ELSEVIER

Immunosuppressive peptides and their therapeutic applications

Kathrin Thell[1], Roland Hellinger[1], Gernot Schabbauer and Christian W. Gruber

Center for Physiology and Pharmacology, Medical University of Vienna, Schwarzspanierstr. 17, A-1090 Vienna, Austria

The immune system is vital for detecting and evading endogenous and exogenous threats to the body. Failure to regulate this homeostasis leads to autoimmunity, which is often associated with malfunctioning T cell signaling. Several medications are available to suppress over-reactive T lymphocytes, but many of the currently marketed drugs produce severe and life-threatening side-effects. Ribosomally synthesized peptides are gaining recognition from the pharmaceutical industry for their enhanced selectivity and decreased toxicity compared with small molecules; in particular, circular peptides exhibit remarkable stability and increased oral administration properties. For example, plant cyclotides effectively inhibit T lymphocyte proliferation. They are composed of a head-to-tail cyclized backbone and a cystine-knot motif, which confers them with remarkable stability, thus making them attractive pharmaceutical tools.

Introduction

The immune system is responsible for detecting and eliminating foreign pathogens and tumor cells, but avoiding self-recognition. Therefore, tolerance mechanisms are continuously under surveillance to retain this homeostasis. If these immunological tools are over-reactive it can lead to autoimmunity, targeting healthy host cells and organs as well as exogenous and endogenous threats. About 5% of the population in Western countries develops an autoimmune disease during life, and these numbers are constantly increasing [1]. The causes of autoimmunity are still ill-defined, but it is known that there are many parameters involved, such as gender, genetic background, environmental factors and, importantly, malfunctioning lymphocyte development [2]. However, autoimmunity is not only a congenital disease; following an infection, certain bacterial proteins can elicit an 'unwanted' immune response. Conversely, there is a link between the decreased appearance of bacterial and parasitic infectious diseases and an increase of allergic reactions, supported by the so-called hygiene hypothesis [3]. Autoimmune diseases can be broadly classified as organ-specific (e.g. multiple sclerosis) or as systemic (e.g. systemic lupus erythematosus). On a molecular level, autoreactive T cells play an important part in disease development and progression [4], and therefore it is a fundamental necessity to take T cell associated pathways into consideration, especially interleukin 2 (IL2) signaling. Gene expression of this T cell growth factor cytokine is induced by the transcription factors NFAT, NFκB or AP-1. IL2 then acts in an autocrine fashion on its own high-affinity cell surface receptor (IL2R) to promote cell proliferation, cell growth and/or inhibition of apoptosis *inter alia* via mTOR [5,6] (for immunological glossary of terms, see Box 1).

Immunosuppressive pharmaceuticals

Various signaling pathways and associated canonical messenger molecules, such as IL2 signaling molecules, offer targets for drug discovery for the treatment of an over-reactive immune response as is the case in autoimmune diseases, allergic reactions and following organ transplantation. Many immunosuppressive agents have already been used in the clinic, demonstrating efficacy while displaying different modes-of-action. Some of these pharmaceuticals target specific kinases and phosphatases, interfere with gene expression, modify DNA via alkylation or inhibit purine or pyrimidine synthesis to block the cell cycle [7].

Corresponding author:. Gruber, C.W. (christian.w.gruber@meduniwien.ac.at)
[1] These authors contributed equally.

1359-6446/06/$ - see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved. http://dx.doi.org/10.1016/j.drudis.2013.12.002

Please cite this article in press as: Thell, K. et al., Immunosuppressive peptides and their therapeutic applications, Drug Discov Today (2013), http://dx.doi.org/10.1016/j.drudis.2013.12.002

BOX 1

Immunological glossary

Abbreviation	Term and/or explanation
CD	Cluster of differentiation
CRAC	Ca^{2+}-release-activated channels
IKCa1	Intermediate conductance calcium-activated potassium channel protein 4 (KCNN4)
$K_v1.3$	Potassium voltage-gated channel, shaker-related subfamily, member 3 (KCNA3)
IP_3	Inositol-1,4,5-triphosphate
IP_3R	Intramolecular inositol-1,4,5-triphosphate Ca^{2+}-release channel
NFAT	Nuclear factor of activated T cells
NFκB	Nuclear factor 'κ-light-chain-enhancer' of activated B cells
AP-1	Activator protein 1 (dimerization of Fos and Jun)
IL2	Interleukin-2
IL2R	High affinity $CD25^+$ interleukin-2 receptor
mTOR	Mammalian target of rapamycin
IFNγ	Interferon γ
TGFβ	Transforming growth factor β

The most commonly used immunosuppressive drugs and historically one of the oldest are the glucocorticoids. They restrict and inhibit the synthesis and secretion of inflammatory cytokines and support the activation of anti-inflammatory signaling cascades. The best-known member of this class of compounds is cortisone, which interacts with and inhibits, for example, NFκB-dependent signaling, resulting in reduced expression of inflammatory cytokines such as tumor necrosis factor (TNF)α or IL1. Besides, glucocorticoid treatment affects many non-immune related signaling pathways; it interferes in gluconeogenesis, lipolysis, protein catabolism and sodium retention. Consequently, the list of side-effects is long, ranging from Cushing's syndrome, hyperglycemia, myopathia, skin atrophy, osteoporosis, hypertonia, weight gain and immunodeficiency [8].

Vitamin D, a fat-soluble secosteroid, has recently been successfully implemented in immunotherapy although it is not a classical anti-inflammatory agent. The physiologically active form of vitamin D [i.e. calcitriol (1,25-dihydroxycholecalciferol)] shows immunosuppressive properties. Activated T and B cells express the vitamin D receptor on their surface, which can be used to downregulate IL2 signaling. Supplementation therapy of vitamin D has already demonstrated protective effects in the treatment of multiple sclerosis [9]. Other immunosuppressive agents interfere with the cell cycle. For example, the enzyme inhibitors azathioprine, mycophenolate mofetil and methotrexate are non-peptide drugs affecting proliferation by targeting and blocking purine and pyrimidine synthesis [10].

The application of monoclonal antibodies (mAbs), which exhibit high specificity towards their appropriate protein epitopes and hence provide the opportunity to inhibit a target molecule and its resulting effects selectively, is the subject of a tremendous amount of research and constitutes an important branch in the field of immunotherapies. The first mAb approved by the FDA in 1986 was muromonab (Orthoclone® OKT3) directed against human CD3 [11]. It was withdrawn from the market for therapeutic application as a result of high toxicity in 2008, but it still has importance in nonclinical applications [10,12]. Anti-TNF or -TNFR mAbs (e.g. infliximab, adalimumab or golimumab) are widely used in the treatment of rheumatoid arthritis, Bechterew's disease, psoriasis or inflammatory bowel diseases [13]. Blocking different immunological cell surface molecules, such as CD52 (alemtuzumab) [12], CD25 (basiliximab) [14] and CD20 (rituximab), is important in the treatment of autoimmune diseases [15]. Together, mAbs account for over US$40 billion in pharmaceutical sales, and anti-TNF antibodies or protein-based inhibitors remain among the most important therapies for the treatment of many autoimmune disorders [16]. Besides the great therapeutic success of mAbs, adverse effects similar to immunodeficiencies or the formation of anti-drug antibodies are a frequently occurring phenomena [13,17]. Furthermore, the lack of homogeneous tissue distribution, limited half-life and enormous production costs decrease the attractiveness of this epitope-specific passive immunotherapy. In summary, all commonly used immunosuppressive drugs demonstrated great success in the treatment of autoimmune diseases. However, many of them cause unwanted and severe side-effects in patients and therefore there is a high demand for less-toxic immunosuppressive pharmaceuticals.

Immunosuppressive peptides

Bioactive peptides and, in particular, ribosomally synthesized peptides often show reduction in cytotoxicity as compared with small organic compounds – obviously as a result of their inherent targeted molecular action [18–21]. However, it is important to note that drug toxicity is a factor that is compound dependent and there is no panacea that guides whether one compound will or will not be toxic in humans. In the following section we would like to provide an overview of natural peptides (i.e. non-ribosomally and ribosomally synthesized ones) that have not been released on the market yet, but that show promising immunosuppressive properties (Table 1).

Non-ribosomally synthesized peptides

Utilization of bioactivity screening led to the identification of a plethora of novel and potentially immunosuppressive compounds in micro- and marine-organisms [22]. Few examples of non-ribosomally synthesized peptides interfering in cytokine signaling are cyclosporine A (CsA), sirolimus and tracrolimus. CsA (Fig. 1a), a cyclic peptide of fungal origin (*Tolypocladium inflatum*), is widely used in the treatment of autoimmune diseases and to prevent allograft rejection of a transplanted organ. CsA antagonizes the activity of calcineurin, a calcium-dependent serine–threonine phosphatase, which dephosphorylates and activates the transcription factor NFAT to stimulate expression of IL2. Therefore, the dephosphorylation of NFAT is inhibited and consequently the IL2-dependent T cell proliferation repressed. Besides, CsA interferes additionally with p38 and JNK signaling cascades [10,23]. Owing to multiple target pathways, the therapeutic potential of CsA is limited, in particular during long-term treatment, because it has several side-effects, such as hepatotoxicity, nephrotoxicity, neurotoxicity and cytotoxicity [24].

FKBP-12 (FK506-binding protein) functions as a chaperone and belongs, together with cyclophilin, to the family of immunophilins. Tacrolimus (FK506), a macrolide lactone first isolated from *Streptomyces tsukubaensis* interferes with calcineurin-dependent IL2 signaling, by inhibiting FKBP-12 50-fold more potently than

Please cite this article in press as: Thell, K. et al., Immunosuppressive peptides and their therapeutic applications, Drug Discov Today (2013), http://dx.doi.org/10.1016/j.drudis.2013.12.002