

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 November 2002 (28.11.2002)

PCT

(10) International Publication Number
WO 02/094845 A2

(51) International Patent Classification⁷: **C07H 21/00**

(21) International Application Number: PCT/EP02/05447

(22) International Filing Date: 17 May 2002 (17.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
A 805/2001 21 May 2001 (21.05.2001) AT

(71) Applicants (*for all designated States except US*): **INTER-CELL BIOMEDIZINISCHE FORSCHUNGS- UND ENTWICKLUNGS AG** [AT/AT]; Rennweg 95b, A-1030 Wien (AT). **CISTEM BIOTECHNOLOGIES GMBH** [AT/AT]; Rennweg 95b, A-1030 Wien (AT).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SCHELLACK, Carola** [DE/AT]; Zeillergasse 23/31, A-1170 Wien (AT). **LINGNAU, Karen** [DE/AT]; Gallgasse 8/10, A-1130 Wien (AT). **SCHMIDT, Walter** [DE/AT]; Steingasse 18/1/10, A-1030 Wien (AT).

(74) Agents: **SONN, Helmut** et al.; Riemergasse 14, A-1010 Wien (AT).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR STABILISING OF NUCLEIC ACIDS

(57) Abstract: Described is a method for stabilisation of nucleic acids by contacting the nucleic acids with a polycationic polymer.



WO 02/094845 A2

Method for stabilising of nucleic acids

The present invention relates to a method for stabilising of nucleic acids.

Nucleic acids have been shown to be promising drugs in special fields of medicine. Especially interesting fields, wherein nucleic acids are pharmaceutically used are vaccination with naked DNA or RNA, using the general immunostimulatory effect of specific polynucleotides, especially in vaccination with specific (recombinant) antigens, antisense treatments and gene therapy treatments.

Vaccination with naked nucleic acids is becoming one of the most promising advances in the development of new generation vaccines. Many advances and specific strategies have been proposed for such vaccination to become useful tools in vaccination technology.

The use of nucleic acids as general immunostimulants is also developing from academic research to medical practice. The immune system recognises lower organisms including bacteria probably due to structural and sequence usage differences between pathogen and host DNA. In particular short stretches of DNA derived from non-vertebrates or in form of short synthetic oligodeoxynucleotides (ODNs) containing non-methylated cytosine-guanine dinucleotides (CpG) in a certain base context, are targeted. CpG motifs are found at the expected frequency in bacterial DNA but are much less frequent in vertebrate DNA. In addition, non-vertebrate CpG motifs are not methylated whereas vertebrate CpG sequences are. The difference between bacterial DNA and vertebrate DNA allow vertebrates to recognise non-vertebrate DNA as a danger signal. Moreover, cells of the innate immune system recognise double stranded RNAs of viruses as danger signal.

Antisense and/or gene therapy treatments are also topics wherein the medical application of nucleic acids plays a central role.

However, in order to achieve a sufficient pharmaceutical efficacy in all these fields of application, nucleic acids to be applied prophylactically or therapeutically should comprise a certain stability, especially in vivo.

In order to enhance effectivity of nucleic acids, especially to prolong the half-life-time in vivo when applied to an individ-

ual several attempts have been made in the prior art. For example in nucleic acid vaccination, sophisticated and complicated packaging systems have been proposed for the nucleic acid, e.g. micro-spheres, liposomes, virosomes, emulsions, micelles, etc. Such packaging systems have their merits mainly in delivering nucleic acids into the cells but not for efficiently and directly stabilising of nucleic acids, especially DNA. On the other hand, modifications of the nucleic acids, especially in the phosphate backbone, have been reported to be effective in prolonging the half-time of such molecules (see e.g. US 5,663,153 and US 5,723,335). Such molecules having at least one phosphorothioate bond, however, are unnatural substances providing a risk, especially when applied to humans, both with respect to potential degradation products as well as with respect to accumulation of such substances, if applied over prolonged time range. Moreover, the potency and safety of such modified nucleic acids in humans remain to be established.

The object of the present invention therefore is to provide a method for stabilisation, especially in vivo stabilisation of nucleic acids, especially DNA, to be applied in a pharmaceutical context.

This object is solved by a method for stabilisation of nucleic acids characterised in that nucleic acids are contacted with a polycationic polymer. Preferred polycationic polymers according to the present invention are polycationic peptides, especially polyarginine, polylysine and similar compounds.

It could surprisingly be shown with the present invention that polycationic polymers, preferably a polycationic peptide, especially a poly amino acid, such as polyarginine have protecting and stabilising effect on nucleic acids, especially nucleic acids with naturally occurring phosphodiester bonds. The present invention is therefore especially suited for nucleic acids lacking an artificial modification in their phosphate backbone. A preferred embodiment of the present invention is therefore aimed at a method for stabilising deoxyribonucleic acids comprising diphosphate groups between the nucleoside residues (i.e. lacking phosphorothioate bonds).

Although also modified nucleic acids may be used according to the present invention, the efficacy of the present invention is especially evident in the application of nucleic acid applica-

tion in medicine with nucleic acids having an unmodified backbone; especially with unmodified DNA and RNA.

According to another aspect the present invention also relates to the use of a polycationic polymer, preferably a polycationic peptide, especially polyarginine and the like for stabilising nucleic acids. As stated above the use according to the present invention is especially suitable for stabilising nucleic acids comprising phosphodiester groups between the nucleoside residues, especially deoxyribonucleic acids.

It has surprisingly been found out that the chemistry of the nucleic acids (e.g. the ODNs) is of significantly higher importance than the sequence (e.g. CpG motifs). Therefore, with the present invention better results in the specific activity of nucleic acids in their natural form are achieved compared to the phosphorothioate modified molecules, although in the prior art the latter ones were held more effective.

The present invention is especially suitable, if stabilisation should occur in an aqueous solution or suspension, preferably in a solution or suspension to be applied ("ready to use") to an individual, especially e.g. in a vaccine as in a gene therapy or antisense drug.

The aqueous solution or suspension can additionally comprise buffer substances, pharmaceutical excipients and carriers, further stabilisers, further drugs, etc., especially those auxiliary substances known in the art to be applied together with the mixture of nucleic acids with polycationic polymers.

It has been shown according to the present invention that the use of a polycationic polymer, preferably a polycationic peptide, according to the present invention allows a prolongation of the in vivo half-life of the nucleic acid applied to an individual, regardless of the specific application, i.e. regardless whether the nucleic acid is applied e.g. as specific "naked" DNA vaccine, as a general immunostimulant, as an antisense drug or gene therapy drug. This enables a long term effectivity of such drugs based on nucleic acids, such as DNA, RNA or PNA.

The polycationic compound(s) to be used according to the present invention may be e.g. any polycationic compound which shows the characteristic effect according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyaminoacids or mixtures

thereof. These polyaminoacids should have a chain length of at least 4 amino acid residues. Especially preferred are substances containing peptidic bounds, like polylysine, polyarginine and polypeptides containing more than 20%, especially more than 50% of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositions are described in WO 97/30721 (e.g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly)peptides may also be polycationic anti-bacterial microbial peptides. These (poly)peptides may be of prokaryotic or animal or plant origin or may be produced chemically or recombinantly or derived from natural sources. Peptides may also belong to the class of defensins.

Such host defense peptides or defensives are also a preferred form of the polycationic polymer according to the present invention. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substance in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (A 1416/2000, incorporated herein by reference), especially antimicrobial peptides derived from mammal cathelicidin, preferably from human, bovine or mouse, or neuroactive compounds, such as (human) growth hormone (as described e.g. in WO01/24822).

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin, especially mouse, bovine or especially human cathelins and/or cathelicidins. Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modi-

fication of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen/vaccine composition according to the present invention. However, these cathelin molecules surprisingly have turned out to be also effective as an adjuvant for an antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KLK-motifs separated by a linker of 3 to 7 hydrophobic amino acids, especially leucine (A 1789/2000, incorporated herein by reference).

In any way, the polycationic compounds, especially the peptides to be used according to the present invention should not be immunogenic by themselves (i.e. not eliciting an immune response) but only assist in immune response.

The invention is further described by means of the following examples and drawing figures, however, not to be restricted thereto.

Fig. 1 shows the generation of specific immune responses with pR and CpG containing ODNs;

Fig. 2 shows the generation of specific immune responses with pR and deoxy I or deoxy U containing ODNs;

Fig. 3 shows the generation of specific immune responses with pR and a cocktail of deoxy-U containing ODNs;

Fig. 4 shows the generation of specific immune responses with pR and ODNs;

Fig. 5 shows the induction of long lasting immune responses with pR;

Fig. 6 shows the generation of specific immune responses with KLK and deoxy I containing ODNs;

Fig. 7 shows the generation of specific immune responses with KLK and deoxy I containing ODNs;

Fig. 8 shows a 1% agarose gel of DNA treated by DNases in the presence or absence of pR.

E X A M P L E S**Example 1**

Generation of specific immune responses against an ovalbumin-derived peptide (OVA₂₅₇₋₂₆₄) with pR and CpG-containing oligodeoxynucleotide CpG 1668 (not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	OVA ₂₅₇₋₂₆₄ -Peptide (SIINFEKL), a MHC class I (H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity Dose: 300µg/mouse
Poly-L-arginine (pR)	Poly-L-arginine with an average degree of polymerization of 43 arginine residues; SIGMA chemicals Dose: 100µg/mouse
CpG-ODN 1668	thiophosphate substituted ODNs containing CpG-motif: tcc atg acg ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse
CpG-ODN 1668 b	ODNs containing CpG-motif (not substituted with thiophosphate): tcc atg acg ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse

Experimental groups (4 mice per group)

1. OVA₂₅₇₋₂₆₄ + pR

2. OVA₂₅₇₋₂₆₄ + CpG-ODN 1668
3. OVA₂₅₇₋₂₆₄ + CpG-ODN 1668 b
4. OVA₂₅₇₋₂₆₄ + CpG-ODN 1668 + pR
5. OVA₂₅₇₋₂₆₄ + CpG-ODN 1668 b + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 μ l (50 μ l per footpad) containing the above-mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph nodes were passed through a 70 μ m cell strainer and washed twice with DMEM medium (GIBCO BRL) containing 5% fetal calf serum (FCS, SIGMA chemicals). Cells were adjusted to the appropriate cell number in DMEM/5%/FCS. An IFN- γ ELISPOT assay was carried out in triplicates as described (Miyahira et al., 1995). This method is a widely used procedure allowing the quantification of antigen-specific T cells. Lymphocytes were stimulated ex vivo in triplicates with medium (background-control), OVA₂₅₇₋₂₆₄-peptide, an irrelevant peptide TRP-2₁₈₁₋₁₈₈ and Concanavalin A (Con A). Spots representing single IFN- γ producing T cells were counted and the number of background spots was subtracted from all samples. The high number of spots detected after the stimulation with Con A (data not shown) indicates a good condition of the used lymphocytes. For each experimental group of mice the number of IFN- γ -producing cells/ 1×10^6 lymph node cells are illustrated in Figure 1, the standard deviation of ex vivo-stimulated triplicates is given.

This experiment shows that the injection of OVA₂₅₇₋₂₆₄ with thiophosphate substituted CpG-ODN 1668 enhances OVA₂₅₇₋₂₆₄-specific immune responses compared to the injection of OVA₂₅₇₋₂₆₄ in combination with poly-L-arginine. The co-injection of poly-L-arginine strongly enhances the CpG-ODN 1668-induced immune response. In contrast, when CpG-ODN 1668 b, which is not substituted with thiophosphates, was used, only upon co-injection of poly-L-arginine a high immune response was induced.

Example 2

Generation of specific immune responses against a melanoma-derived peptide (TRP-2₁₈₁₋₁₈₈) with pR and deoxy-Inosine modified oligonucleotide I-ODN 2b (phosphodiester bonds, not substituted with

thiophosphate) or deoxy-Uridine monophosphate modified oligonucleotide U-ODN 13b (phosphodiester bonds, not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	TRP-2-peptide ₁₈₁₋₁₈₈ (VYDFVWL), a MHC class I (H-2Kb)-restricted epitope of mouse tyrosinase related protein-2 (B16 melanoma, Bloom, M.B. et al., J Exp. Med 1997, 185, 453-459), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity Dose: 100µg/mouse
Poly-L-arginine (pR)	Poly-L-arginine with an average degree of polymerization of 43 arginine residues; SIGMA chemicals Dose: 100µg/mouse
I-ODN 2	thiophosphate substituted ODNs containing deoxy-Inosine: tcc atg aci ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse
I-ODN 2b	ODNs containing deoxy-Inosine (not substituted with thiophosphate): tcc atg aci ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse

- 9 -

U-ODN 13 thiophosphate substituted ODNs containing deoxy-Uridine monophosphate:
tcc atg acu ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen.
Dose: 5nmol/mouse

U-ODN 13b ODNs containing deoxy-Uridine monophosphate (not substituted with thiophosphate):
tcc atg acu ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen.
Dose: 5nmol/mouse

Experimental groups (4 mice per group)

1. TRP-2₁₈₁₋₁₈₈
2. TRP-2₁₈₁₋₁₈₈ + pR
3. TRP-2₁₈₁₋₁₈₈ + I-ODN 2
4. TRP-2₁₈₁₋₁₈₈ + I-ODN 2 b
5. TRP-2₁₈₁₋₁₈₈ + U-ODN 13
6. TRP-2₁₈₁₋₁₈₈ + U-ODN 13 b
7. TRP-2₁₈₁₋₁₈₈ + I-ODN 2 + pR
8. TRP-2₁₈₁₋₁₈₈ + I-ODN 2 b + pR
9. TRP-2₁₈₁₋₁₈₈ + U-ODN 13 + pR
10. TRP-2₁₈₁₋₁₈₈ + U-ODN 13 b + pR

The injections of mice and the analysis of the immune response were performed as described in example 1. Results are shown in Fig. 2. For ex vivo restimulation of lymphnode cells TRP-2₁₈₁₋₁₈₈ was used as specific peptide, OVA₂₅₇₋₂₆₄ as irrelevant peptide.

This experiment shows that the injection of TRP-2₁₈₁₋₁₈₈ with thiophosphate substituted I-ODNs or U-ODNs strongly enhances TRP-2₁₈₁₋₁₈₈-specific immune responses compared to the injection of TRP-2₁₈₁₋₁₈₈ alone. The co-injection of poly-L-arginine with the respective ODN further enhances these responses. In contrast, when I-ODN 2b or U-ODN 13b, which are not substituted with thiophos-

- 10 -

phates, were used, only upon co-injection of poly-L-arginine a strong immune response was induced.

Example 3

Generation of specific immune responses against a melanoma-derived peptide (TRP-2₁₈₁₋₁₈₈) with pR and a cocktail of deoxy-Uridine monophosphate modified oligonucleotides (U-ODN 15b, not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	TRP-2-peptide ₁₈₁₋₁₈₈ (VYDFVWL), a MHC class I (H-2Kb)-restricted epitope of mouse tyrosinase related protein-2 (B16 melanoma, Bloom, M.B. et al., J Exp. Med 1997, 185, 453-459), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity Dose: 100µg/mouse
Poly-L-arginine (pR)	Poly-L-arginine with an average degree of polymerization of 43 arginine residues; SIGMA chemicals Dose: 100µg/mouse
U-ODN 15 b	Cocktail of ODNs containing deoxy-Uridine (not substituted with thiophosphate): nhh hhh wdu dhh hhh hhh wn, were synthesized by Purimex GmbH, Göttingen. (n = GCAT, h = CAT, w = AT, d = GAT) Dose: 5nmol/mouse

Experimental groups (4 mice per group)

- 11 -

1. TRP-2₁₈₁₋₁₈₈
2. TRP-2₁₈₁₋₁₈₈ + pR
3. TRP-2₁₈₁₋₁₈₈ + U-ODN 15 b
4. TRP-2₁₈₁₋₁₈₈ + U-ODN 15 b + pR

The injections of mice and the analysis of the immune response were performed as described in example 1. Results are shown in Fig. 3. For ex vivo restimulation of lymphnode cells TRP-2₁₈₁₋₁₈₈ was used as specific peptide, OVA₂₅₇₋₂₆₄ as irrelevant peptide.

Upon co-injection of TRP-2₁₈₁₋₁₈₈, pR and a cocktail of U-ODNs not substituted with thiophosphates (U-ODN 15b) the TRP-2₁₈₁₋₁₈₈-specific immune responses is strongly enhanced compared to the injection of TRP-2₁₈₁₋₁₈₈ alone or TRP-2₁₈₁₋₁₈₈ with pR or U-ODN 15b, respectively.

Example 4

Melanoma-derived peptide (TRP-2₁₈₁₋₁₈₈)-specific immune responses induced by pR and different oligodeoxynucleotides (phosphodiester bonds, not substituted with thiophosphate) are long lasting.

Mice	C57Bl/6 (Harlan/Olac)
Peptide	TRP-2-peptide ₁₈₁₋₁₈₈ (VYDFFVWL), a MHC class I (H-2Kb)-restricted epitope of mouse tyrosinase related protein-2 (B16 melanoma, Bloom, M.B. et al., J Exp. Med 1997, 185, 453-459), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity
	Dose: 100µg/mouse
Poly-L-arginine (pR)	Poly-L-arginine with an average degree of polymerization of 43 arginine residues; SIGMA chemicals
	Dose: 100µg/mouse

- 12 -

CpG-ODN 1668

thiophosphate substituted ODNs containing CpG-motif:
tcc atg acg ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen.

Dose: 5nmol/mouse

U-ODN 13

thiophosphate substituted ODNs containing deoxy-Uridine monophosphate:
tcc atg acu ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen.

Dose: 5nmol/mouse

U-ODN 13 b

ODNs containing deoxy-Uridine monophosphate (not substituted with thiophosphate):
tcc atg acu ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen.

Dose: 5nmol/mouse

U-ODN 15

Cocktail of thiophosphate substituted ODNs containing deoxy-Uridine:
nhh hhh wdu dhh hhh hhh wn, were synthesized by Purimex GmbH, Göttingen. (n = GCAT, h = CAT, w = AT, d = GAT)

Dose: 5nmol/mouse

U-ODN 15 b

Cocktail of ODNs containing deoxy-Uridine (not substituted with thiophosphate):
nhh hhh wdu dhh hhh hhh wn, were synthesized by Purimex GmbH, Göttingen. (n = GCAT, h = CAT, w = AT, d = GAT)

Dose: 5nmol/mouse

Experimental groups (5 mice per group)

1. TRP-2₁₈₁₋₁₈₈
2. TRP-2₁₈₁₋₁₈₈ + pR
3. TRP-2₁₈₁₋₁₈₈ + CpG 1668
4. TRP-2₁₈₁₋₁₈₈ + U-ODN 13
5. TRP-2₁₈₁₋₁₈₈ + U-ODN 13b
6. TRP-2₁₈₁₋₁₈₈ + U-ODN 15
7. TRP-2₁₈₁₋₁₈₈ + U-ODN 15b
8. TRP-2₁₈₁₋₁₈₈ + pR + CpG 1668
9. TRP-2₁₈₁₋₁₈₈ + pR + U-ODN 13
10. TRP-2₁₈₁₋₁₈₈ + pR + U-ODN 13b
11. TRP-2₁₈₁₋₁₈₈ + pR + U-ODN 15
12. TRP-2₁₈₁₋₁₈₈ + pR + U-ODN 15b

The injections of mice were performed as described in example 1. Results are shown in Fig. 4. For the analysis of the immune response at day 40 after injection single cell suspensions of spleen cells were prepared. Spleen cells were restimulated ex vivo with TRP-2₁₈₁₋₁₈₈ as specific peptide, OVA₂₅₇₋₂₆₄ as irrelevant peptide and ConA.

The injection of TRP-2₁₈₁₋₁₈₈ with pR, CpG 1668, U-ODN 13 or a cocktail of thiophosphate substituted U-ODNs (U-ODN 15) enhances TRP-2₁₈₁₋₁₈₈-specific immune responses compared to the injection of TRP-2₁₈₁₋₁₈₈ alone. The co-injection of poly-L-arginine with CpG 1668 or U-ODN 13 increases this response significantly (only slightly in the case of U-ODN 15). In contrast, when U-ODN 13 or U-ODN 15 b, which are not substituted with thiophosphates, were used, only upon co-injection of poly-L-arginine a high immune response was induced. Remarkably, the response after injection of pR/U-ODN 15b (not substituted with thiophosphate) significantly exceeds the response induced by pR/U-ODN15 (substituted with thiophosphate).

Example 5

Induction of long lasting specific immune responses against a melanoma-derived peptide (TRP-2₁₈₁₋₁₈₈) upon co-injection of TRP-2₁₈₁₋₁₈₈ with pR and CpG-containing oligodeoxynucleotide CpG 1668b

(phosphodiester bonds, not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	TRP-2-peptide ₁₈₁₋₁₈₈ (VYDFFVWL), a MHC class I (H-2Kb)-restricted epitope of mouse tyrosinase related protein-2 (B16 melanoma, Bloom, M.B. et al., J Exp. Med 1997, 185, 453-459), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity Dose: 100µg/mouse
Poly-L-arginine (pR)	Poly-L-arginine with an average degree of polymerization of 43 arginine residues; SIGMA chemicals Dose: 100µg/mouse
CpG-ODN 1668 b	ODNs containing CpG-motif (not substituted with thiophosphate): tcc atg acg ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse

Experimental groups (5 mice per group)

1. TRP-2₁₈₁₋₁₈₈
2. TRP-2₁₈₁₋₁₈₈ + pR
3. TRP-2₁₈₁₋₁₈₈ + CpG 1668 b
4. TRP-2₁₈₁₋₁₈₈ + pR + CpG 1668 b

The injections of mice were performed as described in example 1. Results are shown in Fig. 5. For the analysis of the immune response at day 6, 49 and 98 after injection single cell suspensions of spleen cells were prepared. Spleen cells were restimulated ex vivo with TRP-2₁₈₁₋₁₈₈ as specific peptide, OVA₂₅₇₋₂₆₄ as irrelevant peptide (data not shown) and ConA.

Co-injection of TRP-2₁₈₁₋₁₈₈, pR and CpG 1668b results in significantly higher numbers of peptide-specific IFN-g producing cells compared to injection of TRP-2₁₈₁₋₁₈₈ with pR or CpG 1668b, respectively. This is not only observable at day 6 but also at later time points (day 49, day 98) after single injection, indicating a potent and long lasting immune stimulatory effect of the combination pR/CpG 1668 b.

Example 6

Generation of specific immune responses against an OVA₂₅₇₋₂₆₄-peptide with KLK and deoxy-Inosine modified oligonucleotide I-ODN 2b (phosphodiester bonds, not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	OVA ₂₅₇₋₂₆₄ -Peptide (SIINFEKL), a MHC class I (H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity Dose: 300µg/mouse
KLK	KLKLLLLLKLK-COOH Dose: 168µg/mouse
I-ODN 2 b	ODNs containing deoxy-Inosine (not substituted with thiophosphate): tcc atg aci ttc ctg atg ct, were synthesized by Purimex GmbH, Göttingen. Dose: 5nmol/mouse

Experimental groups (5 mice per group)

1. OVA₂₅₇₋₂₆₄ + KLK
2. OVA₂₅₇₋₂₆₄ + I-ODN 2b
3. OVA₂₅₇₋₂₆₄ + KLK + I-ODN 2b

The injections of mice were performed as described in example 1. Results are shown in Fig. 6. For the analysis of the immune response at day 7 after injection blood was taken and peripheral blood mononuclear cells were isolated by Ficoll-gradient. PBMCs were restimulated ex vivo using OVA₂₅₇₋₂₆₄ as specific peptide, TRP-2₁₈₁₋₁₈₈ as irrelevant peptide and ConA.

Injection of OVA₂₅₇₋₂₆₄ with KLK or I-ODN 2b induces no peptide-specific IFN- γ -production. However, upon co-injection of OVA₂₅₇₋₂₆₄, KLK and I-ODN 2b high numbers of OVA₂₅₇₋₂₆₄-specific IFN- γ producing T cells are detectable.

Example 7

Generation of specific immune responses against a melanoma-derived peptide (TRP-2₁₈₁₋₁₈₈) with KLK and a deoxy-Inosine modified oligonucleotide I-ODN 2b (phosphodiester bonds, not substituted with thiophosphate).

Mice	C57Bl/6 (Harlan/Olac)
Peptide	TRP-2-peptide ₁₈₁₋₁₈₈ (VYDFFVWL), a MHC class I (H-2Kb)-restricted epitope of mouse tyrosinase related protein-2 (B16 melanoma, Bloom, M.B. et al., J Exp. Med 1997, 185, 453-459), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity
	Dose: 100 μ g/mouse
KLK	KLKLLLLLKLK-COOH
	Dose: 168 μ g/mouse

- 17 -

I-ODN 2 b

ODNs containing deoxy-Inosine
(not substituted with thiophosphate):

tcc atg aci ttc ctg atg ct, were
synthesized by Purimex GmbH,
Göttingen.

Dose: 5nmol/mouse

Experimental groups (5 mice per group)

1. TRP-₁₈₁₋₁₈₈
2. TRP-₁₈₁₋₁₈₈ + KLK
3. TRP-₁₈₁₋₁₈₈ + I-ODN 2b
4. TRP-₁₈₁₋₁₈₈ + KLK + I-ODN 2b

The injections of mice were performed as described in example 1. Results are shown in Fig. 7. For the analysis of the immune response at day 7 after injection blood was taken and peripheral blood mononuclear cells were isolated by Ficoll-gradient. PBMCs were restimulated ex vivo using TRP-₂₁₈₁₋₁₈₈ as specific peptide, OVA₂₅₇₋₂₆₄ as irrelevant peptide and ConA.

Injection of TRP-₂₁₈₁₋₁₈₈ alone or in combination with KLK or I-ODN 2b induces no peptide-specific IFN- γ -production. In contrast, upon co-injection of TRP-₂₁₈₁₋₁₈₈, KLK and I-ODN 2b very high numbers of peptide-specific IFN- γ -producing T cells are detectable.

Example 8

Poly-L-arginine prevents degradation of DNA by serum DNases

To investigate the protective effect of pR in preventing the degradation of DNA by DNases the following in vitro experiment was performed. To show a possible in vivo relevance serum was used as source of DNases.

The DNA-plasmid pSP65 (1,58 μ g/2 μ l) was incubated with serum (8 μ l) from naïve mice in the presence or absence of poly-L-arginine (2,4 μ g/2,4 μ l) for 1h at 37°C. Addition of heparin (1,6U / 1,6 μ l) for 20min at RT was used for the dissociation of DNA/pR-complexes allowing DNA to migrate into the gel. Figure 8 shows an

- 18 -

1% agarose gel (30min / 100Volt). As control a 100bp DNA marker (GIBCO BRL) was used.

Typical circular plasmid supercoiled/coiled/relaxed band pattern is observable for untreated pSP65 DNA. Incubation of DNA with serum results in degradation, detected as a smear of various random DNase cleavage products. This degradation by serum DNases is prevented upon addition of poly-L-arginine.

Thus, poly-L-arginine stabilizes DNA against enzymatic degradation and prolongs DNA half life and bioavailability.

Claims:

1. Method for stabilisation of nucleic acids characterised in that nucleic acids are contacted with a polycationic polymer.
2. Method according to claim 1, characterised in that said nucleic acid is a deoxyribonucleic acid comprising diphosphate groups between the nucleoside residues.
3. Use of a polycationic polymer for stabilisation of nucleic acids.
4. Use according to claim 3 for stabilising a deoxyribonucleic acid comprising phosphodiester groups between the nucleoside residues.
5. Use according to claim 3 or 4, characterised in that said nucleic acids are stabilised in an aqueous solution or suspension.
6. Use according to claim 5, characterised in that said polycationic polymer is a polycationic peptide, preferably polypeptides containing more than 20%, especially more than 50% of basic amino acids in a range of more than 8 amino acid residues.
7. Use according to any one of claims 3 to 6, characterised in that the in vivo half-life of a nucleic acid applied to an individual is prolonged.

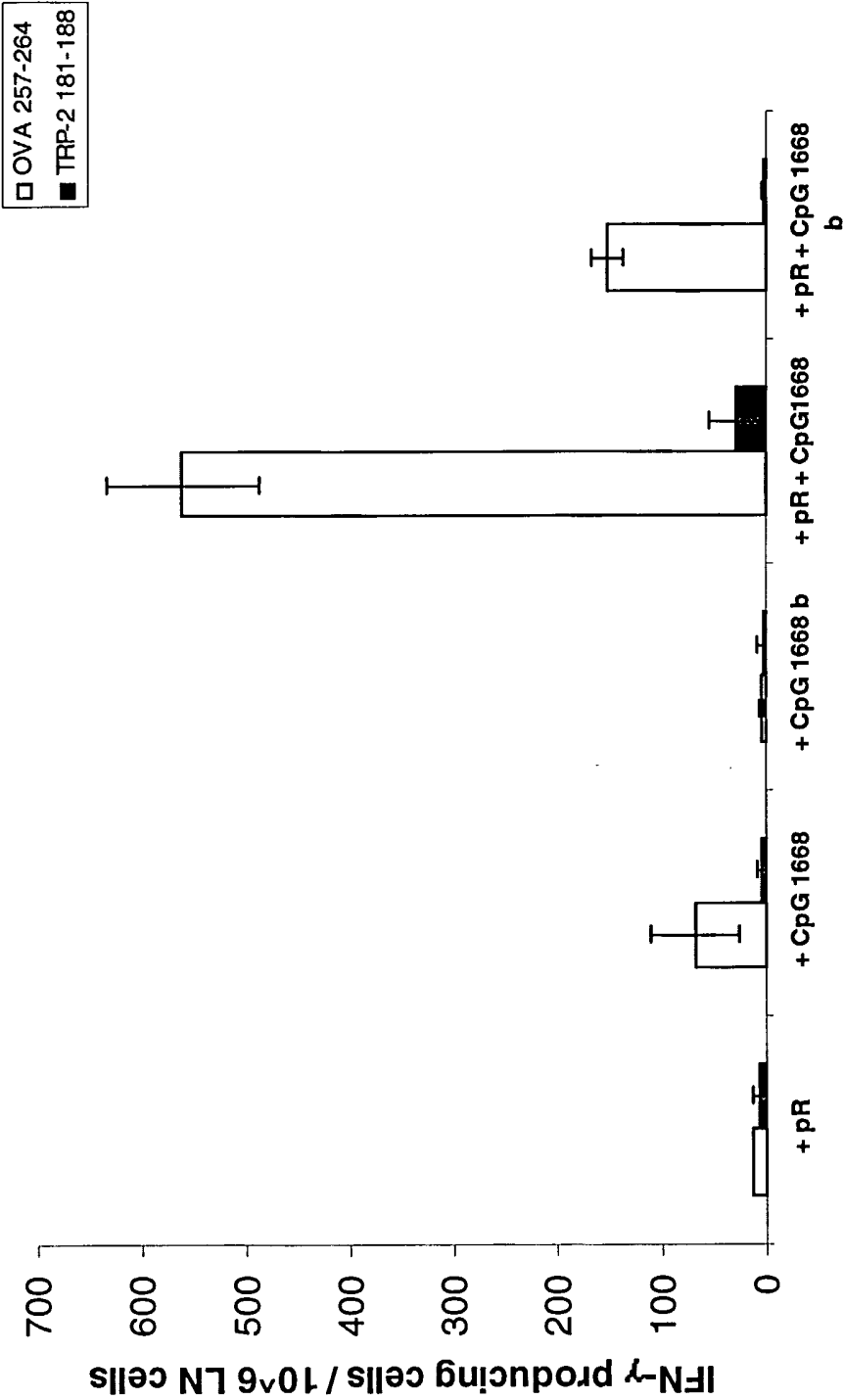


Fig 1
injection of OVA 257-264

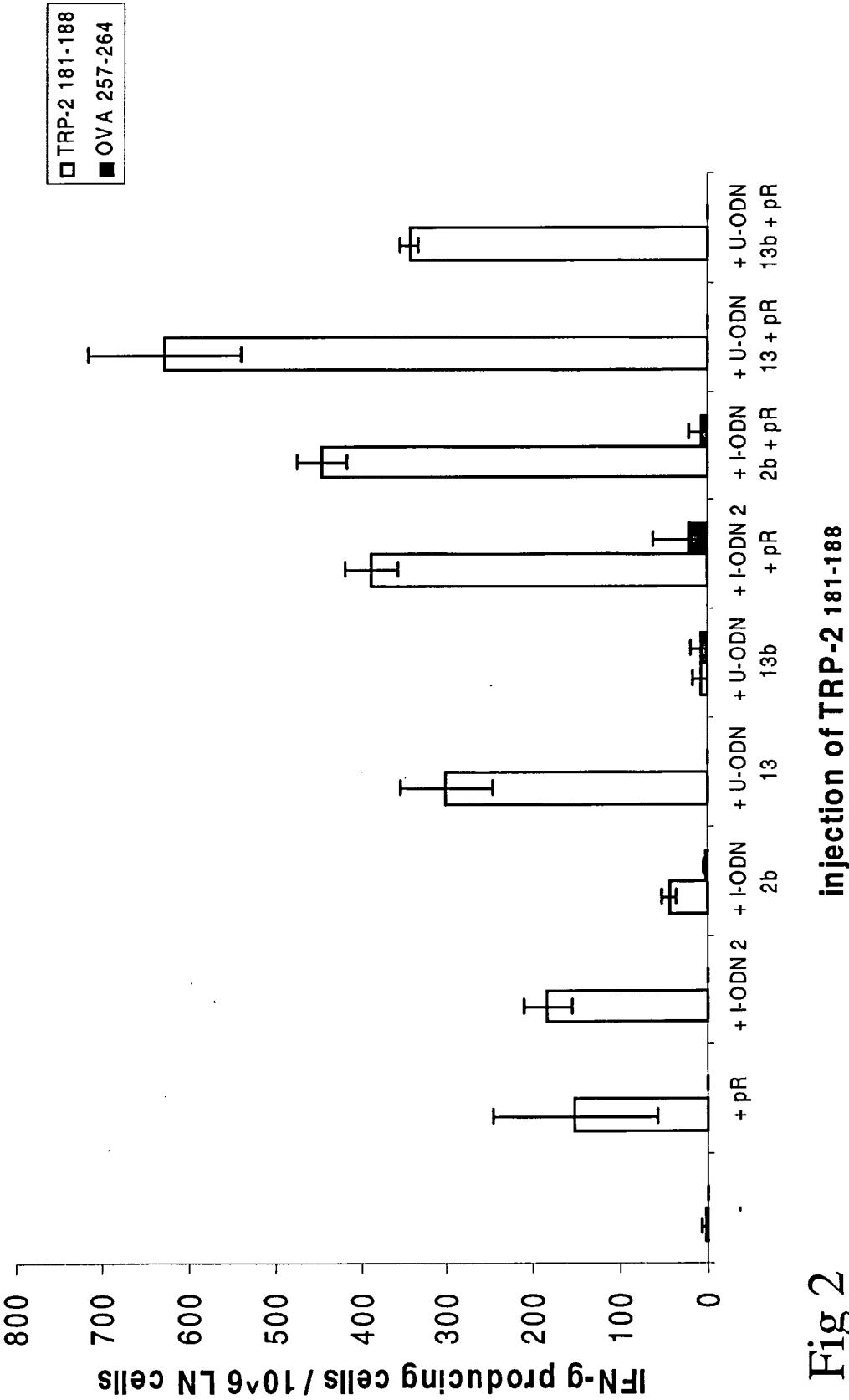


Fig 2

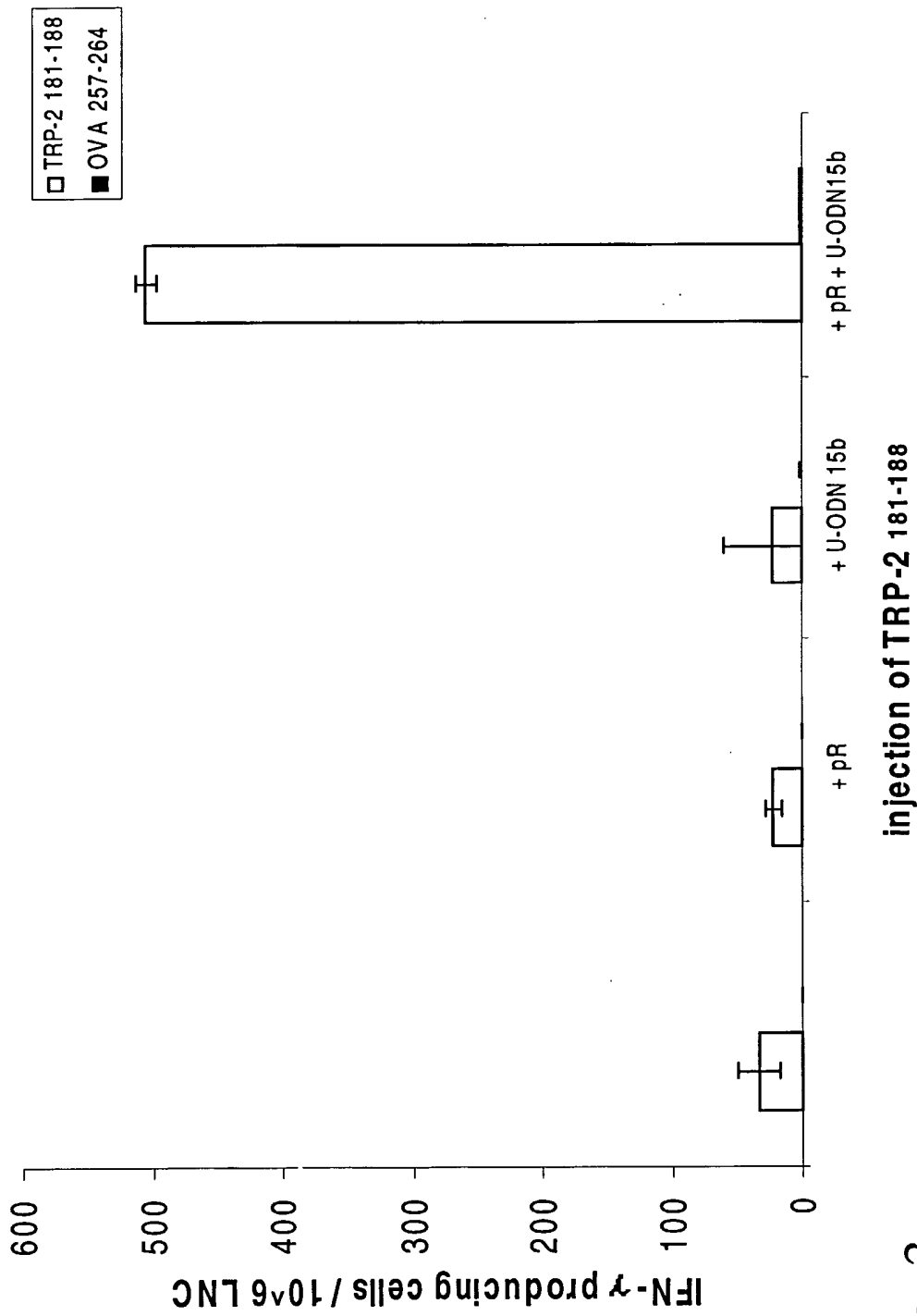


Fig. 3

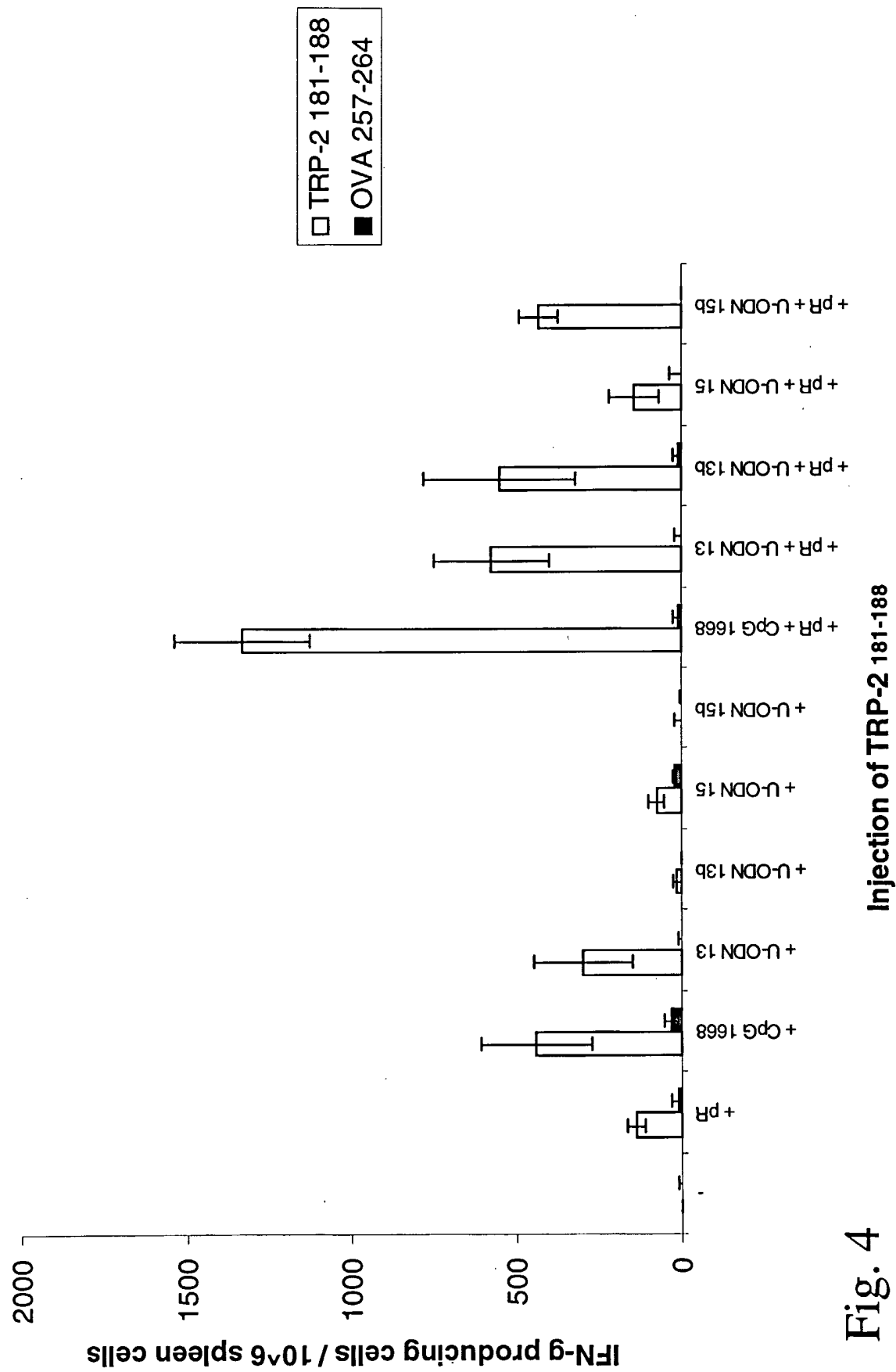


Fig. 4

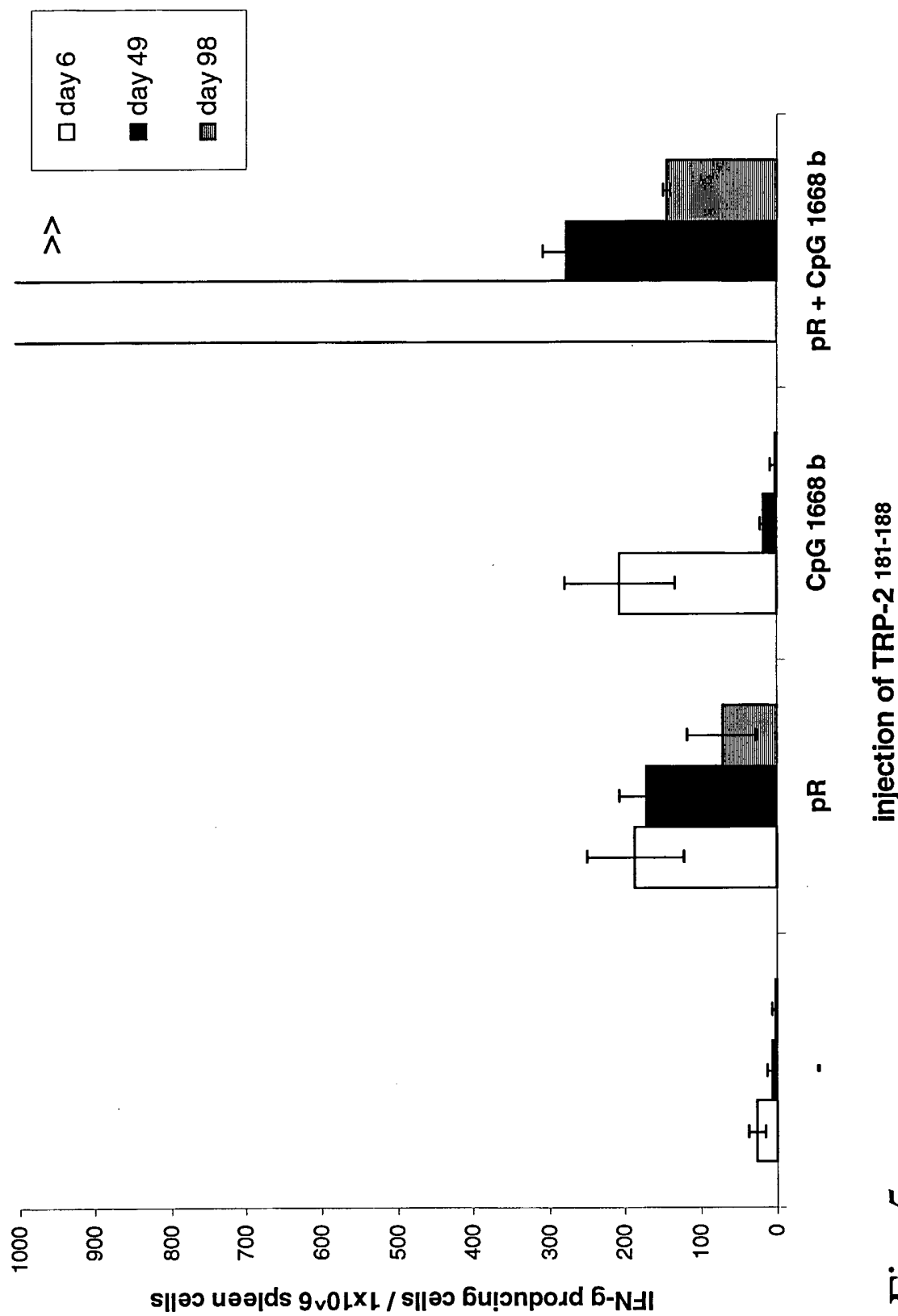


Fig. 5

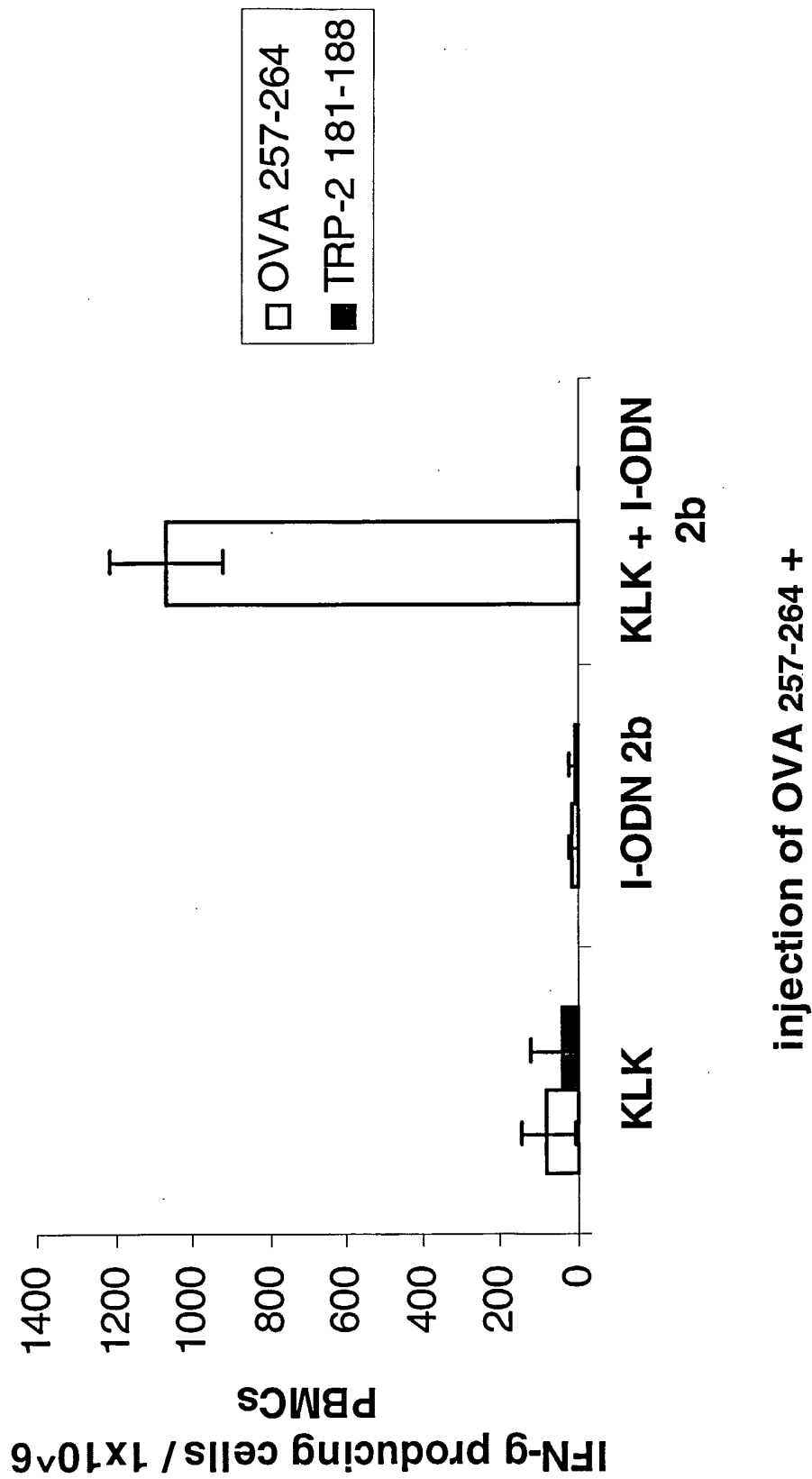


Fig. 6

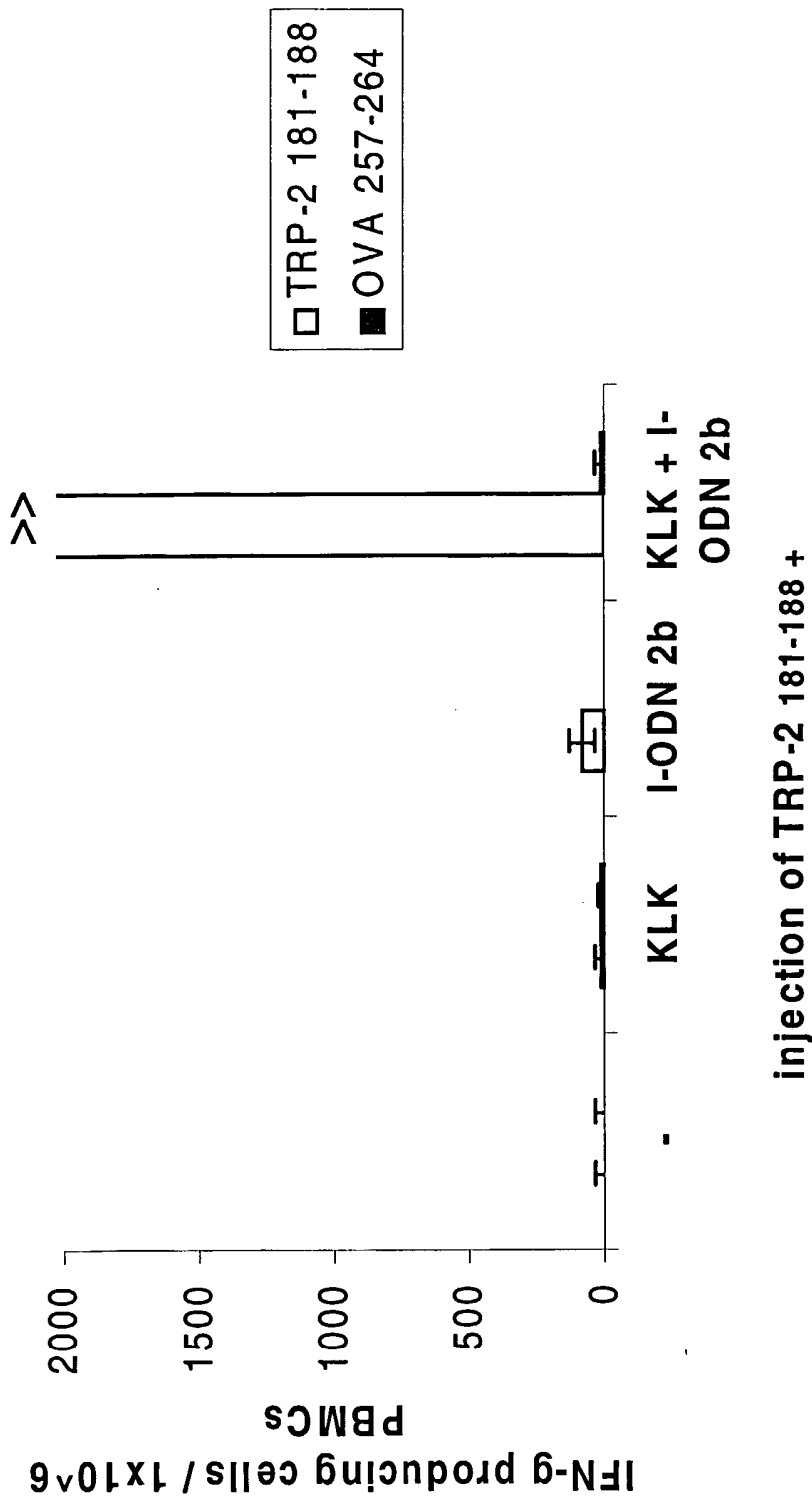


Fig. 7

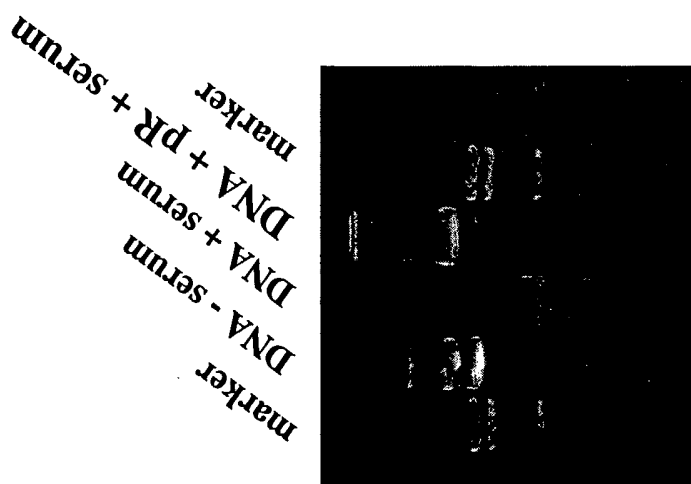


Fig. 8