

C1 - Peptide and peptidomimetics therapeutics

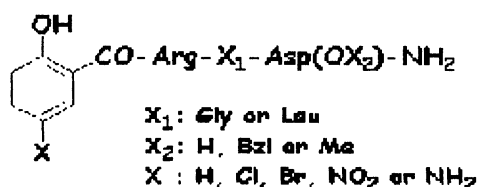
P C29 - Synthetic RGD peptides incorporating salicylic acid derivatives show antiplatelet activity *in vitro*

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Intergrins containing the RGD (Arg-Gly-Asp) tripeptide sequence are found in numerous extracellular matrix proteins such as fibrinogen, fibronectin, osteopontin, etc. Antagonists to intergrin GP IIb/IIIa may be useful as therapeutics in a number of areas including inhibition of platelet aggregation. Many RGD-containing peptides and RGD peptidomimetics have been evaluated as powerful inhibitors of platelet aggregation and thrombus formation *in vitro* and *in vivo*. Furthermore other research groups have revealed the property of RGD containing peptides to inhibit angiogenesis and tumor metastasis.

We have previously reported that the combination in the same molecule of dipeptides amides, containing amino acids of RGD sequence, with salicylic or acetylsalicylic acid moiety at their N-terminal amino group, have shown inhibitory activity on human platelet aggregation. In view of these results we have prepared a series of RGD analogs incorporating salicylic acid derivatives by conventional solution techniques and/or by solid phase peptide synthesis. The synthesized compounds were purified by RP-HPLC (Nucleosil RP-C18 column) and lyophilised to give fluffy solid, identified by FT-IR and ES-MS spectra. These compounds were tested for inhibitory activity on human platelet aggregation *in vitro*, by adding common aggregation reagents (collagen, ADP, thrombin) to citrated platelet rich plasma (PRP). The aggregation was determined using a dual channel electronic aggregometer by recording the increase of light transmission. The IC₅₀ values of the synthesized and tested compounds will be discussed in details.



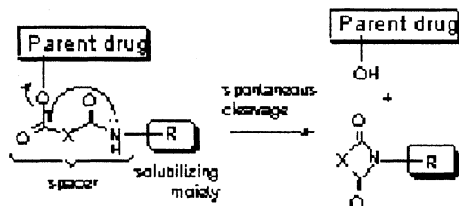
General structure of peptide analogs incorporating salicylic acid derivatives

P C31 - Spontaneously regenerable water-soluble prodrugs: application to HIV protease inhibitors and anti-cancer drug paclitaxel

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The sparing water-solubility of the recently developed HIV-1 protease (PR) inhibitors is one of the major drawbacks in oral administration, causing high pill burden and poor oral bioavailability. In addition, an anti-cancer drug, paclitaxel, which also exhibits sparing water-solubility, is intravenously administered with a detergent to increase its solubility. However, an adverse effect of hypersensitivity to this detergent is known. Hence, new strategies to increase the water-solubility of hydrophobic drugs are required to overcome these problems. In the previous study, we synthesized water-soluble prodrugs of HIV-1 PR inhibitor, KNI-727, in which tandem-linked two auxiliary units, i.e., a self-cleavable spacer and hydrophilic solubilizing moieties, were contained [1]. The biological evaluation of these prodrugs indicated that (1) the introduction of an ammonium ion into the solubilizing moiety was highly effective to increase the water-solubility, (2) the parent drug was released chemically via intramolecular cyclization reaction through the imide formation, and (3) this release was controllable depending on the structure of both spacer and solubilizing moieties, and on the pH of the media. These results suggest that the new water-soluble prodrug strategy is applicable to a variety of sparingly water-soluble drugs. Herein, we report the application of this water-soluble prodrug strategy to a clinically important drug. The water-solubility and drug release time of these prodrugs in physiological condition were determined. Consequently, it was found that synthesized ritonavir prodrugs exhibited more than 10,000 times higher water-solubility than the parent drug. However, the drug release times of these prodrugs were considerably longer than the case of KNI-727 on the whole. The observed difference in the drug release time suggests that the structure of the parent drug is also an important factor affecting the kinetics of cyclization reaction of the auxiliary. On the other hand, the paclitaxel prodrugs exhibited more than 1,000 times higher water-solubility than paclitaxel itself which was regenerated with reasonable $t_{1/2}$ values through chemically triggered intramolecular cyclization reaction. These results suggest that our water-soluble prodrug strategy is one of the versatile systems towards various kinds of sparingly water-soluble drugs, and this system only based on the chemical processing will progressively provide the constant parent drug release rate independent of the processing enzymes with individual difference in the human body.



General structure of water-soluble prodrug, and its intramolecular cyclization-elimination reaction releasing the parent drug and an imide fragment.

Reference

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P C30 - The design and analysis of novel substrate-based peptidomimetic peptidase inhibitors: applications for blood pressure regulation and blood brain barrier permeability.

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We have developed a novel inhibitor of the metalloendopeptidases EC 3.4.24.15 (EP24.15) and EC 3.4.24.16 (EP24.16), N-[1-(R,S)-carboxy-3-phenylpropyl]-Ala-Aib-Tyr-p-aminobenzoate (JA2), in which α -aminoisobutyric acid (Aib) is substituted for an alanine in a well-described but unstable inhibitor, cFP-AAy-pAB. This substitution increases the resistance of the inhibitor to degradation without altering potency.

In the first series of studies, we investigated the effects of JA2 on the responses of mean arterial pressure to bradykinin, angiotensin I and angiotensin II in conscious rabbits. The depressor responses to both low and high doses of bradykinin were increased, during the 30 min after JA2 administration. The hypertensive effects of angiotensins I and II were unaltered, indicating that the bradykinin-potentiating effects were not due to ACE inhibition. Bradykinin potentiation was undiminished 4 hours after JA2 injection. In the second series of experiments we examined the role these enzymes might play in regulating blood brain barrier (BBB) permeability. BBB permeability was assessed using a rat model by determining the leakage of FITC-labelled molecules out of the Pial (brain surface) vasculature via an acute cranial window preparation. Superfusion of the brain surface with bradykinin induced a rapid and reversible increase in leakage of FITC labelled dextrans. Administration of JA2 caused a marked potentiation of this response (which was blocked by pre-administration of a bradykinin B2 receptor antagonist (HOE 140)

Taken together, these data suggest that JA2 is not only a potent and specific inhibitor of EP24.15 and EP24.16, but is also stable *in vivo*. Furthermore, the potentiation of bradykinin-induced hypotension and BBB leakage by JA2 suggests for the first time a role for one or both of these peptidases in the metabolism of bradykinin in the circulation.

P C32 - Synthesis of tetrapeptides analogs of Substance P (SP₆₋₉) and study of their antiproliferative properties *in vitro*

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Synthetic peptide compounds are currently under investigation as possible anti-tumor agents. Thus tetrapeptides, analogs of AS-I toxin, like Cys-Val-Gly-Glu-OH, showed significant antiproliferative activity on three cancer cell lines: HT-29 (human colon cancer), HeLa (human cervical cancer) and T47D (human breast cancer) [1]. Analogs or C-terminal analogs of Substance P (SP) have been studied for their ability to prevent tumor growth or the proliferation of several cancer cell lines. The synthetic SP analog [D-Arg⁶, D-Phe⁷, D-Trp⁸, Leu¹¹]SP (antagonist L) and the C-terminal analog [Arg⁶, D-Trp^{7,9}, MePhe¹⁰]SP₆₋₁₁ (antagonist G) inhibit Small Cell Lung Cancer (SCLC) cell proliferation *in vitro* and *in vivo* [2,3].

Recently we have synthesized the C-terminal analogs [Glp⁶, Glu(Bu)¹¹]SP₆₋₁₁ and [Glp⁵, Glu(Bu)¹¹]SP₅₋₁₁, which showed significant inhibition in the proliferation of the cancer cell lines HeLa and T47D at concentrations of 0.75 mM or higher [4]. In the present study a series of SP₆₋₉ tetrapeptides, like Glp-Phe-Phe-Gly-OH (1), Glp-D-Trp-Phe-Gly-OH (2), Glp-D-Trp-MePhe-D-Trp-OH (3) and Arg-Phe-Phe-Gly-OH (4) have been synthesized, using the stepwise synthesis or the fragment condensation method either in solution or in Solid Phase Peptide Synthesis (SPPS). The analogs were purified (HPLC) and identified (ESI-MS, ¹H-NMR, FT-IR). Subsequently they were tested for their antineoplastic properties in several cancer cell lines. The figure 1 exhibits the potent peptide (3) concentration versus cell survival fraction curve for the cancer cell lines HT-29, T47D and MCF-7 (human breast cancer) at concentrations of 10 μ M or higher. These results with other concerning the rest of the synthesized tetrapeptides will be discussed.

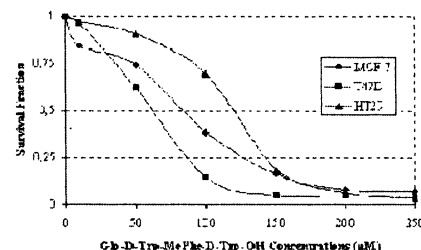


Fig. 1 - Antiproliferative activity of peptide Glp-D-Trp-MePhe-D-Trp-OH

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C1 - Peptide and peptidomimetics therapeutics

P C33 - Vascular endothelial growth factor receptor 2 binding peptide: targeting vascular endothelial cells.

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Vascular endothelial cells (EC) are an attractive target for many therapeutic applications, including targeting endothelium in atherosclerosis, hypercholesterolemia, postangioplasty, restenosis, hypertension and transplantation. Severe pulmonary hypertension (SPH) is a usually fatal disease that is associated with extreme elevation of pulmonary artery pressures (PAP) and right heart failure. Proliferation of pulmonary vascular endothelial cells is characteristically present in pulmonary arteries of patients with SPH. Recently we have demonstrated that in rats, blockade of the Vascular Endothelial Growth Factor receptor 2 (VEGFR-2) in combination with chronic hypoxia induces apoptosis of normal pulmonary ECs and allows for selection of an apoptosis-resistant proliferative type of pulmonary EC. The intravascular growth of ECs present in pulmonary arteries in this rat model of SPH is similar to that present in patients with SPH. We hypothesize that the disruption of normal pulmonary endothelial cell-cell contacts due to the apoptosis causes selection of an apoptosis-resistant EC type which is capable of uncontrolled proliferation.

To target cultured ECs with a VEGFR-2 binding peptide in order to deliver a death-promoting peptide, we have designed and synthesized a peptide (homing peptide) that can specifically target ECs via VEGFR-2. We used a Cytosensor-based assay to demonstrate that this 9 amino acid peptide (IMRIKPHQG-NH₂; V9) binds to human umbilical vein endothelial cells and to porcine aorta endothelial cells (PAEC) stably transfected with human VEGFR-2 (PAEC/VEGFR-2). It does not bind non-transfected PAEC that have no detectable level of the receptors. The increase of extracellular acidification rate upon treatment with VEGF was +85% and with V9 peptide +25% as compared with baseline. Complete internalization of fluorescein-labeled peptide into the cytosol of PAEC/VEGFR-2 cells occurred within the first hour of exposure. When V9 homing peptide is linked to a death-promoting peptide [(klaklak)₂], upon internalization it can induce mitochondria-dependent apoptosis in ECs. We will apply this approach *in vivo* in order to test whether death of normal pulmonary ECs results in SPH. Our overall approach relies on the EC maintenance and survival signals provided by VEGF. This is a novel concept with important ramifications in vascular biology, in particular, conditions where there is abnormal growth of ECs, such as in inflammatory diseases (rheumatoid arthritis) or cancer (tumor angiogenesis).

Acknowledgments: supported by NIH grants HL-66254 and HL-26284)

P C35 - Synthetic antimicrobial peptides designed from sequence templates

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The analysis of numerous antimicrobial peptide (AMP) sequences from different families has led to the definition of sequence templates and of the cationicity, hydrophobicity and amphipathicity ranges which characterise them, and has facilitated the design of simplified AMPs as lead compounds for the development of peptidic anti-infective agents. Using a database that contains most known eukaryotic antimicrobial polypeptide sequences (www.bbcm.units.it/~tossi/antimic.html), we have successfully applied this approach to obtaining synthetic α -helical AMPs, β -defensins and frog brevinin-like peptides. Synthetic, template generated AMPs are generally potent and broad spectrum antibiotics, and information derived from the sequence analyses have been used to guide SAR studies that probed the roles of structural and physico-chemical characteristics in modulating biological activity. Over 30 different α -helical AMPs have been synthesised in which side-chain characteristics of proteinogenic and non-proteinogenic amino acids were used to systematically vary the helix forming propensity, cationicity, amphipathicity, global hydrophobicity and hydrophobic and polar sector depths. The aim was to determine their individual role in modulating antimicrobial potency and specificity, and selectivity with respect to host cells. The most promising AMPs were then tested *in vitro* in a standardised assay against a wide set of clinical indicator strains, including highly antibiotic resistant Gram-positive and Gram-negative bacteria as well as fungi, and those peptides showing MIC values in the 0.5–4 μ M range against most of the tested micro-organisms have been short-listed for further evaluation. The membrane permeabilising activity of selected peptides towards erythrocytes and mouse leukocytes was evaluated by flow cytometry and SEM. As it was found to correlate with the helix forming propensity and helix sense, sequence modifications for a fine-tuning of the structure are leading to a reduced cytotoxicity. The information obtained from these studies is being used to screen template-generated *in silico* libraries for new potential AMPs. A template-based β -defensin has also been synthesised that folds to the correct β -sheet structure and shows a salt-sensitive antimicrobial activity. Fragments of this peptide containing only one or two disulphide bridge have been prepared in an attempt to identify the regions responsible for this activity. These studies indicate that the correct disulphide connectivity and folding requires the presence of all cysteine residues, but that antimicrobial activity does not seem to depend on the complete sequence, or the formation of a stable β -sheet structure.

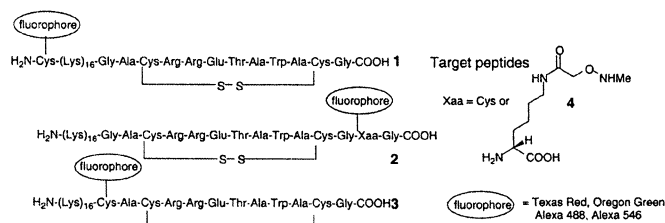
P C34 - Fluorescently labelled peptides as tools for probing the structure and function of a non-viral gene delivery vector

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A significant advance in the field of non-viral gene delivery vectors has been the introduction of three-component lipid/peptide/DNA complexes. The LID vector, which consists of Lipofectin (L: DOTMA:DOPE in a 1:1 ratio), a peptide (I: combining a receptor targeting motif with a Lys₁₆ tail to allow for DNA binding and compaction), and plasmid DNA (D), has demonstrated high transfection efficiency *in vitro* and *in vivo* and low toxicity. Direct information about the stoichiometry and structure of such macromolecular complexes has previously been difficult to obtain. Analysis of this vector using fluorescence correlation spectroscopy (FCS) and confocal microscopy was therefore undertaken. For these experiments, a range of regioselectively fluorescently labelled peptide components (1–3) was needed. This required the development of methodology capable of labelling one side-chain in the presence of sixteen Lys and two Cys residues, and compatible with sensitive fluorophores. Strategies based on differential Cys protecting groups were unsuccessful. Instead, the desired labelled peptides were synthesised using a novel N-methylaminoxy amino acid (4) which selectively reacts at pH7 in the presence of Lys residues; an unprecedented rearrangement was observed during this reaction, which gave a high yield of a stable covalent conjugate.

For the FCS experiments, LID complexes containing peptides labelled at various positions along the sequence were then formulated. Varying the fraction of labelled peptide enabled the stoichiometry to be determined by titration. Similar FCS experiments, with the LID complex incorporating regioselectively labelled DOTMA or DOPE in the complex, were then carried out and the stoichiometry with respect to the lipid determined. The size of the particles was also determined from these experiments, along with some preliminary information about the organisation of the macromolecular complex.



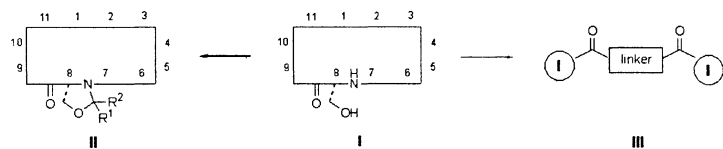
P C37 - Structure-activity studies of novel D-Ser⁸-cyclosporine a derivatives as potential anti-HIV drugs

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Since the discovery of the immunosuppressive activity of cyclosporine A (CsA), considerable work has been devoted to the chemical synthesis of potent analogues [1]. More recently, the finding of potential anti-HIV 1 activity of CsA[2] raised the interest for the design of CsA-derived compounds devoid of immunosuppressive activity [3]. With this objective, the highly immunosuppressive CsA analogue D-Ser⁸-CsA [4] (I) was taken as a versatile candidate for modulating the binding mode to its receptors cyclophilin A (CypA) and calcineurin A (CnA). To this end, the functional group at position 8 is used for the chemoselective transformation of I to a series of derivatives including C-2 substituted pseudo-proline systems (II) and dimers disposing variable linkers (III) (Figure 1).

The chemical synthesis, characterisation and structure-function studies result in CypA binding compounds exhibiting strongly reduced CnA affinities serving as potential leads for the development of anti-viral, non-immunosuppressive drugs.



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P C39 - Synthesis and antigenic properties of the identified epitopes of the Helicobacter Pylori CagA protein

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The discovery of *Helicobacter Pylori*, a spiral, gram-negative bacterium widely distributed among the human population is an important breakthrough in the field of gastroenterology [1]. *Helicobacter pylori* is one of the major causes of acute gastritis, peptic ulcer and it is associated with gastric ulcer and stomach cancer. One of the major pathogenic factors that permits the colonization of gastric mucosa by *Helicobacter pylori* and therefore plays an important role in the pathogenesis is the toxin CagA [2]. Against this toxin are developed antibodies. In order to contribute to this field we predicted the antigenic and hydrophilic sequences of toxin CagA using theoretical algorithms. From the correlation of the most antigenic and hydrophilic regions it was found that the region 598-650 and particularly the incorporated 608-622 (KKAQKDLEKSLRKRE) could be a highly antigenic region of CagA. Based on these results the region was separated in five 15-peptides overlapped by five amino acids which were synthesized. The synthesis was carried out by the Fmoc strategy using as a solid support the Wang resin. The peptides were purified by semi-preparative HPLC and were identified by ESI-MS. The antigenicity of these peptides was evaluated by ELISA using human sera. It was thus demonstrated that the anti-CagA antibodies in sera do recognize the region 608-622. Preliminary 1H-NMR experiments of the sequence 608-622 showed a possible helicoid conformation in DMSO-d₆ solution.

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P C38 - New synthetic peptides related to laminin YIGSR fragment

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The anti-adhesion therapy is a new experimental method of tumor metastasis inhibition. It was found that the polypeptides derived from adhesion molecules were able to inhibit tumor metastases formation. Laminin, a major constituent of basement membrane, is one of the cell adhesion proteins. It is composed of three polypeptide chains (A, B1 and B2). The B1 chain contains a Tyr-Ile-Gly-Ser-Arg (YIGSR) sequence that has been found to inhibit experimental metastasis by blocking the binding of tumor cell to basement membrane.

We report the synthesis and results of tumor cell attachment assay of new YIGSR-related peptides. The native sequence YIGSR was modified by the replacement of Tyr residue by desaminotyrosine or uracil derivatives, and Arg residue by homoarginine, 4-guanidino-2-aminobutyric acid and 3-guanidino-2-aminopropionic acid. All new synthetic peptides attached to the culture wells coated with laminin equally or better than parental YIGSR. Moreover, all of the peptides were able to inhibit (30-50%) the adhesion of tumor cells (mouse transplantable lung cancer - LLC and melanoma B16) to laminin.

P C40 - Reduction of mouse fertility by antibody against P12 in females

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We have purified P12, a Kazal-like protease secreted from male accessory gland contains 57 amino acid residues, and showed it as a calcium transporter inhibitor that gives a strong affinity to a receptor on the anterior acrosome of mouse spermatozoa. Residues 11-40, which is less likely in the formation of an antigenic determinant, locate three-dimensionally distant from the three potential epitopes predicted in residues 1-10, 41-48 and 50-57. We examined the change in cell morphology associated with immunoagglutination of P12 bound to mouse sperm and evaluated the contraceptive potential of the presence of antibodies to P12 in female mice. We found that P12-sperm binding did not cause spermatozoal capacitation and an acrosomal reaction. *In vitro* incubation of mouse epididymal spermatozoa (2x10⁶ cells/mL) with 3.0 μM P12 and antibody partial purified from rabbit antiserum (1.0 mg/mL) in a modified tyrode's solution caused the removal of the acrosome from the cells. The antibody against P12 was detectable in the genital tract of female mice after they had been intrasplenically immunized with p12. The fertility of these females was markedly reduced. The higher the antiserum titer in the immunized female, the longer the delay in its parturition compared with the control animals after coitus. Intravenous injection of the P12 antibodies purified from the antiserum of immunized female mice also showed a dosage-dependent delay in parturition.

P C41 - An allergy vaccine based on solvent-exposed non-anaphylactic peptides of the major birch pollen allergen, Bet v 1

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We present an approach for the rational design of peptide allergy vaccines with low risk of inducing anaphylactic side effects. Almost 100 million allergic patients are sensitized to the major birch pollen allergen, Bet v 1, a 17 kDa protein containing most of the IgE epitopes present in pollens of trees belonging to the Fagales order and plant-derived food. According to the sequence and the three-dimensional (3-D) structure of Bet v 1 solvent-exposed non-allergenic peptides were selected for induction of protective IgG antibodies which block IgE antibody binding to the wildtype allergen. The boundaries of the six Bet v 1-derived peptides (2809-3484 Da) were chosen to include solvent-exposed amino acids but according to NMR analysis the peptides lacked secondary structure. None of the peptides exhibited IgE binding capacity and allergenic activity when studied by basophil histamine release and skin prick testing in allergic patients. Peptide immunization of mice and rabbits induced IgG antibodies which reacted with the complete Bet v 1 allergen, Bet v 1-related pollen allergens and strongly inhibited the binding of allergic patients' IgE antibodies to Bet v 1. The presented approach should allow the rational design of safe and defined peptide vaccines for treatment of IgE-mediated allergies whenever the structure of the disease-eliciting allergen is known.

P C42 - Encapsulation of new biologically active peptides into liposomes and their immunoadjuvant effect

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We have been examining the encapsulation of the new immunomodulators of peptidoglycan structure and synthetic adamantyltripeptides into the liposomes with the aim to possibly increase and optimize their therapeutic efficacy. Our studies concern two groups of immunomodulators: a) peptidoglycan monomer (PGM, β -D-GlcNAc-(1 \rightarrow 4)-MurNAc-L-Ala-D-isoGln-mesoA2pm(ϵ NH₂)-D-Ala-D-Ala) b) synthetic adamantyltripeptides (D- and L-(adamant-2-yl)-Gly-L-Ala-D-isoGln) comprising a part of peptidoglycan structure. All mentioned compounds were shown previously to exhibit immunostimulating activity [1,2].

The immunostimulators tested were incorporated in large multilamellar negatively charged vesicles using egg lecithin and synthetic dipalmitoyl phosphatidylcholine (DPPC). The entrapment of tested compounds and the stability of the respective preparations were followed in two ways: by the use of ¹⁴C-labelled peptidoglycans or by HPLC.

The immunoadjuvant activity was tested *in vivo*, in mice, using ovalbumin (OVA) as an antigen. The production of OVA-specific antibodies was monitored by ELISA. In comparison to the nontrapped tested compounds the encapsulated preparations showed the better anti-OVA antibody production. Hence, incorporation of immunoadjuvants into liposomes improved their adjuvant activity. In conclusion, liposomes containing immunoadjuvants were prepared, quantity of incorporated material determined and the improved adjuvant effect of immunoadjuvants was demonstrated.

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P C43 - Antibody recognition of synthetic antigens from MUC1

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MUC1 mucin glycoprotein can be an ideal antigen for active immunotherapy of adenocarcinoma related cancer. Expression of the protein is greatly up-regulated on almost all human epithelial cell tumors. More importantly the tumor-associated mucin is underglycosylated and therefore the protein core is more exposed for immune recognition [1]. The MUC1 mucin is composed of 20-amino acid (VTSAPDTRPAPGSTAPPAHG) tandem repeat unit, which may be replicated 20-125 times. APDTRP sequence as core epitope within this unit recognized by several mucin reactive antibodies appears to be the immunodominant domain [2]. For the detection of MUC1 specific antibodies related to epithel cell tumors we have developed several linear and cyclic peptide constructs containing the immunodominant domain. The dimer or trimer versions of the full length tandem repeat unit were built up from the 20 amino acid unit by fragment condensation with formation of either amid [3] or thioether bond as well as disulfide bridge between the fragments. In case of the two sulphur based conjugation the C-terminal Gly of the sequence was replaced by Cys and its thiol group was used for the non-peptide bond formation. We have also prepared the linear (APDTRPAPGC) and cyclic (c[CH₂CO-APDTRPC]PGC) peptides, which are available for attachment of multiple copies of the core epitope to carrier molecules. The antigenicity of the multiple repeat and the cyclic peptides was studied with the help of a MUC1 specific monoclonal antibody (C595) in competitive ELISA assay. The ability of the peptides to inhibit the binding of the antibody to a MUC1 synthetic target antigen was determined. Differences in the antibody binding properties of the linear and cyclic peptides will be discussed.

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Acknowledgements: these studies were supported by grants from the Hungarian Research Fund (OTKA No. T 03838 and No. F 034886).

P C44 - Synthesis and in vitro T cell immunogenicity of oligopeptides corresponding to the 91-110 region of 16 kDa protein of *Mycobacterium tuberculosis*

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The 16kDa protein of *Mycobacterium tuberculosis* provokes specific immune response, it is a targets for the development of peptide-based diagnostic reagents and subunit vaccines. The antigenic structure of the protein has been analysed previously and these studies have demonstrated the presence of regions containing murine as well as human T-cell epitopes [1]. Based on these results we selected the 91-110 region from 16 kDa protein for detailed analysis. Three groups of peptides (N- and C-terminal truncated variants of the epitope) corresponding to the 91-110 region of 16 kDa protein were prepared. The aims of the work in the present project are: 1) to identify the functional T-cell epitope core (the minimal size region) using truncated synthetic peptides 2) to analyse the role of flanking regions (the amino acid residues on both sides of the core region) 3) to determine the minimal size of synthetic peptide containing the epitope core and native or non-native flanks. Peptides were prepared by standard procedures on MBHA resin (1.04 mequiv/g). Amino acids were coupled as Boc-derivatives with the HOBt/DCC coupling method using 3 molar excess over the resin's capacity in DMF/DCM 1:4 (v/v). The primary structure of peptides and the homogeneity were checked by amino acid analysis, analytical RP-HPLC and ESI-MS methods. The solution conformation of peptides was studied by CD spectroscopy in order to establish correlation between epitope structure and specific T-cell responses. Spectra were recorded in water, in trifluoroethanol (TFE) and in water:TFE mixture (1:1, v/v). The T-cell stimulatory activity of synthetic compounds was investigated using *in vitro* assays (proliferation and cytokine (IFN- γ , IL-4) production) on a) epitope specific T-cell clones b) PMBC (peripheral blood mononuclear cells) from patients and PPD⁺ healthy individuals. Studies with BV2⁺ 91-110 human T-cell clones showed that all the peptides lacking amino acid 91 and 92 were inactive. Peptides lacking amino acids 105-110 are able to stimulate T cell clones to produce IFN- γ and IL-4. Studies on the human PMBC cells showed that the peptides containing 91 and 103 amino acids (and acetylated 92 glutamic acid) were active and initiated IFN- γ production. The peptide ⁽⁹²⁾AFAYGSFVRTVSL⁽¹⁰⁴⁾ with alanine at position 92 was inactive in PMBC assay. These results are considered for the development of synthetic peptides related to *M.tuberculosis* with optimised T-cell response provoking capacity.

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Acknowledgements: these studies were supported by grants from COST Chemistry Action "Peptide based synthetic antigens against infectious diseases" (D13/0007/00), Hungarian Research Fund (OTKA No. F 034886) and from Ministry of Culture (0153/2001).

P C45 - Enhanced immunogenicity of single-dose antigen based on nanospheres with antigen presenting cell-targeting function

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Effective vaccine and immunotherapies have long been sought by many researchers. Immunization with antigen alone fails to induce the effective immune response. Better immune response can be elicited by administration of antigens with adjuvants. However, these adjuvants often have serial side effects including severe inflammation and only alum is approved for human use in spite of a relative weak potentiator. Microsphere or nanosphere particles have been shown to be potential for single-dose vaccine. To improve the vaccine of antigen-conjugated particles, this study examined the effect of antigen presenting cell (APC)-targeting molecules on immunogenicity of model antigen. F2 motif was a CTLA4 binding domain-mimicking peptide, which was isolated from random peptide phage display library by panning toward anti-CTLA4 monoclonal antibody, as described in our previous report (Nat. biotechnol. 16, 267, 1998). This motif could bind to CD80 but not CD86 on APC and inhibit the binding between CD80 and its counter receptors CD28 and CTLA4 (CD152) on T cell. CTLA125 is extracellular domain of CTLA-4, a negative regulator on T cell and can bind to CD80 and CD86 on APC. So that, these two molecules were chosen as targeting molecule for APC. Furthermore, we prepared F2-lysozyme that was a genetically engineered fusion protein of lysozyme (model antigen) and F2 motif. The nanosphere used here was in a diameter with 590 nm on average, its core was composed of polystyrene and surface was covered with poly (methacrylic acid) (PMAA) layer. Both with CTLA125 and model antigen (lysozyme), or F2-lysozyme alone was covalently conjugated to the nanosphere by EDC-activated amine coupling method. These antigen-conjugated nanosphere was intraperitoneally immunized on Balb/c mice at a single dose and the antibody response in the serum was examined at 2 and 3 weeks after immunization or 1 week after re-stimulation with soluble antigen. The mice immunized with the nanoparticle conjugate with lysozyme alone (4.8 ug) did not induce antigen specific IgG production. However, the challenge of the particles attached with F2-fused lysozyme or of the particle together with CTLA125 and lysozyme showed the enhanced antibody response even under the low antigen-dose condition (3.2-4.2 ug), as shown in Fig. Therefore, we concluded that APC-targeting molecules used here could successfully function on induction of strong immune response in particle immunization, even under the low and single dose of antigen.

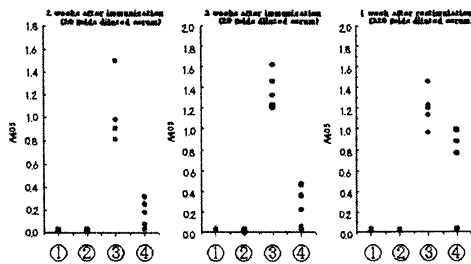


Figure 2. Enhancement of antigen-specific IgG production by antigen-conjugated nanosphere attached with APC targeting molecule. ① HEL-conjugate, ② CTLA4-conjugate, ③ HEL/CTLA4-conjugate, ④ F2HEL-conjugate.

P C47 - Synthesis of the citrullin-containing epitope peptides of flaggrin

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Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. It is the most common inflammatory joint disease, affecting 1-2% of the world population. Anti-flaggrin antibodies (AFA) directed against the epidermal protein, flaggrin, belongs to the most specific markers of RA. Epitopes, containing citrullin within the sequence of flaggrin, have been recently identified as major antigenic sites recognised by AFA. The aim of our study was to localise these epitopes in the flaggrin by the use of synthetic peptides and by RA specific antibodies from patients.

Peptides corresponding to the most antigenic six sequences of flaggrin (1) and the N- and C-terminal shortened version of the peptide ³⁰⁶SHQESTRGRSRGRSGSGS³²⁴ were synthesized. We used conventional solid-phase peptide synthesis (Fmoc strategy) carried out on „MULTIPIN NCP” non-cleavable kit. The peptides were prepared in duplicates. As a negative and positive control GLAQGGGGG and PLAQQGGGGG peptides were also synthesised. In the “indirect” ELISA experiments the presence of AFA was determined using serum samples of RA patients and healthy blood donors.

In conclusion our results provide evidence that not simply the presence of citrullin, but also the nature of its surrounding amino acids have an important role in the creation of autoantigenic epitope reactive with anti-flaggrin antibodies. Substitution of arginine 312 by citrullin plays a major role in the antigenicity of flaggrin derived sequences. When the 19 amino acid peptide with citrullin (X) in position 312 were shortened from its N-terminal, the 14-mer peptide showed the highest reactivity. Further shortening of this sequence from its C-terminal showed that TXGRS motif seems to be essential to comprise the autoantigenic epitopes. It must be emphasized that the use of surface-attached peptides is a screening procedure can lead to the identification of sequences but for detailed studies such peptides have to be produced on larger scale, and purified. The peptides selected from the screening procedure - with Cys on the there N- or C-terminal - were synthesized on Rink-amid resin by solid-phase peptide synthesis, using Fmoc strategy. The peptides were purified by FPLC, and analysed by MS. Then the peptides were covalently coupled to sulphidryl-bind microplates, and AFA was determined in ELISA.

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Acknowledgments: this work was supported by the Ministry of Health and the „Foundation for Hungarian Peptide and Protein Research”, Budapest

P C46 - MSP-1 pseudo-peptides: a possible malaria vaccine component

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Naturally occurring proteins are made up of L-amino acids which can form either structural functional domains (such as in enzymes) or contain immuno-stimulating regions in antigenic proteins (so-called epitopes). Proteins formed by L-amino acids usually provide binding sites specifically recognising only L-enantiomeric forms of their natural ligands. Chiral specificity was demonstrated when a solid phase synthetic HIV-PR D-form was found to hydrolyse only the D-enantiomer of its peptide substrate. The development of peptide drugs and vaccines is strongly impaired by their susceptibility to proteolysis.

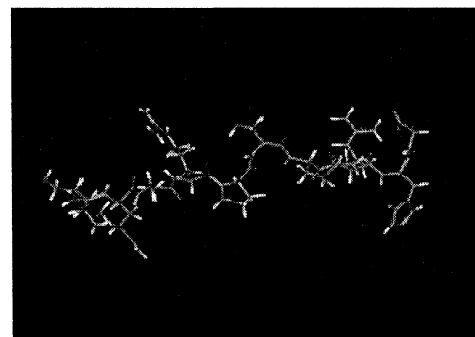
Peptides are the target for immune recognition by T cells; these peptides bind peptide receptors (so-called major histocompatibility (MHC) molecules). Class I MHC bind peptides presented to cytotoxic T cells, whilst class II MHC molecules bind peptides presented to helper T cells. In each case, these peptides are the product of proteolytic enzymes present in the cytosol or in endosome compartments, respectively. The antigenic processed peptide is normally presented in a complex with a MHC molecule. It is then presented to T cells through its T cell receptor (TCR) leading to specific T cell stimulation by proliferating or modulating certain immune responses in the host. Mass spectroscopy analysis has revealed that a single MHC molecule expressed on the cell surface can present up to 10⁴ different peptides; this great amount of peptides has also led to the idea that modulating T cell activation may occur using specific TCR antagonists and partial agonists. Peptide antagonists or partial agonists mimic the conformation of a known antigenic peptide and thereby maintain satisfactory binding affinity for a particular MHC allotype. However, they differ sufficiently from the original peptide to change T cell signalling when the MHC-peptide complex interacts with TCR (being the peptide size) and structure important components for a suitable immune response. This forms a ternary complex provoking intracellular signalling and an immune response effect. Our laboratory has gained experience and accumulated important evidence about testing 20-mer peptide synthetic polymers as sub-unit malarial vaccine candidates *in vitro* as well as *in vivo* during the last twenty years. The role of malarial MSP-1 protein Reduced Amide Pseudo-peptide analogues has been assessed in *in vitro* studies. These pseudo-peptides represent conformational B cell epitopes capable of mimicking possible structural antigens by modulating the molecule's backbone and also modulating immune response in animal models. Since T cell activation is essential for the induction of protective immune responses, this has raised the question of whether pseudo-peptides can serve as both B cell and T cell epitopes for the development of human vaccines. In this work, we describe the development of a particular family of modified peptides (Reduced Amide Pseudo-peptides - RAP) and their place within the wider realm of peptide modification. Pseudo-peptide chemistry offers the possibility of mimicking certain peptide conformations or antigenic protein transient stages by inducing local as well as global structural constraints. Pseudo-peptides thus represent structural probes having broad molecular interaction possibilities. The role of a Reduced Amide Pseudo-peptide could thus be that such peptide analogues represent both B and T cell epitopes with their subsequent potential application as components of synthetic sub-unit vaccines.

P C48 - Design, synthesis and conformational properties of linear analogues based on Human Myelin Basic Protein epitope MBP1-11.

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Experimental Allergic Encephalomyelitis (EAE) is induced in experimental animals immunized with myelin proteins and is commonly used as a model system useful in evaluating potential therapies for Multiple Sclerosis (MS). In EAE, residues 1-11 of myelin basic protein are the dominant disease-inducing determinants in PL/J and (PL/JxSJL/J)F1 mice. Analogues of disease-associated epitopes have been identified which alter disease progression upon co-immunization. Based on the Human MBP1-11 sequence [Ala1-Ser2-Gln3-Lys4-Arg5-Pro6-Ser7-Gln8-Arg9-His10-Gly11] (analogue 1) we designed and synthesized linear analogues in which Lys at position 4 was replaced with Ala, [Ac-Ala1-Ser2-Gln3-Ala4-Arg5-Pro6-Ser7-Gln8-Arg9-His10-Gly11] (analogue 2) or Tyr, [Ac-Ala1-Ser2-Gln3-Tyr4-Arg5-Pro6-Ser7-Gln8-Arg9-His10-Gly11] (analogue 3). In these analogues the N-terminus was acetylated. For the synthesis of linear MBP analogues, we resorted to the Fmoc/tBu methodology utilizing the 2 chlorotrityl chloride resin. In bioscreening assays we found that these linear analogues were not effective in inducing or inhibiting EAE in Lewis rats in contrast to PL/J mice, where these analogues were found active. However, it was noticed that analogue 2 when co-injected with guinea pig MBP72-85 give a maximum clinical score for two additional days, in contrast to analogues 1, 3 which didn't alter effect caused by MBP72-85.



Low-energy conformers of MBP1-11 epitope derived by dynamics experiment.

P C49 - Preparation of synthetic antigens and bioconjugates containing oligo-tuftsin carrier molecule

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Biodegradable and well-characterised carrier molecules can be useful as a part of synthetic antigens or bioconjugates in cancer therapy. We have developed a new group sequential oligopeptide carriers based on tuftsin like sequence. In this pentapeptide (TKPKG) two lysine residues are available for connection of drug molecules. As tandem repeat unit oligomers were prepared (H-(Thr-Lys-Pro-Lys-Gly)_n-NH₂, where n = 2 (T10), 4 (T20), 6 (T30), 8 (T40)). The carriers were non toxic and non antigenic, but they showed immunostimulatory activity [1].

Epitope peptides from glycoprotein D of herpes simplex virus type 1 were selected to prepare synthetic antigens using tetratuftsin derivatives as carrier molecules. Side chain of every second lysine residues in the T20 oligopeptide was modified by chloroacetylation. Epitope peptides elongated by cysteine at the C- or N-terminal were attached to the carriers by thioether bond formation. For this study peptides corresponding to the 9-22 (LKNleADPNRFRGKDL), the 272-279 (DPEDSALL) or to the 276-287 (SALLEDPVGTVA) sequences were applied.

The ε-amino group of lysine residues of oligo-tuftsin derivatives were also modified either by succinylation or by the incorporation of Gly-Phe-Leu-Gly tetrapeptide as degradable spacer in lysosomes. These compounds were used as carrier molecules for antitumor drug (eg. GnRH-analogue or daunomycine) conjugation.

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Acknowledgements: This study was supported by grants from the Hungarian Research Fund (OTKA) N°T032425 and COST Chemistry A13.

P C51 - Antimicrobial activity of N-terminal fragments of Bac7, a cathelicidin-derived PR-rich peptide

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Antimicrobial peptides (AMPs) play a major role in host defence, particularly in the early phases of infection. From a functional point of view, AMPs can be divided into two groups according to their mechanism of action. The vast majority act by permeabilizing bacterial membranes, while a restricted group inactivates bacteria without membrane permeabilization. PR-rich peptides of mammals and insects are members of the second group. The mammalian peptides are derived from precursors belonging to the cathelicidin family and have linear structures characterised by the presence of tandem repeated sequences. Among the PR-rich peptides that have been identified in bovine leukocytes, the 60 residue Bac7 has a modular structure comprising an Arg-clustered region at the N-terminus followed by three identical tandem repeats of a tetradecamer. Chemical synthesis of numerous Bac7 fragments, covering the whole sequence, indicated that the highly cationic N-terminal region is essential for antibacterial activity and that the minimum required length is 16 residues. In contrast with membrane active peptides, e.g. α-helical peptides, for which no significant effect of chirality was observed, all-D enantiomers of insect members of the PR-rich peptide family are virtually inactive. The interaction with stereospecific targets was considered as a possible explanation of this behaviour. Following these observations, the present study is aimed at investigating whether the activity of the N-terminal 1-35 fragment of Bac7, whose activity is comparable to that of the parent peptide, is also based on an interaction with a stereospecific target. For this reason, its activity was compared to that of its all-D enantiomer. The results showed that a 4 to 10-fold higher concentration of the all-D peptide was required to obtain an effect comparable to that of the all-L isomer. Moreover, a complete inhibition of the killing activity was observed when the activity of both enantiomers was determined at 4°C, suggesting an energy-dependent mechanism of bacterial killing. This is in contrast to a membranolytic peptide such as SMAP-29, which maintained a partial activity under those conditions. Concerning the effect on cellular morphology, both enantiomers induced relevant alterations on the affected cells, that shranked dramatically and emitted long protrusions. The number of cells with this morphology increased with time, but even after 120-240 minutes of peptide treatment, a significant percentage of cells with an apparently normal morphology was still present, although parallel experiments indicated that at these time intervals, there was a 3-4 log decrease in cell viability. What is interesting is that the cells did not show blebbing of their surface, which is typical of membrane-active AMPs. This suggests a mechanism of action in which the primary event is not bacterial membrane permeabilization. Such a mechanism is currently under investigation.

P C50 - A stimulatory monoclonal antibody that induces G-CSF release from macrophages

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Maturation of hematopoietic cells is known to be controlled by various hematopoietic cytokines. Granulocyte-colony stimulating factor (G-CSF) is one of such cytokines. G-CSF is produced and secreted from monocytes/macrophages, endothelial and stromal cells and fibroblasts, and promotes proliferation and maturation of neutrophils from promyelocytic and myelocytic cells. G-CSF has been clinically applied to treat patients suffering from agranulocytosis due to chemotherapy and aplastic anemia. It has been demonstrated that cytokines such as interleukin-1 and tumor necrosis factor (TNF), cell adhesion molecules such as integrins and fibronectin, and endotoxin lipopolysaccharide caused the secretion of G-CSF. However, these stimuli also promote the production of inflammatory cytokines such as interleukin-6 and TNF-α. If substances that induce G-CSF release specifically, they have great advantage as alternative therapeutic reagents of G-CSF against neutropenia. In this study, we cloned and identified a mouse monoclonal antibody (mAb), 3-4H7, which binds to a cell surface antigen of mouse macrophage cells and promotes not only the induction of G-CSF gene expression but also the secretion of G-CSF. This mAb has the striking feature that selectively stimulates G-CSF release, whereas other substances that produce G-CSF are pleiotropic. This mAb facilitates not only identification of membrane receptors for production of G-CSF and their corresponding ligands but also development of peptidergic substances that specifically induce the secretion of G-CSF in monocyte/macrophage cells.

P C52 - Synthesis and biological activity of N-(tert-butylloxycarbonyl)-(adamant-2-yl)-DL-glycyl-peptidoglycan monomer

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Peptidoglycan monomer (PGM, β-D-GlcpNAc-(14)-MurpNAc-L-Ala-D-isoGln-[L-meso-A₂pm(εNH₂)-D-Ala-D-Ala, PLIVA), disaccharide pentapeptide, is the basic repeating unit of *Brevibacterium divaricatum* cell wall peptidoglycan. It exhibits, *inter alia*, strong immunomodulating activity [1]. Semisynthetic derivatives of peptidoglycan monomer, N-tert-butylloxycarbonyl-L-tyrosyl-PGM and (adamant-1-yl)-acetyl-PGM are good substrates [2] for N-acetylmuramyl-L-alanine amidase present in mammalian blood. Neither of them affected [2] the immunostimulating properties of the parent compound. The aim of this work was to prepare the new derivative of PGM containing (N-tert-butylloxycarbonyl)-(adamant-2-yl)-DL-glycyl-residue and to investigate the structure-activity relationship. It might be expected that even small structural changes can alter the biological activity of the parent compound. Derivatives of PGM containing the adamantyl residue might combine immunostimulating activity of PGM with antiviral activity of the moiety comprising adamantyl residue. The novel adamantylacyl derivative of PGM was prepared by acylation of ε-amino group of diaminopimelic acid with symmetrical anhydride of N-(tert-butylloxycarbonyl)-(adamant-2-yl)-DL-glycine. The anhydride was prepared utilizing (a) DCC and (b) EDC. The reaction of the PGM with anhydride (a) gave a mixture of two products N-(tert-butylloxycarbonyl)-DL-(adamantan-2-yl)-glycyl-peptidoglycan monomer (product A) and [N-(tert-butylloxycarbonyl)-DL-(adamantan-2-yl)-glycyl]₂-peptidoglycan monomer (product B) that have different chromatographically mobilities. The reaction with anhydride (b) resulted in product A only. Products were isolated by gel filtration on Sephadex LH 20 and their structures were confirmed. Activity in relation to the structure of the product A was examined in the two different models: *in vitro* (the susceptibility of A to the N-acetylmuramyl-L-alanine amidase) and *in vivo* (immunomodulating activity was tested in an experimental model in mice, using ovalbumin as antigen [1]). Product A was a good substrate for N-acetylmuramyl-L-alanine amidase which cleaves it yielding the disaccharide and the respective peptide. Immunostimulating activity of product A was compared with the activity of PGM and (adamant-1-yl)-acetyl-PGM. Product A didn't exhibited immunostimulating effect in used experimental model.

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P C53 - β -Amino acid peptides elicit a spectrum of T cell responses

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β -Amino acids are versatile peptidomimetics that can be included in α -amino acid containing peptides to engender increased stability to proteases and altered biological activity [1]. Recently β -amino acid containing peptides have been shown to stimulate T cells specific for natural all α -amino acid containing peptides [2,3]. Here we have exploited a model system of cytotoxic T lymphocyte (CTL) recognition of the chicken ovalbumin immunodominant epitope ²⁵⁷SIINFEKL²⁶⁴ in C57/BL6 mice. By substituting each amino acid for its corresponding β -amino acid analogue we demonstrate pleiotropic effects on the serum stability, binding of each analogue to the murine major histocompatibility complex molecule H-2K^b and the recognition of these peptides by specific T cell clones. Both agonist and antagonist activities were observed with different β -amino acid analogues. We propose that substitution of α -amino acids with their corresponding β -amino acid will provide a portal to the rational design of immunotherapeutics based on peptides and peptidomimetics.

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P C54 - Constrained peptide mimetics and virus-like particles in synthetic vaccine design

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An approach to synthetic vaccine design is illustrated [1], using as an example the immunodominant (NPNA)_n repeat region of the circumsporozoite (CS) protein of the malaria parasite *Plasmodium falciparum*. Modelling suggests that the NPNA motif may adopt a helical β -turn, which is tandemly repeated in the CS protein to generate a novel supersecondary structure. Cyclic peptidomimetics of this NPNA motif were synthesized and shown by NMR to populate helical turns in aqueous solution. When incorporated into Immunopotentiating Reconstituted Influenza Virosomes (IRIV), humoral immune responses were generated in mice that cross-react with native CS protein on sporozoites. IRIVs are a human-compatible delivery system that appear generally suitable for inducing antibody responses against conformational epitopes using constrained peptidomimetics. This approach may offer great potential for the design of molecularly defined synthetic vaccines, including those targeted against multiple antigens and development stages of *P. falciparum*, or against other infectious agents.

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P C55 - Immunogenic properties of the main T-cell epitopes of gp63 anchored to the sequential oligopeptide carrier Soc4

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The major surface glycoprotein, gp63 *Leishmania major*, of the outer cellular form of the parasite, which causes leishmaniasis, is responsible for the worldwide distribution of the disease. As major T-cell epitopes of the gp63 have been proposed the sequences 467-482 (H-GNVQAAKDGNTAAGR-NH₂) and 154-169 (H-YDQLVTRVTHEMAHA-NH₂) [1]. These T-cell epitopes were synthesized in order to study their immunological response in immunized animals as well as their conformational characteristics by 1H NMR spectroscopy. They were covalently attached to the Lys-N^εH₂ groups of a sequential oligopeptide carrier SOC4, (Lys-Aib-Gly)₄ (Fig. 1), which adopts a 3₁₀ helical conformation and it has been successfully applied in our lab. This helical conformation allows to the antigens to maintain their initial conformation, whereas induces favorable molecular recognitions. In order to generate antibodies against the parasite of leishmania in immunization experiments, the SOC4 carrier was synthesized by the solid phase and the T-cell epitopes gp63 (467-482) and gp63 (154-169) were covalently attached to the Lys-N^εH₂ groups in position 1/3 and 2/4 respectively. It was also studied the in vitro T-cell proliferation of CBA and BALB/c mice immunized with the free forms of T-cell epitopes, as well as the production of antibodies against the SOC4 conjugate. Conformational study of T-cell epitopes, by ¹H NMR spectroscopy, is in progress.

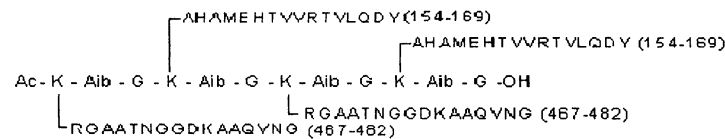


Fig. 1 - The SOC4 carrier bearing the main T-cell epitopes of gp63.

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P C56 - The Ro60KD zinc-finger motif: design, synthesis and molecular interactions

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The humoral autoimmune response in Systemic Lupus Erythematosus (SLE) and Sjogren's Syndrome (SS) is primarily directed against the protein components (Ro60KD, Ro52KD and La/SSB) of the Ro/La RNP complex [1]. It has been proposed that the zinc-finger motif (Cys2/His2) of the Ro60KD protein spanning the sequence 301-327 is mainly responsible for interacting with Ro52KD within the Ro/La RNP complex [2]. This work includes the design, synthesis and molecular interactions of the H-VSLVCEKLCNEKLLKKARHPFHIAIA-NH₂ (301-327) (zif-1), the H-VCEKLCGGGHPFHI-NH₂ (zif-2) peptides and their biotinylated derivatives. The zif-2 peptide derives from the substitution of the 310-320 loop of zif-1 by three glycines corresponding to the minimum distance between Cys309 and His321. We examined the conditions for the formation of the zinc-finger complexes at different ratios with mass spectroscopy (ESI-MS). We also studied the recognition of these zinc-finger models from patients sera with SS, SLE and normal sera and from r.Ro52KD in the presence or absence of Zn⁺². The future target of this work is to test the ability of immunosuppression in animals immunized with r.Ro52KD and the zif models of Ro60KD. This approach will offer a new alternative in immunoregulation, leading to new therapeutical applications.

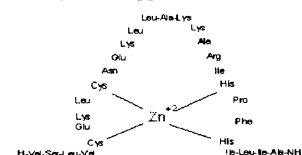


Fig. 1 - Structure of zif1

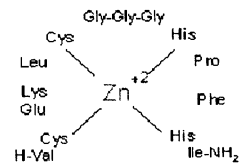


Fig. 2 - Structure of zif2

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Acknowledgements: this work was supported by the Greek General Secretariat of Research and Technology.

C2 - Peptides in immunology/vaccines

P C57 - Antipeptide antibodies to develop diagnostic tests for bacterial toxins

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Antipeptide antibodies are widely used for research applications for example in the identification and/or isolation of a protein of which only the primary structure is known based on the DNA sequence or when investigating different splice variants of a protein.

We believe that antipeptide antibodies could also offer advantages, both technical and economic, in routine applications such as diagnostic tests.

The staphylococcal enterotoxins (SEs), (designated SEA, SEB, SEC1, SEC2, SEC3, SED, SEE and SEH) are a family of low molecular mass proteins (26-30 Kda) produced by some strains of *Staphylococcus aureus*. The SEs commonly cause food poisoning in the humans, and are also associated to a toxic shock-like illness. In addition, SEs act as superantigens, stimulating T-cell proliferation. In order to develop diagnostic tests for these bacterial toxins using antipeptide antibodies, we have further investigated the antigenic properties of SEA and SEB. Relationships between the known three dimensional structure of these SEs and their antigenic determinants will also be discussed.

P C58 - Identification of T-cell epitopes using spot synthesised peptides

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Positionally addressable parallel SPOT synthesis on continuous cellulose membranes is a extensively used tool to rapidly synthesize thousands of matrix-bound peptides [1,2]. However, there is a need for testing high numbers of soluble peptides directly in biological test systems. The identification of CD8 T-cells stimulating peptides derived from the proteome of the Cytomegalovirus is used as a model system in the study. Synthetic peptides bearing a C-terminal carboxy-function are essential for loading the MHC I molecules of the antigen presenting cells.

The efficient, reproducible and automatic assembly of cleavable peptides is limited due to chemical problems in the formation of the ester bond between C-terminal amino acid and the hydroxy-functions of the cellulose. Based on the rapid, efficient and chemoselective formation of thioethers, a novel handle for the automatic spot synthesis of cleavable peptides on cellulose membranes was developed. The present communication describes detailed analytical data to verify this strategy. Several peptides, derived from the UL46 and UL85 proteins of the HCMV AD 169 were synthesized via spot synthesis technique, deprotected, cleaved from the membrane by hydrolysis of the ester bond and analyzed by HPLC and mass spectrometry. This approach is useful for monitoring the preparation of soluble peptide sets gained automatically by spot synthesis. These peptide sets then can be used for biological screening assays to identify T-cell epitopes of whole protein sequences [3].

References

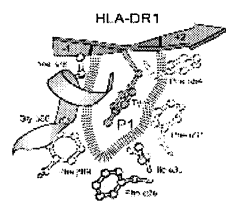
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P C59 - Specificity in the P1 pocket of the human class II MHC protein HLA-DR1

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The P1 pocket of peptide binding groove of human class II major histocompatibility complex protein HLA-DR1 is deep and mostly hydrophobic. It is known that for high affinity binding to MHC molecule P1 pocket has to be occupied by one of aromatic residues such as tyrosine, tryptophan or phenylalanine. To investigate the role of P1 pocket in peptide binding, we used a group of analogues of Ha (306-318) a tightly binding viral peptide (K_D 14) and A2 (103-117) a tightly binding endogenous peptide (K_D 10). Tyrosine and tryptophan in the P1 position were substituted

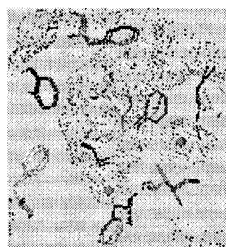


The diagram of the P1 pocket for HLA-DR1 drawn from respective crystal structure, illustrating the tyrosine, originating from Ha peptide, accommodation.

by unnatural amino acid residues and the peptide chain length was reduced from 13 to seven and four residues. Binding affinities of analogue peptides were determined by a competition immunoassay using biotinylated Ha and A2 peptides (time resolved Europium fluorescence).

For peptides showing high binding affinity K_D was also determined in a direct binding assay.

Data collected show that P1 pocket can accommodate phenylalanine derivatives with bromine in position 4 of aromatic ring as well as chlorine in position 3. The P1 pocket is big enough to accommodate 3-nitro-tyrosine residue. The described analogs loose their high binding affinity upon truncation of the peptide chain.



Side view of pocket 1 in the A2 and Ha peptide complexes. The A2 peptide places a tryptophan sidechain into pocket 1, and Ha peptide places a tyrosine sidechain into this pocket

P C60 - Study on structure activity relationship of the immunosuppressive fragment of ubiquitin

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Ubiquitin is a small protein (8.5 kDa) present in all eukaryotic cells, which plays an important role in tagging proteins for destruction. Our previous studies revealed that some of the fragments of the 48-59 domain of the ubiquitin molecule possess an unusually high immunosuppressive potency, comparable with that of cyclosporine [1]. The active sequence is located in the loop of the molecule exposed toward the solvent and therefore may serve as an important functional epitope for intermolecular binding.

To find the shortest immunosuppressive fragment of the ubiquitin 48-59 loop, we synthesized a series of its analogs with the reduced peptide chain, both from the N and C terminus. Furthermore, the contribution of the side chains to the activity of the shortest immunosuppressive ubiquitin fragment was tested using a series of its synthetic analogs, in which each particular amino acid residue was substituted by alanine. The synthesized peptides were characterized by CD spectroscopy and investigated for their activity in humoral and cellular immune response.

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Acknowledgement: this work was supported by grant No. 6 P05A 013 21 from the State Committee for Scientific Research (KBN, Poland).