Peptide-based therapies for arthritis

Marina Ali &
Nicholas Manolios[†]

[†]*Author for correspondence*
University of Sydney, Rheumatology Dept, Westmead Hospital, Westmead 2145, Sydney, Australia
Tel.: +61 298 458 099; Fax: +61 298 458 317; nickm@westgate.wh.usyd.edu.au

In this review we focus on peptide and peptidomimetic-based approaches in the treatment of inflammatory arthritis and outline what is in the pipeline for future therapeutic possibilities. Since immunomodulation is the key process influencing an inflammatory response, this review highlights strategies aimed at interfering with the immune synapse. Peptides used to block cytokines, and newer promising therapies on the horizon for arthritis are discussed. Finally, we review the mechanistic-based strategy used to develop transmembrane peptides and outline their potential use in arthritis and other fields of medicine.

Keywords:
immunosuppression, peptides, rheumatoid arthritis, T cells, therapy, transmembrane peptides, vaccination

future medicine part of fsg

To date, therapeutic drugs used in arthritis have largely consisted of the use of small molecules involved with analgesia, reducing inflammation or disease modification. These small molecules are easy to manufacture, are stable and can be produced at a relatively low cost per unit of production. With advances in biotechnology the use of monoclonal antibodies (mAbs) over the last two decades has grown in application and newer therapies with specific targets are becoming increasingly available for the treatment of autoimmune diseases. The use of mAbs in the treatment of rheumatoid arthritis (RA) has led the way in medicine for the clinical application of these compounds [1] and they now occupy a central role in the management of these patients. Currently, there is a large and growing number of biologic agents on the market or in clinical trials not only for arthritis (Table 1), but for use in a wider range of medical diseases. With the increase in understanding of the pathophysiology of diseases, combined with the ability to synthesize new compounds on a large scale with low costs, there has been a change in approach and newer therapeutic strategies are leading us into a new era of therapeutics. By and large, gone are the days where the traditional small molecules were derived from natural products (e.g., plants and tree bark) and tested in multiple assay systems for efficacy. The new era of drug design, based on better understanding of disease processes, molecular recognition of cellular and protein events and the ability to disrupt such crucial interactions, has come of age.

Peptides as therapeutic agents have trailed small molecules and mAbs in application largely owing to the problems listed in Box 1. These include: delivery problems, low metabolic stability and the high costs involved with manufacturing. However, trends are changing and with better delivery systems, including aerosols, transdermal patches and the identification and application of modified amino acids that stabilize peptides for oral delivery, there is an increasing awareness of their use as therapeutic agents and an increase in the number of companies developing peptides as therapeutic agents. Peptides are now considered viable treatment options, with a number of lead compounds being developed and tested in cancer, hepatitis, viral infections and inflammation [2–10]. Manufacturing techniques have reduced the overall costs of peptide-based drug development and the advantages of low toxicity and high specificity appear to outweigh the disadvantages, leading to the recent approval of products such as Fuzeon™ and ziconotide for the treatment of AIDS and nonaddictive pain relief, respectively. In this review, we briefly focus on the different strategies and current approaches used in the treatment and prevention of arthritis by the use of peptides. We also outline a novel approach developed in our laboratory that has applications not only in treating arthritis but also a wider range of clinical diseases.

Pathophysiology of RA

Unabated, persistent chronic inflammation is the cornerstone of RA. The perpetrators include cells (T cells, B cells, macrophages, synoviocytes, endothelial cells and mast cells) combined with chemical mediators (cytokines, chemokines and metalloproteinases) to cause joint damage. Central to this schema is the recognition of an inciting agent by T cells that triggers the process leading to a cascade of events that has no effective negative feedback.

Table 1. Current monoclonal antibody therapeutics for rheumatoid arthritis.

Therapeutic agent	Mechanism of action	Stage of development
Cytokine targets		
Infliximab	TNF-α inhibitor	US FDA approved
Etanercept	TNF-α inhibitor	US FDA approved
Adalimumab	TNF-α inhibitor	US FDA approved
Anakinra	IL-1 receptor antagonist	US FDA approved
IL-1 TRAP	IL-1 inhibitor	Phase II trials
Tocilizumab	IL-6 receptor inhibitor	Phase III trials
IL-15 mAb (HuMax IL15/AMG 714)	IL-15 inhibitor	Phase II trials
IL-12 mAb	IL-12 p40 subunit inhibitor	Preclinical
IL-18-binding protein	IL-18 antagonist	Phase II trials
Anti-MCP1 mAb (ABM912)	MCP-1 inhibitor	Phase II trials with negative results
CCR1 antagonist	CCL3/CCL5 chemokine blocker	Phase II trials
C5a receptor mAb	Inhibit C5aR	Preclinical
B-cell targeting		
Rituximab	$CD20^+$ B-cell-depleting agent	Awaiting US FDA approval
Belimumab	BLyS inhibitor	Phase II trials
T-cell targeting		
Abatacept	Inhibitor of T-cell activation	US FDA approved
Osteoclast inhibitors		
Zolindronic acid	Inhibitor of pyrophosphate synthase	Phase II trials
RANKL monoclonal antibody (AMG 162)	Inhibitor of RANKL-induced osteoclast activity	Preclinical
Small molecules		
p38 MAP-kinase inhibitor (VX-702 and BIRB796)	Inhibits expression of proinflammatory cytokines	Phase II and III trials
HMGCoA-reductase inhibitors	Inhibits Th1 responses	Phase II trials

CCL: CC chemokine ligand; CCR: CC chemokine receptor; HMGCoA: 3-hydroxy-3-methyl-glutaryl-coenzymeA; IL: Interleukin; mAb: Monoclonal antibody; MAP: Mitogen-activated protein; MCP: Monocyte chemoattractant protein; RANKL: Receptor activator for nuclear factor-κB ligand; Th: T helper; TNF: Tumor necrosis factor.
Modified from [66,67].

Of particular interest has been the immune synapse, which has been a crucial point of focus in the development and approach to interrupting inflammation. Figure 1, outlines the pro-inflammatory cascade leading to arthritis and identifies points of intervention that have been targeted and are discussed below. These strategies include: peptide mimetics, T-cell receptor (TCR) vaccines, human leukocyte antigen (HLA) peptide vaccines and others.

Peptides that effect the immune synapse

Antigen mimetics

Tolerance induction by administration of antigen has long been considered. Collagen given in the form of deer antler by the Chinese has been used over the centuries for the treatment of arthritis. Trentham and colleagues, in a randomized, double-blind trial involving 60 patients with severe active RA, demonstrated a decrease in the number of swollen and tender joints in subjects fed chicken type II collagen for 3 months [11]. A similar finding was noted in patients with juvenile RA [12]. By contrast, a double-blind, randomized study by Cazzola and colleagues showed that the oral treatment of RA patients with chicken collagen was ineffective and resulted in only a small and inconsistent benefit [13]. Other researchers have also intimated that a subset of RA patients sensitive to oral collagen may need to be identified for this therapy to be useful.

The use of solubilized class II major histocompatibility complex (MHC) molecules loaded with disease autoantigenic peptide [14], post-translationally modifying the antigenic peptide by galactosylation [15], or synthesizing altered antigenic peptides that may compete with the

original peptide for occupancy of the MHC groove subsequently leading to altered T-cell responses, have all been considered as possible means to induce tolerance. These approaches are practical if the autoantigen is known, for example, myelin basic protein (MBP) in multiple sclerosis (MS). Glatiramer acetate, a synthetic copolymer with an amino acid composition based on the structure of MBP, is a worldwide-approved drug for the treatment of relapsing MS, believed to enter the brain and, through cross-reactivity with MBP, produce suppression and anti-inflammatory effects [4]. A recent review of the benefit of glatiramer in MS disease progression and risk of relapse is discussed by Munari and colleagues [16,17]. Unfortunately the antigenic peptide in arthritis is unknown and little progress has been made in this area.

In RA, more than 90% of patients carry at least one of the class II MHC DRB1 alleles, *DRB*0101*, *0401*, *0404* and *0405*. Residues in the DRβ chain of the shared epitopes are reported to influence binding of antigen peptides. Woulfe and colleagues have reported the ability of a peptidomimetic inhibitor (SC-67655) to specifically block the peptide binding site of the RA-linked HLA-encoded *DRB1*0401* allele, and thereby block functional immune responses (Figure 2) [18]. This inhibitor bound to purified *DRB1*0401* molecules with an affinity similar to the original peptide ligand. In a similar approach of identifying peptidomimetic ligands to DR molecules, Falcioni and colleagues have identified a heptapeptide with high affinity to RA class II MHC molecules [19]. Using information regarding the binding-site interactions of this peptide, a variety of mimetics containing unnatural amino acids and dipeptide mimetics were found to be appropriate substitutes with comparable binding affinity to the original peptide. These compounds were found to be up to 1000-fold more potent than the original peptide in inhibiting T-cell responses to protein antigens presented by the target MHC molecule. Although the above approaches using peptidomimetics as selective MHC-antagonists are promising, there has been little progress noted in the literature regarding their clinical application.

TCR peptides

In the 1990s, when it was recognised that there was a subclass of arthritogenic T cells with preferential Vβ usage causing disease, patients were immunized with attenuated activated autoreactive T cells in order to suppress disease and provide protection [20,21]. This approach failed and instead of whole T-cell immunization, TCR peptides were used. One such example was the Vβ17 TCR peptide used for immunization of RA patients. In a Phase I, open label study this approach was found to be safe and feasible with a decrease in joint score [22]. A double-blind, placebo-controlled, multicenter, Phase II trial was subsequently reported using the peptide vaccine (IR501), which consisted of a combination of three peptides derived from the TCR (Vβ3, -β14 and -β17) regions. IR501 was found to be well-tolerated, immunogenic and produced clinical improvement in RA patients [22–24]. At present, investigational new drug applications with the US FDA are currently under consideration for the above TCR peptide-based therapeutic vaccines (RaVax™, The Immune Response Corporation, Carlsbad, CA, USA) [101].

HLA peptides

Peptide-based strategies of immunosuppression have also focussed on the HLA locus. As discussed above, predisposition to RA has been linked to

Box 1. Advantages and disadvantages of therapeutic peptides.

Advantages

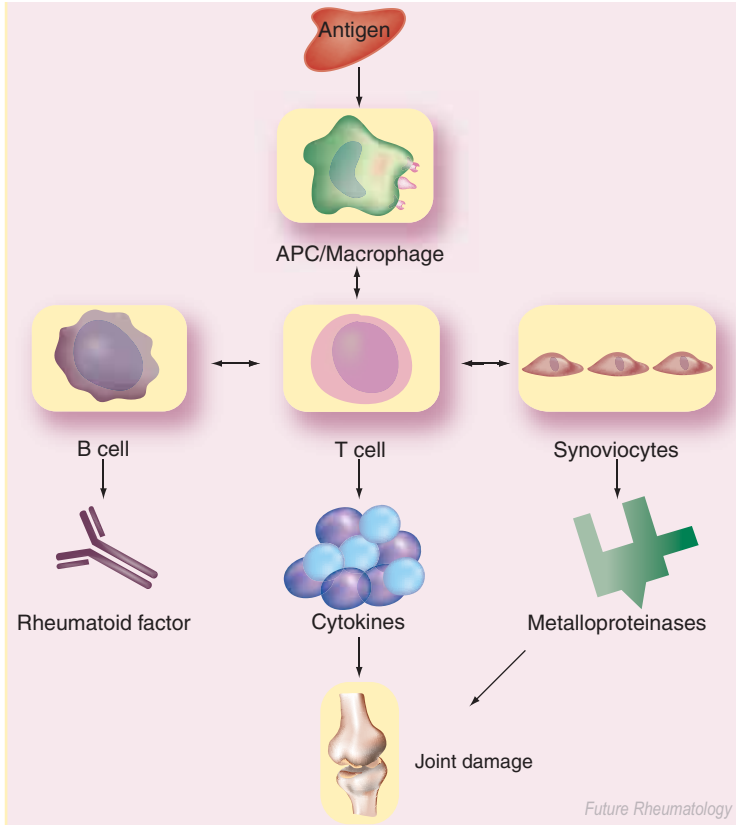
- Broad range of potential clinical benefits:
  - Antibiotic/antifungal diseases
  - Viral indications
  - Immune system disorders
  - Cardiovascular disease
  - Neurological disorders
  - Cancer
- High activity/specificity
- No accummulation in organs
- Low toxicity
- Less immunogenic than antibodies
- Less drug–drug interaction
- Less accumulation in tissue
- Biological and chemical diversity

Disadvantages

- Oral delivery not effective due to stomach enzymes
- Nasal/pulmonary delivery requires high dosage
- Low metabolic stability i.e., short half-life
- Cost of manufacturing
- Injection required
- Solubility challenges
- Delivery challenges
- Clears from the body quickly

Taken from [10,68].

Figure 1. Etiopathogenesis of RA.



APC: Antigen-presenting cell; RA: Rheumatoid arthritis.

particular MHC class II alleles, especially at the HLA-DRB1 locus. The shared epitope hypothesis predicts that similar motifs within the third hypervariable (HV3) region of HLA-DRB1 alleles are responsible for the class II-associated predisposition to RA. Using a line of transgenic mice expressing the *DQB1*0302/DQA1*0301* (*DQ8*) genes in the absence of endogenous mouse class II molecules, Zanelli and colleagues have analyzed the antigenicity of peptides covering the HV3 regions of RA-associated and nonassociated DRB1 molecules [25]. Their studies found that a correlation exists between the proliferative response to peptides derived from the HV3 regions of DRB1 chains and nonassociation of the corresponding alleles with RA predisposition. While HV3 peptides derived from nonassociated DRB1 molecules are highly immunogenic in *DQ8* transgenic mice, all the HV3 peptides derived from RA-associated DRB1 alleles fail to induce a *DQ8*-restricted T-cell response. That is, a peptide derived from the HV3 region (HV3 65–79) of the HLA-DR allele, *DRB1*0402*, binds to DQ8 with high affinity and has protective properties on collagen-induced arthritis in HLA-DQ8 mice. This implies that [26],

"the use of self MHC peptides that can induce the production of regulatory cells, offers a unique approach towards the development of potential therapeutic modalities for the treatment of human RA" .

Peptide analogs of epitopes critical for normal T-cell activation derived from various regions of HLA class I and II molecules have demonstrated immunomodulatory effects both *in vitro* and *in vivo*. Although reviewed in the context of transplantation this approach also has relevance to autoimmune diseases.

Heat shock proteins (chaperonins)

This is an exciting new area of development that is gaining momentum since the publication by Vanag and colleagues demonstrating that chaperonin 10 has antiarthritic activity in humans [27]. It has long been suspected that heat shock proteins (hsp's) may be the autoantigens causing RA and that immunization with these proteins inhibits arthritis [28–30].

Chaperonins are mitochondral proteins involved in intracellular protein folding. In addition to acting as scaffold molecules, hsp10 and hsp60 are linked to a number of extracellular immunomodulatory and anti-inflammatory activities mediated via cell-surface receptors. Johnson and colleagues demonstrated that purified recombinant human hsp10 incubated with cells *in vitro* reduced lipopolysaccharide-induced nuclear factor (NF)κB activation and secretion of several inflammatory mediators (e.g., tumor necrosis factor [TNF]-α and interleukin [IL]-6) from murine macrophages and human peripheral blood mononuclear cells [31]. The current paradigm is that once these proteins are released from the cell, such as might occur following cell damage due to inflammation, they have a direct effect on macrophages to cause release of IL-10; and, concurrently act on surrounding T cells via Toll-like receptor-4 to inhibit NFκB, thereby inhibiting the production of TNF-α and IL-6 [31]. Therapeutic efficacy and safety of using chaperonin 10 in patients with RA has been demonstrated by Vanags and colleagues in a double-blind, randomized trial [27].

The stress protein and endoplasmic reticulum chaperone immunoglobulin-binding protein (BiP) is a member of the hsp70 family and an

autoantigen in RA. Brownlie and colleagues had reported that intravenous or subcutaneous administration of a single dose of BiP at the onset of collagen-induced arthritis in DBA/1 mice leads to resolution of disease [32]. Studies by Corrigall and colleagues showed that there was free BiP present in the synovial fluid of RA patients [33]. Peripheral blood monocytes responded to BiP with secretion of anti-inflammatory cytokines including, IL-10 and IL1-RA. The presence of extracellular BiP may stimulate immunomodulatory and anti-inflammatory pathways and have relevance in the future treatment of RA.

While human studies are progressing, there are complementary animal studies pushing the field closer towards a vaccine. Quintana and colleagues demonstrated that DNA vaccination with the human *hsp60* gene can inhibit adjuvant arthritis [29]. In a later study they searched for regulatory epitopes using DNA vaccination with *hsp60* gene fragments [34]. They found that specific hsp60 DNA fragments can serve as effective vaccines. Using overlapping hsp60 peptides, they identified a regulatory peptide (Hu3) that was specifically recognized by the T cells of DNA-vaccinated rats. Vaccination with Hu3, or transfer of splenocytes from Hu3-vaccinated rats, inhibited the development of adjuvant arthritis. Vaccination with the mycobacterial homolog of Hu3 had no effect. Effective DNA or peptide vaccination was associated with enhanced T-cell proliferation to a variety of disease-associated antigens, along with a T helper (Th)2/3-like shift (downregulation of interferon [IFN]-γ secretion and enhanced secretion of IL-10 and/or tumor growth factor [TGF]β1) in response to peptide Mt176–190 (the 180–188 epitope of hsp65). The regulatory response to hsp60 or its Hu3 epitope included both Th1 (IFN-γ) and Th2/3 (IL-10/TGFβ1) secretors. These results demonstrate that regulatory mechanisms can be activated by immunization with relevant self-hsp60 epitopes.

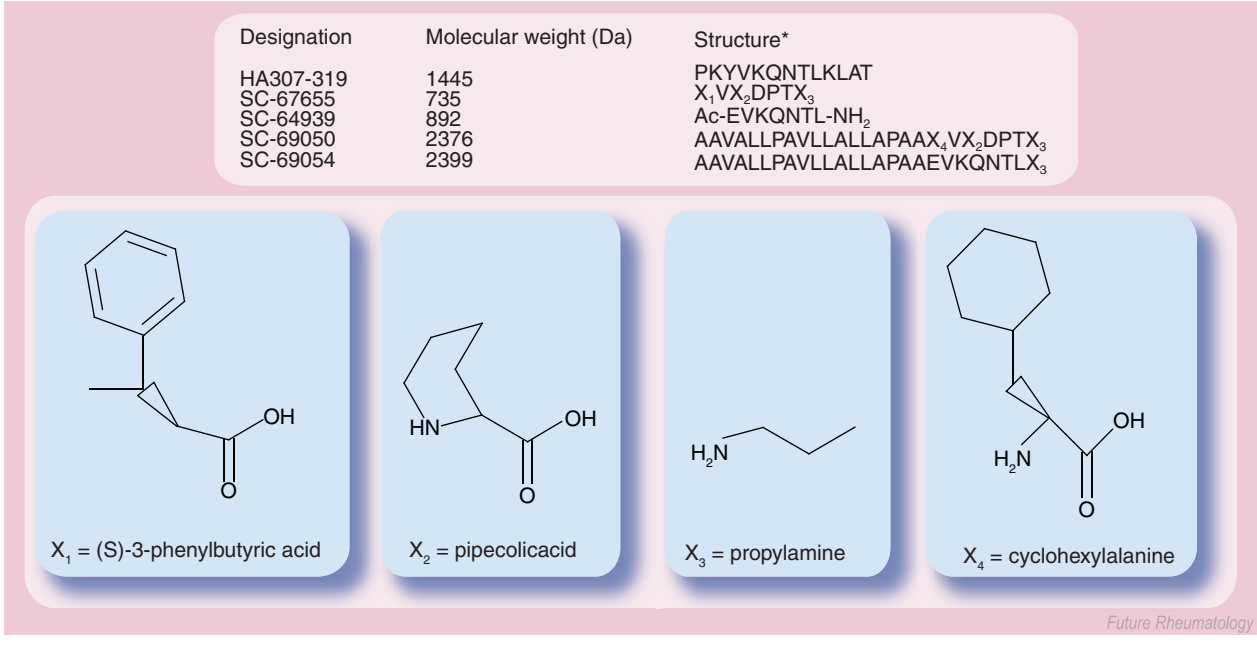
Other peptide immunomodulators

Cytokine peptides

Efforts have begun to identify peptides that retain either the active or passive vaccinating properties of the native proteins involved in the immunoregulation of inflammation. Such peptides are derived from the active segments of IL-1β [35], IL-1R [36], IFN-inducible protein 10 [37], IL-2Rβ [38] and TNF-α.

Figure 2. Composition of peptide-based inhibitors.

Designation	Molecular weight (Da)	Structure*
HA307-319	1445	PKYVKQNTLKLAT
SC-67655	735	$X_1VX_2DPTX_3$
SC-64939	892	Ac-EVKQNTL-NH_2
SC-69050	2376	$AAVALLPAVLLALLAPAAX_4VX_2DPTX_3$
SC-69054	2399	$AAVALLPAVLLALLAPAAEVKQNTLX_3$



*Expressed using single-letter amino acid code.
Ac: Acetyl.
Reproduced from [18].

Cytokine modulators

Cyclosporine and FK506 are both potent inhibitors of T-cell activation and cytokine gene regulation. This mode of action is attributed to inhibition of calcineurin, which, in turn, effects a number of transcriptional regulators referred to as nuclear factor of activated T cells (NFAT) [39]. Recently, an NFAT-inhibitor peptide, VIVIT, has been developed that interferes selectively with calcineurin–NFAT interactions, making it a useful therapeutic agent [40]. To date, it has not been applied to arthritis.

HIV glycoprotein-41 fusion peptide

The fusion peptide in the N terminus of the HIV envelope glycoprotein (gp)41, together with other gp41 domains, is essential for viral fusion with the host cell membrane. In the study by Quintana and colleagues, they reported that this fusion peptide colocalizes with CD4 and TCR molecules and inhibits antigen-specific T-cell proliferation and pro-inflammatory cytokine production [41]. When this peptide was tested in an autoimmune disease model of adjuvant arthritis it inhibited the activation of arthritogenic T cells and reduced the disease-associated IFN-γ response.

Measles-derived peptides

Another interesting source of antiarthritic peptide is the measles virus. Patients with Still's disease have been reported to experience long-term remission of symptoms and sometimes outright cures following measles infections [42,43]. Two peptides with significant homologies to peptides that are recognized by synovial fluid antibodies to RA have been found and are currently under investigation. These peptides are discussed in a recent review by Root-Bernstein and colleagues and are under investigation [44].

Peptide carriers for protein transduction

Several proteins and small peptides have the ability to travel through biological membranes independent of classical receptor- or endocytosis-mediated pathways. Sequence alignments of these protein segments, termed post-transduction domains (PTDs), show a high basic amino acid content (Lys and Arg) that may facilitate interaction of these regions with negatively-charged lipids in the membrane. The first protein reported with transductional properties was the HIV transactivator protein TAT [45,46] in which the 11-amino acid protein transduction domain was identified by virtue of its cationic content [47]. Cross-linking of the TAT PTD to either β-galactosidase or horseradish peroxidase was able to produce efficient internalization of the marker proteins into cells. Since the original discovery, others have demonstrated that PTDs are able to confer efficient protein uptake to a wide variety of cell types, including synovial cells, and can be fused to other macromolecules, peptides or proteins to successfully transport these molecules into a cell [48]. The significance of these peptides becomes apparent when one considers that they can be used to transfer therapeutic proteins and/or DNA for the treatment of a wide range of diseases, including arthritis.

Mi and colleagues have identified a novel specific synovial-targeted transduction peptide, Huntingtin-associated protein (HAP)-1, which is able to facilitate internalization of large protein marker complexes into synovial cells in culture and *in vivo* in a specific manner [49]. Attaching a proapoptotic peptide to HAP-1 induced extensive apoptosis when injected intra-articularly in arthritic rabbit joints with hyperplastic synovium, while reducing the leukocytic infiltration and synovitis.

We evaluated the ability of core peptide (CP; H-GLRILLLKV-OH) and its conjugate, lipophilic peptide (LP; 2-aminododecanoic acid) to enter cells and influence oligodeoxynucleotide (ODN) delivery in human retinal pigment epithelial cells. The results revealed good transfection efficiency for both CP and LP compared with currently available transfection agents. These results suggest that CP, and particularly its lipophilic analog LP, similarly to the other PTDs, may have a role as a delivery agent [50].

Peptides to costimulatory/adhesion molecules

Another exciting approach for intervening in human inflammatory disorders is the development of peptides for blocking the interactions between costimulatory molecules and/or adhesion molecules. Linear and cyclic peptides derived from the sequences of lymphocyte function-associated antigen [LFA]-1, intracellular adhesion molecule-1 and -2, vascular cell-adhesion molecule-1 and fibronectin, have been demonstrated to have inhibitory effects *in vitro* and *in vivo*. Yusuf-Makagiansar and colleagues have reviewed the structural basis and clinical relevance of LFA-1 and α4β1 integrin (VLA-4) inhibition by peptides and

small molecules as adhesion-based therapeutic strategies for inflammation and autoimmune diseases [51]. For further information on adhesion molecules as targets for therapeutic interventions the reader is directed to the additional review articles [51–54].

Transmembrane peptides

We have demonstrated that peptides with sequences derived from the transmembrane segments of the TCR, are capable of inhibiting the immune response both *in vitro* and in animal models of T-cell mediated inflammation [55,56]. In the *in vivo* animal models tested, inhibition of inflammation was noted in adjuvant-induced arthritis, contact dermatitis, experimental allergic encephalomyelitis and non-obese diabetic mouse models [57]. Although the exact molecular mechanism of action is still not clear, we hypothesize that these peptides become incorporated into the receptor leading to loss of TCR function, either by steric hindrance of the TCR-chains or effecting the assembly/oligomerization process involving coaccessory molecules in the immune synapse necessary for signaling.

Gollner and colleagues have examined these peptides in rodents and humans and noted that, in the latter, although the numbers were small and uncontrolled, it was effective in the treatment of several skin conditions, including psoriasis [58]. The substitution of amino acids with D-forms may, in the future, allow the use of these compounds as oral agents. Gerber and colleagues demonstrated that there was no loss of activity of D-enantiomer peptide of the TCR-α transmembrane domain to inhibit T-cell activation *in vitro* and *in vivo* [59]. They found that both L-CP and D-CP colocalize with TCR in the membrane and inhibited T-cell activation *in vitro* making this possibility a reality.

Other delivery systems may increase the scope of application of these peptides [58]. Mahnke and colleagues have transfected dendritic cells with an adenovirus-expressing CP and demonstrated that transfected dendritic cells injected back into the animal were able to induce antigen-specific immunosuppression [60]. In a CD8-driven allergy model, the injection of dendritic cells transduced with TCR peptides markedly reduced inflammation, and in a $CD4^+$ T-cell dependent model of MS, injection of TCR peptide-secreting dendritic cells abrogated symptoms and prolonged survival. This suggests that the expressed peptides are stable and remain intact and do not undergo problems with folding. The possibility of transfecting other different cells remains a therapeutic challenge, for example, human β-islet-cells for the treatment of juvenile diabetes. Gene therapy is another biological strategy for treatment of arthritis that emerged in the early 1990s and is reviewed elsewhere [61–63].

Transmembrane peptides are useful new agents for *in vivo* immune suppression [60] and their current application is directed to conditions such as transplantation, RA, MS, pernicious, anemia, thyroid disease, diabetes and allergy. However, the receptor from which these peptides were derived is an autoimmune receptor and the principles used to derive these peptides can easily be applied to any other multisubunit receptor that has transmembrane charges or site-specific points of protein–protein interactions. The ability of peptides to interfere with these interactions may lead to the receptors loss of function. This type of approach may have wider applications to include infectious agents, for example, HIV, cancers and many other critical receptors, which depend on transmembrane protein–protein charge interactions or specific site of protein–protein interactions.

Future perspective

There has been increasing appreciation and application of peptides as therapeutic agents in the last decade [44,64–66]. The low cost of production relative to recombinant techniques required for mAbs, the high specificity offered and wide range of applications makes this group of peptides very attractive for clinical use. Peptide-based therapeutic strategies are increasing and will continue to increase as the clinical need grows to find a cure for a large number of devastating and crippling medical diseases. With novel applications and new manufacturing techniques to lower the cost of production and resolve pharmacological issues, such as stability, half-life and delivery, the use of these compounds will become front runners for future medicines.

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Executive summary

- There is an increasing interest in peptide therapies and peptide-based therapeutic approaches in clinical medicine.
- A number of peptide and peptidomimetic-based approaches for the treatment of inflammatory arthritis are currently under investigation and are at different stages of development.
- The T cell, and in particular the T-cell receptor (TCR), remains a point of focus in targeting new therapies to effect the immune response.
- There are a variety of human leukocyte antigen peptidomimetics and inhibitors under investigation.
- TCR-peptide vaccines are being considered for therapeutic application by the US FDA.
- Peptides derived from heat shock protein are emerging as a new form of therapy for rheumatoid arthritis.
- New targets using peptides as therapeutic agents are continuously emerging.
- Peptides designed to interfere with protein–protein interactions have been shown to be feasible therapeutic agents with widespread clinical applications.

Bibliography

1. Elliott MJ, Maini RN, Feldmann M *et al.*: Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor-α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 344(8930), 1105–1110 (1994).
2. McClesky EW, Fox AP, Feldman DH *et al.*: ω-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc. Natl Acad. Sci. USA* 84, 4327–4331 (1987).
3. Proksch P, Edrada R, Ebel R: Drugs from the seas – current status and microbiological implications. *Appl. Microbiol. Biotechnol.* 59, 125–134 (2002).
4. Dhib-Jalbut S: Glatiramer acetate (Copaxone 1) therapy for multiple sclerosis. *Pharmacol. Ther.* 98, 245–255 (2003).
5. Borghardt J, Rosien B, Gortelmeyer R, Lindemann S, Hartleb M, Klingmuller M: Effects of a spleen peptide preparation as supportive therapy inoperable head and neck cancer patients. *Arzneimittelforschung* 50, 178–184 (2000).
6. Shield CFI, McGrath MM, Goss TF: Assessment of health-related quality of life in kidney transplant patients receiving tacrolimus (FK506)-based versus cyclosporine-based immunosuppression. *Transplantation* 64, 1738–1743 (1997).
7. Mutchnick MG, Lindsay KL, Schiff ER *et al.*: Thymosin α1 treatment of chronic hepatitis B: results of a Phase III multicentre, randomized, double-blind and placebo-controlled study. *J. Viral Hepat.* 6, 397–403 (1999).
8. Sehgal A: Recent developments in peptide-based cancer therapeutics. *Curr. Opin. Drug Discov. Dev.* 5, 245–250 (2002).
9. Wang H-L, Kurtz A: Breast cancer growth inhibition by delivery of the MDGI-derived peptide P108 space. *Oncogene* 19, 2455–2460 (2000).
10. Latham PW: Therapeutic peptides revisited. *Nat. Biotechnol.* 17, 755–757 (1999).
11. Trentham DE, Dynesius-Trentham RA, Orav EJ *et al.*: Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 261(5129), 1727–1730 (1993).
12. Barnett ML, Combitchi D, Trentham DE: A pilot trial of oral type II collagen in the treatment of juvenile rheumatoid arthritis. *Arthritis Rheum.* 39(4), 623–628 (1996).
13. Cazzola M, Antivalle M, Sarzi-Puttini P *et al.*: Oral type II collagen in the treatment of rheumatoid arthritis. A six-month double blind placebo-controlled study. *Clin. Exp. Rheumatol.* 18(5), 571–577 (2000).
14. Spack EG: Treatment of autoimmune diseases through manipulation of antigen presentation. *Crit. Rev. Immunol.* 17, 529–536 (1997).
15. Dzhambazov B, Nandakumar KS, Kihlberg J *et al.*: Therapeutic vaccination of active arthritis with a glycosylated collagen type II peptide in complex with MHC class II molecules. *J. Immunol.* 176, 1525–1533 (2006).
16. Munari L, Lovati R, Boiko A: Therapy with glatiramer acetate for multiple sclerosis (Cochrane Review). *Cochrane Database Syst. Rev.* 4, CD004678 (2003).
17. Munari LM, Filippini G: Lack of evidence for use of glatiramer acetate in multiple sclerosis. *Lancet Neurol.* 3, 641 (2004).
18. Woulfe SL, Bono CP, Zacheis ML *et al.*: A peptidomimetic that specifically inhibits human leukocyte antigen *DRB1*0401*-restricted T-cell proliferation. *J. Pharmacol. Exp. Therap.* 281(2), 663–669 (1997).
19. Falcioni F, Ito K, Vidovic D *et al.*: Peptidomimetic compounds that inhibit antigen presentation by autoimmune disease-associated class II major histocompatibility molecules. *Nat. Biotechnol.* 17, 562–567 (1999).
20. Panayi GS: The immunopathogenesis of rheumatoid arthritis. *Rheumatol. Rev.* 1, 63–74 (1992).
21. Stinissen P, Medear R, Raus J: Myelin reactive T-cells in the autoimmune pathogenesis of multiple sclerosis. *Mult. Scler.* 4, 203–211 (1998).
22. Moreland LW, Heck LWJ, Koopman WJ *et al.*: Vβ 17 T cell receptor peptide vaccination in rheumatoid arthritis: results of Phase I dose escalation study. *J. Rheumatol.* 23(8), 1353–1362 (1996).
23. Moreland LW, Morgan EE, Adamson TC *et al.*: T cell receptor peptide vaccination in rheumatoid arthritis. *Arthritis Rheum.* 41(11), 1919–1929 (1998).
24. Bridges SLJ, Moreland LW: T-cell receptor peptide vaccination in the treatment of rheumatoid arthritis. *Rheum. Dis. Clin. North Am.* 24(3), 641–650 (1998).
25. Zanelli E, Krco CJ, Baisch JM, Cheng S, David CS: Immune response of HLA-DQ8 transgenic mice to peptides from the third hypervariable region of HLA-DRB1 correlates with predisposition to rheumatoid arthritis. *Proc. Natl Acad. Sci. USA* 93, 1814–1819 (1996).
26. Das P, Bradley DS, Geluk A, Griffiths MM, Luthra HS, David CS: An HLA-*DRB1*0402* derived peptide (HV3 65-79) prevents collagen-induced arthritis in HLA-DQ8 transgenic mice. *Human Immunol.* 60, 575–582 (1999).

27. Vanags D, Williams B, Johnson B *et al.*: Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. (Clinical report). *Lancet* 368(9538), 855–863 (2006).
28. Anderton SM, can der Zee R, Prakken B, Noordzij A, van Eden W: Activation of T-cells recognizing self 60-kD shock protein can protect against experimental arthritis. *J. Exp. Med.* 181, 943–952 (1995).
29. Quintana FJ, Carmi P, Mor F, Cohen IR: Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J. Immunol.* 169, 3422 (2002).
30. Ulmansky R, Cohen CJ, Szafer F *et al.*: Resistance to adjuvant arthritis is due to protective antibodies against heat shock protein surface epitopes and the induction of IL-10 secretion. *J. Immunol.* 168, 6463–6469 (2002).
31. Johnson BJ, Le TTT, Dobbin CA *et al.*: Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. *J. Biol. Chem.* 280(6), 4037–4047 (2005).
32. Brownlie R, Sattar Z, Corrigall VM, Bodman-Smith MD, Panayi GS, Thompson S: Immunotherapy of collagen-induced arthritis with BiP. *Rheumatology* 42(Suppl. 1), S13 (2003).
33. Corrigall VM, Bodman-Smith MD, Brunst M, Cornell H, Panayi GS: Inhibition of antigen-presenting cell function and stimulation of human peripheral blood mononuclear cells to express an anti-inflammatory cytokine profile by the stress protein BiP: relevance to the treatment of inflammatory arthritis. *Arthritis Rheum.* 50(4), 1164–1171 (2004).
34. Quintana FJ, Carmi P, Mor F, Cohen IR: DNA fragments of the human 60-kDa heat shock protein (hsp60) vaccinate against adjuvant arthritis: identification of a regulatory hsp60 peptide. *J. Immunol.* 171, 3533–3541 (2003).
35. Bertin-Maghit SM, Capini CJ, Bessis N *et al.*: Improvement of collagen-induced arthritis by active immunization against murine IL-1β peptides designed by molecular modelling. *Vaccine* 23, 4228–4235 (2005).
36. Jouvenne P, Fossiez F, Banchereau J, Miossec P: High levels of neutralizing autoantibodies against IL-1α are associated with a better prognosis in chronic polyarthritis: a follow-up study. *Scand. J. Immunol.* 46, 413–418 (1997).
37. Salomon I, Netzer N, Wildbaum G, Schif-Zuck S, Maor G, Karin N: Targeting the function of IFN-γ-inducible protein 10 suppresses ongoing adjuvant arthritis. *J. Immunol.* 169(5), 2685–2693 (2002).
38. Parissi T, Chatzantoni K, Deraos S, Matsoukas I, Mouzaki A: Synthetic peptides mapping to epitopes of the extracellular domain of the interleukin-2 receptor β-chain to inhibit T-cell activation. *Transplan. Proc.* 36, 1723–1727 (2004).
39. Rao A, Luo C, Hogan PG: Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* 15, 707–747 (1997).
40. Noguchi H, Matsushita M, Okitsu T *et al.*: A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. *Nat. Med.* 10, 305–309 (2004).
41. Quintana FJ, Gerber D, Kent SC, Cohen IR, Shai Y: HIV-1 fusion peptide targets the TCR and inhibits antigen-specific T cell activation. *J. Clin. Investig.* 115(8), 2149–2158 (2005).
42. Simpanen E, van Essen R, Isomaki H: Remission of juvenile rheumatoid arthritis (Still's disease) after measles. *Lancet* 2, 987–988 (1977).
43. Urbach J, Schurr D, Abramov A: Prolonged remission of juvenile rheumatoid a rthritis (Still's disease) following measles. *Acta Paediatr. Scand.* 72(6), 917–918 (1983).
44. Root-Bernstein R: Peptide vaccines against arthritis. *Future Rheumatol.* 1(3), 339–344 (2006).
45. Green M, Loewenstein PM: Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. *Cell* 55, 1179–1188 (1988).
46. Frankel AD, Pabo CO: Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* 55, 1189–1193 (1988).
47. Fawell S, Seery J, Daikh Y *et al.*: Tat-mediated delivery of heterologous proteins into cells. *Proc. Natl Acad. Sci. USA* 91, 664–668 (1994).
48. Mi Z, Mai J, Lu X, Robbins PD: Characterization of a class of cationic peptides able to facilitate efficient protein transduction *in vitro* and *in vivo*. *Mol. Ther.* 2(4), 339–347 (2000).
49. Mi Z, Lu X, Mai J *et al.*: Identification of a synovial fibroblast-specific protein transduction domain for delivery of apoptotic agents to hyperplastic synovium. *Mol. Ther.* 8(2), 295–305 (2003).
50. Chan YEN, Amon M, Marano RJ *et al.*: Novel cationic lipophilic peptides for oligodeoxynucleotides delivery. *Bioorg. Med. Chem.* doi:10.1016/j.bmc.2007.03.078 (2007) (Epub ahead of print).
51. Yusuf-Makagiansar H, Anderson ME, Yakovleva TV, Murray JS, Siahaan TJ: Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. *Med. Res. Rev.* 22(2), 146–167 (2002).
52. Hilden TJ, Nurmi SM, Fagerholm SC, Gahmberg CG: Interfering with leukocyte integrin activation – a novel concept in the development of anti-inflammatory drugs. *Ann. Med.* 38(7), 503–511 (2006).
53. Ulbrich H, Eriksson EE, Lindbom L: Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacolog. Sci.* 24(12), 640–647 (2003).
54. Delgado M, Abad C, Martinez C *et al.*: PACAP in immunity and inflammation. *Ann. NY Acad. Sci.* 992, 141–157 (2003).
55. Manolios N, Bonifacino JS, Klausner RD: Transmembrane helical interactions and the assembly of the T-cell receptor complex. *Science* 249, 274–277 (1990).
56. Manolios N, Letourneur F, Bonifacino JS, Klausner RD: Pairwise, cooperative and inhibitory interactions describe the assembly and probable structure of the T cell antigen receptor. *EMBO J.* 10(7), 1643–1651 (1991).
57. Manolios N, Collier S, Taylor J, Pollard J, Harrison L, Bender V: T-cell antigen receptor transmembrane peptides modulate T-cell function and T-cell mediated disease. *Nat. Med.* 3, 84–88 (1997).
58. Gollner GP, Muller G, Alt R, Knop J, Enk AH: Therapeutic application of T cell receptor mimic peptides or cDNA in the treatment of T-cell mediated skin diseases. *Gene Ther.* 7, 1000–1004 (2000).
59. Gerber D, Quintana FJ, Bloch I, Cohen IR, Shai Y: D-enantiomer peptide of the TCRα transmembrane domain inhibits T-cell activation *in vitro* and *in vivo*. *FASEB J.* 19(9), 1190–1192 (2005).
60. Mahnke K, Qian Y, Knop J, Enk AH: Dendritic cells, engineered to secrete a T-cell receptor mimic peptide, induce antigen-specific immunosuppression *in vivo*. *Nature Biotech.* 21, 903–908 (2003).

61. Evans CH, Ghivizzani SC, Kang R *et al.*: Gene therapy for rheumatic diseases. *Arthritis Rheum.* 42(1), 1–16 (1999).
62. Evans CH, Ghivizzani SC, Robbins PD: Gene therapy for arthritis. What next? *Arthritis Rheum.* 54(6), 1714–1729 (2006).
63. Robbins PD, Evans CH, Chernajovsky Y: Gene therapy for arthritis. *Gene Ther.* 10, 902–911 (2003).
64. Hauff K, Zamzow C, Law WJ *et al.*: Peptide-based approaches to treat asthma, arthritis, other autoimmune diseases and pathologies of the central nervous system. *Arch. Immunol. Ther. Exp.* 53, 308–320 (2005).
65. Mouzaki A, Deraos S, Chatzantoni K: Advances in the treatment of autoimmune diseases; cellular activity, type-1/type-2 cytokine secretion patterns and their modulation by therapeutic peptides. *Curr. Med. Chem.* 12, 1537–1550 (2005).
66. Savage C, St Clair EW: New therapeutics in rheumatoid arthritis. *Rheum. Dis. Clin. N. Am.* 32, 57–74 (2006).
67. Köller MD: Targeted therapy in rheumatoid arthritis. *Wien Med. Wochenschr.* 156(1–2), 53–60 (2006).
68. Marx V: Watching peptide drugs grow up. C & EN northeast news bureau. *Business* 83(11), 17–24 (2005).

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Affiliations

- *Marina Ali, PhD, Henry Langley Research Fellow*
University of Sydney, Department of Rheumatology, Sydney, Australia
Tel.: +61 29 845 8099;
Fax: +61 29 845 8317
- *Nicholas Manolios, MBBS, PhD, MD, FRCPA, FRACP, Director of Rheumatology and Associate Professor of Rheumatology*
University of Sydney, Rheumatology Dept, Westmead Hospital, Westmead 2145, Sydney, Australia
Tel.: +61 29 845 8099;
Fax: +61 29 845 8317;
nickm@westgate.wh.usyd.edu.au