allowing the passage of smaller, non-polar molecules through passive diffusion. The recent discovery and characterization of numerous peptides/small proteins, generally referred to as cell-penetrating peptides (CPP) or protein transduction domains¹⁰, allow for rapid, non-invasive transport of conjugated macromolecules through the membrane (Table 3).

In 1988, both Green and Frankel identified the first CPP in the HIV-1 transactivation factor TAT protein^{34, 40}. Subsequently it was discovered that the minimal 13-amino-acid peptide sequence (amino acids 48-60) was also able to transduce the membrane¹³⁵. The antennapedia protein^{25, 105} and, later, the shortened penetratin peptide²⁴ and the herpes simplex virus structural protein VP22²⁷ represent some of the better characterized peptides that are currently being used for the transport of larger biomolecular cargoes. Comprehensive reports of other identified CPPs have been compiled in several recent reviews^{54, 64, 143}.

Table 3. Bio-molecular cargoes delivered by cell-penetrating peptides

CPP	Sequence	Cargo
TAT	GRKKRRQRYKC	nanoparticles ⁷⁰
	YGRKKRRQRRR	apoptin ⁴⁴
	RQIKIWFQNRRMKWKK and YGRKKRRQRRR (respectively)	liposomes ⁸⁵
TAT and Antennapedia	CRQIKNRRMKWKK	siRNA ⁹⁴
Penetratin	KKWKMRNQFWVKVQRG	Mdm2-binding do- mains of p53 ⁵⁷
	34 carboxy-terminal amino acids VP22	IxB α ¹²⁹
VP22	full-length VP22	p53 protein ¹³⁷
Poly-arginine	RRRRRRRRR	anti-CEA (carcinoem- bryonic antigen) immunotoxin ⁴⁸
	RRRRRRR	cyclosporin A ¹¹⁷

Several CPPs appear to share similar structural characteristics, suggesting a common mechanism of membrane transduction. The most notable commonalities appear to be the cationic character and diminutive length of the peptides. In support of this it has been demonstrated that short poly-arginine and, to a lesser extent, poly-lysine peptides have exhibited efficient cellular uptake⁴⁶. Despite these structural similarities, the exact mechanism of CPP transduction through the cell membrane remains unclear. Early studies demonstrated that peptide transduction could occur at low temperatures and in the presence of endocytic inhibitors, suggesting an endocytic-independent mechanism of membrane transduction²⁵. These findings have been recently debated along the premise that harsh fixation of cells may have resulted in artifactual observations¹¹⁴. Regardless, the mode of entry appears to be dependent on an interaction between the positively charged CPP and the negatively charged surface of the cell membrane. In support of this it has been shown that negatively charged heparan sulfate proteoglycans, which are associated with the cell membrane, are involved in the internalization of the TAT protein¹³². Ultimately, further experimentation is warranted to determine the mechanism(s) of CPP entry into the cell.

Despite the lack of detailed knowledge of a mechanism of entry into the cell, numerous studies have demonstrated the potential applications of CPP as carriers of biomolecular cargoes. The cargoes may consist of, but are not restricted to, peptides, nucleic acids, and lipids and can be attached either covalent-

ly or non-covalently. A recent review by Zhao and Weissleder¹⁴³ presented several cargoes that have been utilized to date.

Based on the amount of published experimental data that describes the usage of CPPs to deliver larger cargoes, the potential therapeutic applications of these cellular ferries as transporters of large, pharmacologically active agents that target cancer, autoimmune pathologies, and other diseases are significant. For example, the p53 tumor suppressor protein is mutated in over half of all cancer cells⁴¹, which has made it an attractive model to employ CPP-based strategies. Targeted delivery of functional p53 or other agents to cells, thereby correcting disabled signaling pathways, would have significant potential for the development of new therapies for cancer, asthma, autoimmune pathologies including OA, and other diseases. Initial studies using: TAT⁷², VP22¹⁰⁶, poly-arginine¹³⁰, and penetratin⁹¹ as transport vectors conjugated to the p53 protein, or derived peptides, have resulted in varying degrees of success. The CPP-p53 model, which consists of targeted recovery of a defective molecular pathway, exemplifies the potential of CPP-based transport of large cargoes in the treatment of cancer, inflammation, and other diseases.

CONCLUSION

Dysregulation of apoptosis contributes to a multitude of human diseases, and drugs that modulate apoptotic signaling pathways may have many clinical applications in the future⁷⁶. Although the development of caspase inhibitors for clinical use has a long way to go, many promising results have already been gleaned from animal studies. As the compounds used in many of these studies are not optimal for *in vivo* caspase inhibition, new inhibitors with improved cell penetration and pharmacokinetic properties could well yield better results⁶¹. In time, agents designed to enhance caspase activity, rather than inhibit it, will probably also be developed for therapeutic application¹⁰⁷. With due attention to the potential risks outlined above, therapeutic modulation of caspase activity is likely to offer significant relief to patients with not only autoimmune conditions, but also neurodegenerative disorders.

Given the progress in understanding cytokine pathways and their effect on the pathophysiology of inflammatory-related diseases, several possibilities towards a new approach in the treatment of osteoarthritis and asthma exist. Studies have established a central role for IL-1 β and a slightly lesser role for IL-18 in osteoarthritis, rheumatoid arthritis, and intestinal inflammatory disease. The approval of

some disease-modifying drugs used for rheumatoid arthritis could intensify the research on such treatment procedures not only for osteoarthritis, but also for other diseases. Furthermore, modulation of the type of cell death (apoptotic – no activation of immune response, necrotic – activation of immune response against antigens released from the dying cell), may open completely new treatment strategies for cancer and autoimmune diseases^{16, 38, 77}. This approach is becoming more feasible than ever since *in vivo* assays that can discriminate between both forms of cell death, apoptotic or necrotic are already in place⁵.

Our knowledge of the immediate mechanisms of inflammatory-caspase activation advances along with that of the components of inflammasomes, of which many have been defined⁸⁴. Thus, targeting their components, such as NALPs or ASCs, may prove to be even more effective than targeting the caspases or cytokines themselves. Furthermore, better understanding of inflammasome functions may lead to the development of entirely new treatment strategies that would, for example, activate one's own immune system to better combat cancer or HIV-infection, or even ordinary viral infections related to the "common cold".

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