Development in Lipid Drugs

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Abstract: Lipopeptide lipid moieties induce dendritic cell (DC) internalization and epitopes are recognized by MHC, the major histocompatibility complex. HIV-1 (human immunodeficiency virus type 1) lipopeptide vaccine candidate elicits immune responses, and sustains HIV control after highly active antiretroviral therapy (HAART). Mp- and Dp-MART (anti-melanoma lipopeptides) induce strong CTL (cytolytic T lymphocyte) response. New BGTC, BGDA, TGKC lipoplexes mediate gene delivery, e.g., into mouse pancreatic tumor nodules. Triterpene glycyrrhizic acid (GL) inhibits SARS-CoV (severe acute respiratory syndrome associated coronavirus) replication. Compared to CDV (cidofovir), CDV ether lipid esters have enhanced activity against vaccinia (VV) and cowpox (CV) viruses *in vitro*. Oral treatment of VV and CV infected mice with CDV ether lipid esters, as effective as i.p. CDV, may be useful against orthopoxvirus infections in humans.

Keywords: BGTC, dendritic cells, HIV, gene transfection, lipopeptides, lipoplexes, orthopoxvirus, SARS.

INTRODUCTION

As emphasized in a recent review [1]. ''In the twenty years since the isolation of HIV-1, modern drug discovery and development have transformed HIV-1 disease into a treatable, chronic infectious disease, although issues of resistance to current therapy and viral reservoirs remain''...''despite all of the setbacks and obstacles, new agents such as fusion, entry and integrase inhibitors, improved drug discovery processes and better understanding of both HIV-1 virology and general immunology are likely to accelerate the pace of development of more potent therapeutic regimens and, eventually, of a prophylactic vaccine''.

The present article is essentially aimed at reviewing two large domains of medicinal chemistry, both involved in lipid prodrug therapy, though different in construction and mechanism:

i., lipopeptides, candidates for retroviral vaccinotherapy, ii., supramolecular constructs, already recognized as particularly efficient for gene transfection, which also show features for other therapeutic purposes. Recent development in other lipophilic drugs and prodrugs will be also mentioned.

In a previous review [2] concerning interrelationships between retroviral and cellular lipids, it was assumed that it may be presumptuous to expect more from lipid antiviral therapy, at a time when gene therapy could efficiently complement available anti-HIV therapy. However, according to H. Gras-Masse (personal communication), "the very peculiar trafficking properties of lipopeptides, which among others, confer the capacity to vectorize antigens into *ad hoc* presentation pathways are linked to interactions with cellular lipids''. Immunogenicity can be obtained from anti-retroviral peptides, made up either of a single chain peptide or of numerous long amino acid sequences derived from regulatory and structural HIV-1 proteins. Further, a vigorous virus-specific CTL (cytolytic T lymphocytes) response was elicited, simply by adding a lipid tail at one end of the sequences [3-5]. Consequently, the potential selective delivery of these antigens to dendritic cells (DCs), the professional antigen-presenting cells (APCs), is a promising approach for the improvement of vaccine efficacity.

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I. LIPOPEPTIDES

1.1. Presentation Pathways

The presentation pathway of lipopeptides derived from an HLA-A2.1-restricted HIV-1 reverse transcriptase (RT) epitope was studied in human DCs using confocal microscopy and functional T lymphocyte recognition assays. To follow entry into human monocyte derived-DCs, rhodamine-labeled analogs of the RT 476-484-peptide and -lipopeptide were used. The lipid moiety of the short lipopeptide derived from the HIV-1 RT epitope with N-terminal addition of palmitoyl-lysine, induced its internalization by endocytosis into human DCs, and the epitope was recognized in association with HLA-A2.1, presumably through an intracellular cross-presentation pathway [6].

To define the cross-presentation pathway and to generalize these previous results to more than one MHC class I molecule, longer lipopeptides were then used, derived from the HIV-1 Pol 461-484 and Nef 66-97 sequences by terminal addition of palmitoyl-lysine. To determine the intracellular pathway of lipopeptide presentation and degradation by proteasome, antigen-processing inhibitors were used: monensin, brefeldin, epoxomycin and lactacystin. Like the short model lipopeptide [6], the longer Pol 461-484 lipopeptide was internalized by endocytosis and then degraded by proteasome, following an endosome-to-cytosol pathway, a classical cross-presentation pathway. This pathway seems to be restricted to DCs, filling a requirement for presentation of exogenous antigens to CD8(+) effector T lymphocytes. The pathway used by the Nef 66-97 lipopeptide was different. This lipopeptide did not depend

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on proteasome for processing. This independence could be an intrinsic property of the polypeptide sequence - though the palmitoyl moiety attachment at the Pol peptide N-terminus, or at the C-terminus of the Nef construct can make a difference. Therefore, the association with MHC class I molecules is possible in late endosomes in DCs, which contain MHC class I as well as MHC class II molecules [7].

1.2. Clinical Trials

1.2.1. Tolerability as a Vaccine Candidate

The clinical tolerability of a HIV-1 vaccine candidate was determined. This HIV-1 vaccine candidate was based on six different 24- to 32-amino acid sequences derived from regulatory or structural HIV-1 proteins (Nef, Gag and Env). The six sequences were modified in the C-terminal by adding a palmitoyl-lysylamide group and formulated as lyophilized mixed-micelles [3,4, and Appendix]. Vac 04 ANRS was a phase I, randomized, dose-escalating trial in seronegative volunteers. Intramuscular injection started with 100µg, then 250µg and finally 500µg of each of the six lipopeptides, at 0, 4 and 16 weeks. Sera were tested for IgG antibodies to Nef (N1, N2, N3), Gag (G1, G2), and Env (E) peptides. Specific CTL responses were observed in volunteers whether they received low or high doses of lipopeptides [8].

1.2.2. Booster Injections

Booster immunization efficiency was analyzed after a fourth injection, and the long-term immune response measured two years after the first injection. The lipopeptide vaccine contains immunodominant HIV-1 CD4(+) epitopes [9]. The amino acid sequences of the six lipopeptides are presented, as well as proliferation responses to Nef, Gag, and Env long peptides obtained from vaccinated subjects' PBMCs (peripheral blood mononuclear cells). Immunology data are reported for induced and sustained T cell responses to vaccination in HIV uninfected volunteers. After three injections (20 wk), IgG, CD4(+) and CD8(+) T-cells were detected in sera of 71-89% of the volunteers. After four injections (52 wk), 85% of the volunteers had a positive response to at least one of the immunological parameters tested. Distribution of anti-HIV immunoglobulin isotypes after lipopeptide vaccination was also determined. At 20 wk, antibodies elicited by three immunizations belonged predominantly to the IgG1 subclass. At 52 wk, IgG1, IgG2, IgG3, IgG4 and IgA were detected, indicating the production of cytokines from Th1 and Th2 cells. At 104 wk, a sustained CD8(+) T cell response was observed in more than 50% of the volunteers, with no significant difference between the two groups, with or without QS21 saponin adjuvant. CD4(+) and CD8(+) T cell epitopes included in the HIV-1 lipopeptide vaccine were efficiently processed in humans, and this might have important implications in immunotherapy [9].

1.2.3. Vaccinotherapy

Results concerning lipopeptide vaccinotherapy trials on HIV seropositive individuals (ANRS 093) were presented [10] at the 10th Conference on Retroviruses and Opportunistic Infections (Boston, Feb. 2003). Patients treated for at least one year with HAART (highly active antiretroviral therapy) were randomized to continue either HAART alone (37 patients) or combined with Lipo-6T (33 patients), a lipopeptide preparation of antigenic HIV fragments. ALVAC-VIH 1433, a recombinant canarypox vector, was administered at 0, 4, 8 and 12 wk and followed by 3 cycles of IL-2 at 16, 24, 32 wk to improve immunological function. At 36 wk, patients with low HIV RNA, were proposed to stop HAART (63 stopped at 40 wk). Twelve weeks after stopping, 5% of the HAART alone patients remained off antiretroviral therapy, compared to 24% of the vaccinated patients [10-12]. These results were reported at the 2nd Int. AIDS Society Conference on HIV Pathogenesis and Treatment (Paris, July 2003). Y. Lévy reminded us that HIV-1-specific immune responses are not entirely destroyed in primary infection during antiretroviral therapy. A lipopeptide vaccinotherapy can allow immune response to develop after interrupting HAART.

1.3. Immunization *via* Mucosal Route

Immunization *via* the mucosal surfaces, without adjuvant and without the use of needles, would greatly increase ease of vaccination [13]. The simple application of lipopeptides in mice to the nasal and buccal cavities resulted in efficient delivery to the central lymphoid system, evidenced by specific antibody and T cell responses in distant lymphoid organs. Compared to the dominant Th2 cytokines and IgG1 production obtained by the subcutaneous route, antibodies and T cell responses preferentially promoted Th1 cytokines and IgG2a antibody production and were of higher intensity after lipopeptide intranasal or sublingual administration.

1.4. Inhibition of HIV-1 Protease Dimerization

Active HIV-1 protease has a homodimeric structure, and subunits are connected by an interface beta-formed by its NH2- and COOH-terminal aminoacid segments. An approach to inhibit the dimeric HIV protease was to target this interface region to prevent subunit dimerization. Structure of the lipopeptides targeting the enzyme dimer interface has been rationally studied: preferably, C-terminal amino acid residues should be thyroxine and thyronine, plus 2-aminopalmitic acid, the N-terminal blocking group. The high inhibitory power of some lipopeptides [e.g., palmitoyl-Tyr-Glu-Leu-OH; palmitoyl-Tyr-Glu-(L-thyronine)-OH; palmitoyl-Tyr-Glu-(L-biphenyl-alanine)-OH], with low nanomolar Ki values in enzyme tests, suggests their good bio-availability. By targeting the enzyme hydrophobic pocket and interface antiparallel beta-sheet, relatively free of mutations contrary to its active site, these new lipopeptides appear to be efficient inhibitors of HIV-1 protease dimerization [14-16].

II. CATIONIC LIPID SUPRAMOLECULAR CONSTRUCTS

2.1. BGTC, a Combination of Bis-Guanidinium Group with Cholesterol

Synthetic nonviral vectors are an attractive alternative to viral vector gene therapy. As recently mentioned, nonviral vectors display a lower transfection efficiency, compared to viral systems, though various cationic lipids have been developed and display efficiency for gene transfection *in*

vitro. In contrast, their *in vivo* efficiency is often less satisfactory [17].

To alleviate this drawback, Lehn's group combined a bis-guanidinium group with cholesterol, and obtained BGTC (bis-guanidinium-tren-[tris(2-aminoethyl)amine]-cholesterol) (Fig. (**1**)) [18]. The spontaneous formation of DNA/lipid aggregates *in vitro* is due to ionic interactions between the positively charged cationic lipid and the negatively charged DNA phosphate groups. The guanidinium group appeared particularly well-suited for interaction with polynucleotide phosphate residues with which it is able to establish a characteristic pair of hydrogen bonds. At the molecular level, the rational underlying this approach was that the cationic lipid should combine the membrane-compatible features of the cholesterol subunit and the favorable features of the guanidinium group for DNA binding. As it is highly basic, a guanidinium group remains protonated over a wide range of pH, so DNA binding should be relatively insensitive to pH variations during *in vitro* generation of lipoplexes and their intracellular traffic. Guanidinum groups in arginine amino acid residues play a key role in DNA-binding proteins such as histones and protamines [18, 19]. Therefore, BGTC was assessed for transfection of various mammalian cell lines.

2.2. Advantages of the BGTC

Multiple studies indicate the usefulness of such transfection reagents, e.g., BGTC can be directly suspended in aqueous solutions. *In vitro*, for gene transfection of recombinant adenoviruses, BGTC can be used as a micellar solution and added to primary human airway epithelial cell cultures. *In vivo*, BGTC and DOPE (dioleoylphosphatidyle-thanolamine) liposomes are used for gene delivery to the mouse airway, because of DOPE fusogenic properties. Columnar cells were principally transfected in surface epithelium, but transgene expression was also detected in submucosal glands [20].

2.3. DNA Internalization. Endosomal Escape. Nuclear Entry

It is generally recognized that DNA complexes are internalized *via* receptor-mediated endocytosis. The lipid/DNA complexes have to overcome various barriers, from the extracellular medium to the nucleus of the target cell [21].

Lipoplexes have to escape from the endosomal compartment to avoid lysosomal degradation, and to be released into cytoplasm. DOPE, but not DOPC (dioleoylphosphatidylcholine) enhances DNA endosomal escape by facilitating endosomal disruption, possibly *via* fusion between the lipoplex and endosomal membranes [19]. When the lipoplex surface boundary is interacting with the inner endosomal leaflet, this can induce formation of (transient) pores or sites of membrane destabilization, which create a way for the lipoplex and/or DNA to escape from the endosomal compartment. The membranous nature of the lipoplex seems to be crucial, in that it allows an exchange of lipids between the endosomal membrane and the lipoplex,

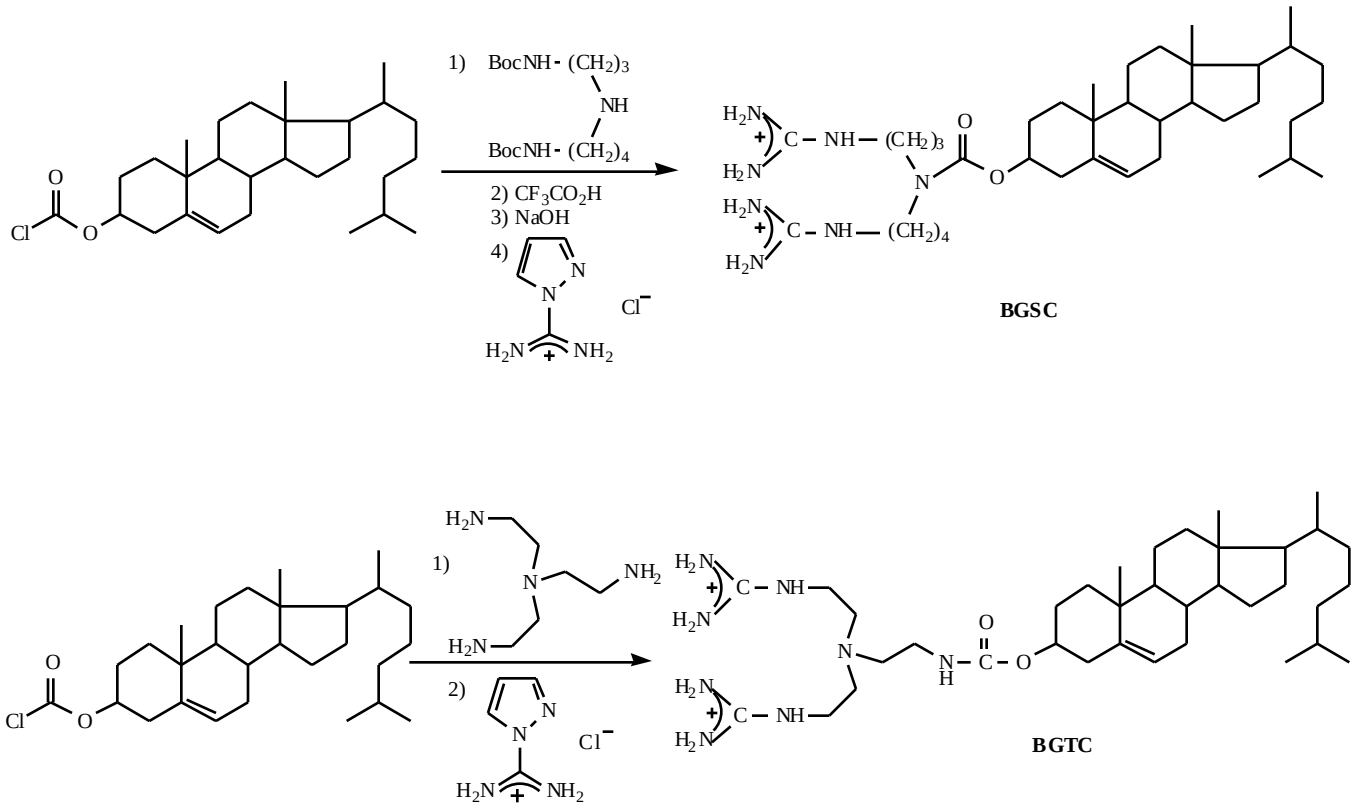

Fig. (1). Scheme for the synthesis of BGSC and BGTC.

Source: Vigneron *et al.*, *Proc. Natl Acad. Sci. USA*, **1996**, *93*, p. 9683 [18].

which results from perturbations that are the prerequisite in DNA endosomal escape [22, 23]. Typical BGTC/DOPE-DNA and BGTC-DNA structures are mainly detected in intracytoplasmic vesicles of transfected HeLa cells, and these concentric multilamellar lipoplexes may escape from the vesicles by membrane disruption. It cannot be excluded that plasmids arriving at the nuclear membrane were still partly coated with lipids [22]. However, the uncoated DNA found in nuclei might be the result of cationic lipid competitive distribution between DNA issued from exogenous plasmids and that from the large pool of chromatin [19-24].

2.4. Stabilization

Stability of lipid/DNA aggregates, a major requirement for cationic lipid-mediated transfection, is particularly difficult at the high DNA concentrations used for *in vivo* delivery. A PEG (polyethylene-glycol) coating - therefore colloidal stabilization of BGTC/DOPE lipoplexes [DNA-complexed BGTC/DOPE/PEG formulation (0.54/0.36/0.19 molar ratio)] (Fig. (**2**)) - enhances gene transfection *via* intra-nasal instillation. Cryo-TEM (cryo-transmission-electron microscopy) allows imaging of bioassemblies close to their native state, and PEG-stabilized lipoplexes display typical DNA-coated structures. Stabilized BGTC/DOPE/ Cholesterol-PEG lipoplexes further improved transfection of mouse lung airways [25].

2.5. Hydrophobic Moiety and Cationic Lipid Properties

Cationic lipid properties are not limited to their head groups but are influenced by their hydrophobic moiety. Cholesterol units can influence transfection efficiencythrough their packing arrangement within the membrane lipid bilayers. Therefore, BGDA (bis-guanidinium diacetylene) was constructed with a bis-guanidinium head group identical to that of BGTC, but with a diacetylene unit instead of cholesterol (Fig. (**3**)). BGDA, and BGDA with the neutral diacetylene co-lipid HEDA (hydroxyethylenediacetylene) showed no transfection activity, while BGDA/DOPE liposomes are efficient for gene transfer into various mammalian cells. Lipids containing diacetylene units, characterized by their two ends positioned apart, offer potential for polymerization. Polymerized domains may aid site directed gene delivery by providing means to immobilize targeting ligands on the gene delivery system [26].

2.6. Aminoglycoside-Based Systems

The positive headgroup structure could also have an impact on cationic lipid efficiency. Therefore, the transfection potential of lipophilic aminoglycoside derivatives was explored. A cationic cholesterol conjugate (KanaChol), characterized by a kanamycin A headgroup was synthesized, as well as its polyguanidinylated TGKC derivative (3β-[6'-(1, 3, 3''-triguanidino)kanamycin-carbamoyl]-cholesterol)

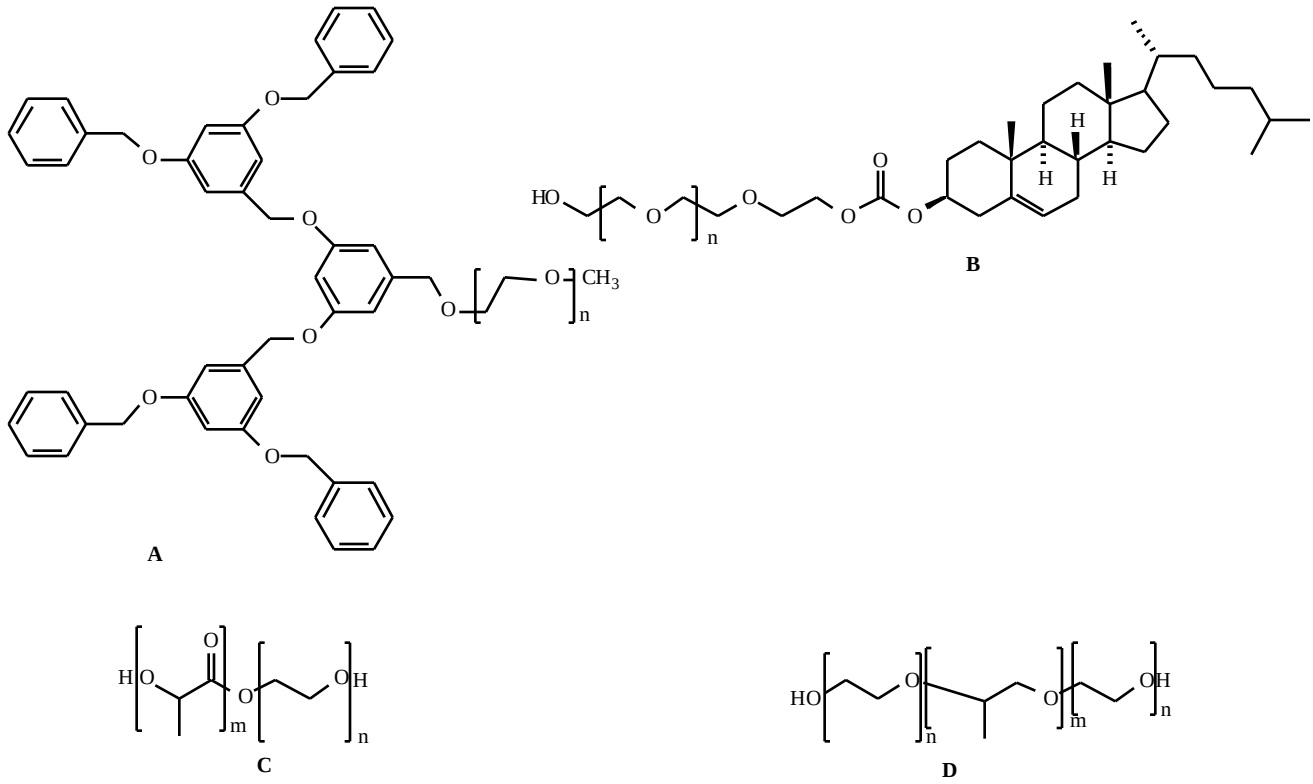

Fig. (2). Structure of the PEG conjugates used for steric stabilization of BGTC/DOPE lipoplexes. (A) G2D-PEG: second-generation poly(benzylether) dendrimer-poly(ethyleneglycol)n (n=100); (B) Chol-PEG: cholesterol-poly(ethyleneglycol)n (n=100); (C) PLA-PEG: poly(lactic acid) m-poly(ethyleneglycol)n (m-8 and n=45); (D) Pluronic F68: poly(ethyleneglycol)n-poly(oxypropylene)m-poly(ethyleneglycol)n (m=30 and n=75).

Source: Pitard et al., *J. Gene Med.,* **2001**, *3*, p. 481 [25].

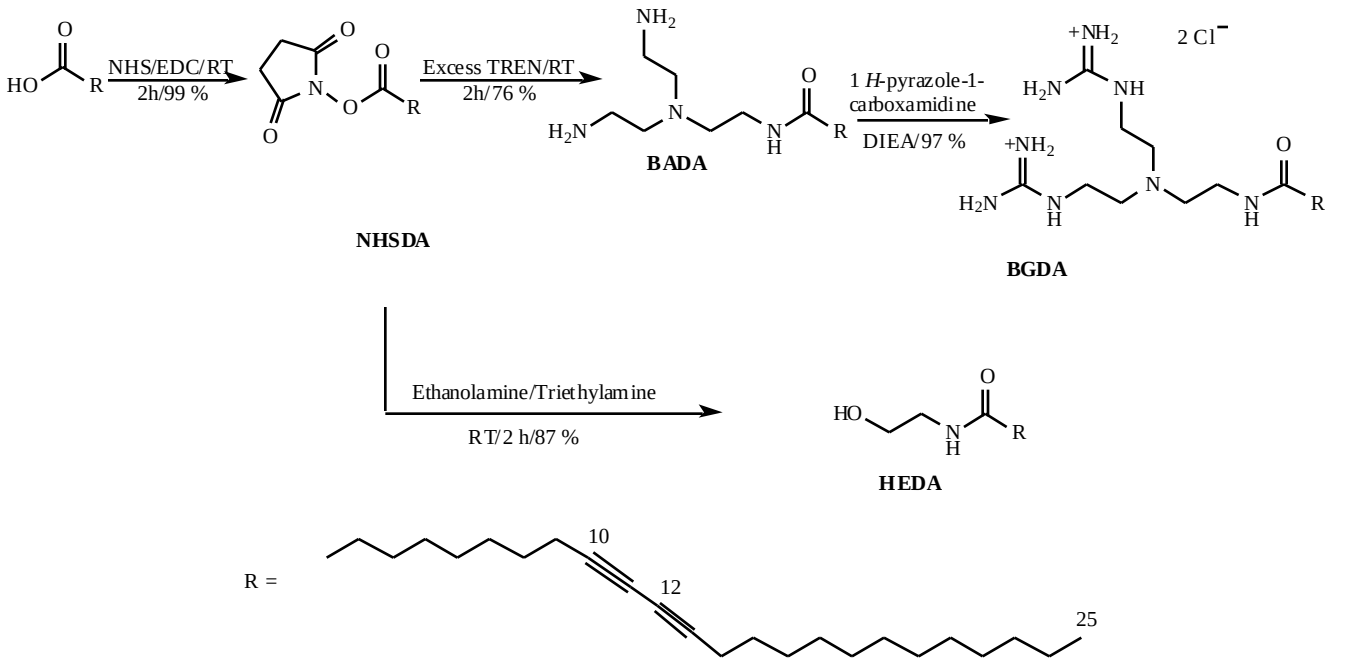

Fig. (3). Scheme for the synthesis of the diacetylene-based lipids HEDA and BGDA.

Source: Patel *et al. Biochem. Biophys. Res. Comm.*, **2001**, *281*, p. 538 [26].

(Fig. (**4**)). This aminoglycoside-based cationic lipid was efficient for gene transfection into a variety of mammalian cells *in vitro*, either alone or as a liposomal formulation, and its polyguanidinylated derivative was also found to mediate gene transfection *in vitro*. *In vivo*, KanaChol/DOPE liposomes were efficient for gene transfection into mouse airways. Intranasally instilled, Chol-PEG-stabilized KanaChol/DOPE lipoplex mediated gene delivery in mice. According to literature, this is a first step towards the design of multimodular aminoglycoside-based systems for *in vivo* gene delivery [27].

III. LIPOPLEXES AND LIPOPEPTIDES - CANDIDATES FOR ANTI-TUMOR TRIALS

3.1. Pancreatic Carcinomatosis: BGTC-Mediated Lipofection in Mice

A novel, nonviral gene therapy approach, consisting of BGTC-mediated lipofection of a combined suicide gene system, was evaluated in a mouse model of pancreatic peritoneal carcinomatosis. Human BxPC-3 pancreatic cells secreting the carcinoembryonic antigen (CEA) tumor marker were injected into the peritoneal cavity of nude mice. Eight days later, i.p. lipofection was performed using BGTC/DOPE cationic liposomes complexed with plasmids encoding two prodrug-activating enzymes, HSV-TK (herpes simplex virus thymidine kinase) and E.coli cytosine deaminase. The lipoplex administration was followed by treatment with the corresponding prodrugs, ganciclovir (GCV) and 5-fluorocytosine (5-FC). HSV-TKmRNA was then detected in tumor nodule tissues. The transgene expression, preferential for tumor nodules, lasted for two weeks. Mice receiving the full treatment (BGTC liposomes, suicide genes and prodrugs) had lower serum CEA than controls, indicating a strong reduction of peritoneal carcinomatosis progression [28].

3.2. MART-1, Melanoma Target Antigen. Lipopeptide, a Vaccine Candidate

Cytotoxic T lymphocytes (CTLs) play a central role in anti-tumor immune responses by recognizing small antigenic peptides bound to MHC class I molecules on cancer cells. To induce a strong melanoma specific CTL response, a formulation was developed where the CD4 epitope was included in a lipopeptide construct, which resulted in a simple vaccine formulation. MART-1 (melanoma antigen transmembrane protein) was selected because of its frequent expression in melanoma, as MART 27-35, the immunodominant peptide of this antigen, is expressed in 45% of the Caucasian population. Mp-MART and Dp-MART lipopeptides were constructed with 1 or 2 palmitic acid chains (Fig. (**5**)). Both included HLA-A2-restricted MART 27-35 CTL epitope covalently linked to tetanus toxoid TT 830-843 HTL (helper T lymphocyte) epitope and made water-soluble by insertion of spacers between the peptide and the lipid tail. Lipopeptide constructs were strongly recognized *in vitro* by human CTLs derived from tumor-infiltrating lymphocytes. The high cytotoxic activity observed shows that the covalent linkage of MART 27-35 peptide inside the lipopeptide construct did not impair TCR-MHC peptide recognition [29].

IV. NEW LIPID COMPOUNDS AGAINST HIV, SARS AND ORTHOPOXVIRUSES

New viral infection and proliferation inhibitors under current investigation include lipid prodrugs (e.g., anti-RT and lipid conjugates), HIV protease inhibitors (PI), inhibitors of HIV entry into host cells (i.e., CD4 attachment, chemokine receptor binding, membrane fusion) and viral integrase inhibitors - to which may be added inhibitors of the SARS-CoV (coronavirus associated with severe acute

respiratory syndrome), as well as cowpox (CV) and vaccinia (VV) orthopoxvirus inhibitors.

4.1. Lipid Prodrugs

Synthetic phospholipid analogs display potent activity against HIV infection when used alone or conjugated with therapeutic agents. Data indicate that hepatoma-derived cells activate lipid-AZT prodrugs to release therapeutic drugs from their carrier lipids. AZT-phospholipid conjugates can double target the virus replication cycle by inhibiting RT by AZT and inducing the production of defective virus particles lacking gp120 expression [30]. Besides, due to the pH-dependent lipophilicity of the anti-protease indinavir, practically all molecules are incorporated into the lipid phase when formulated at pH 7.4 and 5:1 lipid-to-drug molar ratio. No elaborate purification procedure is necessary. Lipid association greatly enhances delivery of this anti-HIV drug to lymph nodes, at much higher level than its soluble form, and provided significant virus load reduction in a HIV-2-infected *Macaca nemestrina* primate model [31].

CDV (cidofovir) is effective against orthopoxvirus infection in murine models of CV and VV infections. However, its usefulness in humans is limited by its nephrotoxicity and lack of activity when administered orally. To obtain better oral bioavailability, CDV ether lipid esters

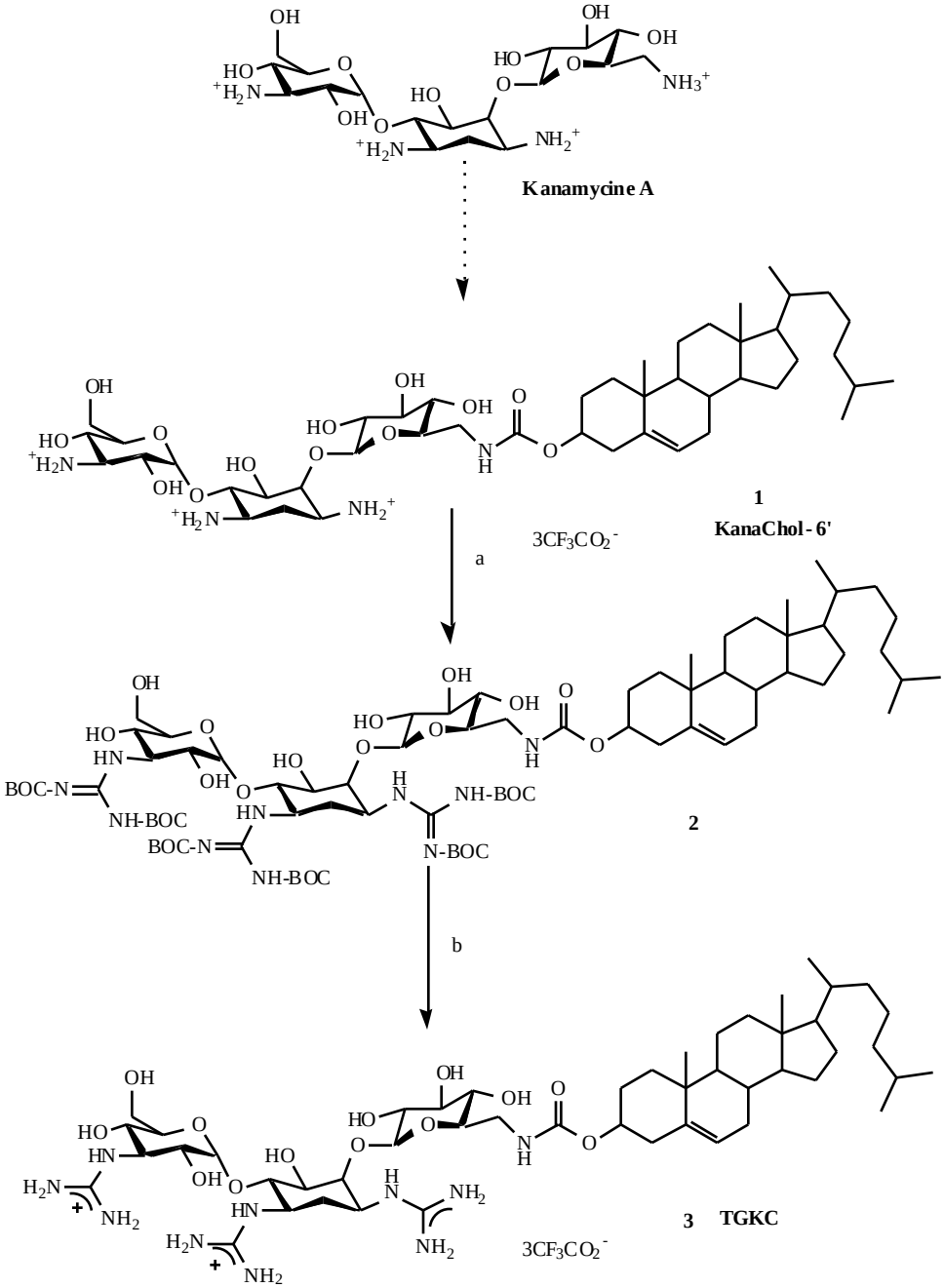

Fig. (4). Structure of the cationic lipid KanaChol (compound **1**) and scheme for the synthesis of its polyguanidinylated derivative TGKC (compound **3**).

Source: Belmont *et al., J. Gene Med.*, **2002**, *4*, p. 519 [27].

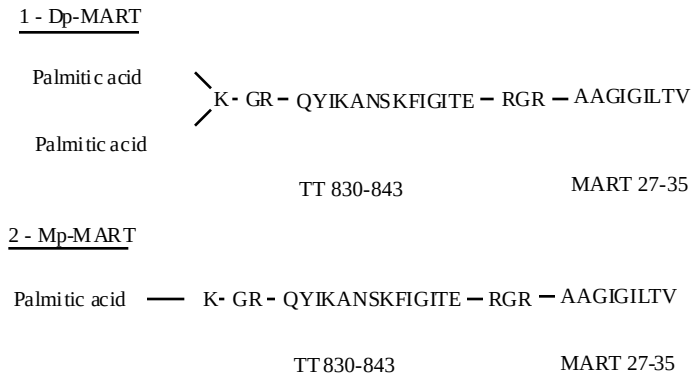

Fig. (5). Sequences of the Mp-MART and Dp-MART. The amino acid position in the HTL epitope was identified with respect to the tetanus toxoid protein and the CTL epitope from the MART antigen. A palmitoyl-lysylamide group was added in the NH2-terminal position.

Source: Le Gal, Gahéry-Ségard *et al*., *Int. J. Cancer,* **2002**, *98,* p. 223 [29].

were synthesized and provided as dry powder. HDP-CDV (hexadecyloxypropyl-CDV), HDP-cCDV (HDP-cyclic CDV), ODE-CDV (octadecyloxyethyl-CDV), OLP-CDV (oleoyloxypropyl-CDV), and OLE-CDV (oleoyloxyethyl-CDV) were found to have enhanced activity. Compared with parent nucleotide activity to inhibit virus replication, HDP-CDV and HDP-cCDV analogs are active at levels 50- to 200-fold less than levels required from CDV and cCDV molecules. HDP-CDV and ODE-CDV are both bioavailable, have high degrees of antiviral efficacy, and persist in tissues for relatively long periods of time. Cellular uptake of the HDP-[2-14C] CDV analog was 11- to 23-fold greater than that of [2-14C] CDV, and the CDV-diphosphate intracellular level of this key molecule, the active antiviral agent, was 100 times greater [32].

4.2. Triterpenes

The inhibitory efficiency of naturally occurring triterpenes and derivatives on the retroviral life cycle was largely studied in the last few years [33], and the impact of these hydrophobic compounds was extended to inhibition of virus entry into host cells and viral integrase. For instance, chemical modifications of glycyrrhizic acid (GL), the major bioactive triterpene of the licorice *Glycyrrhiza radix* root, elicit new bioactive derivatives for clinical purposes [34]. Also, betulinic acid, a triterpenoid isolated from *Syzigium claviflorum* leaves, displayed potent inhibitory activity against HIV-1. A betulinic acid derivative, YK-FH 312, might affect virion assembly and/or host cell budding [35].

The outbreak of SARS-CoV, the severe acute respiratory syndrome associated coronavirus, warrants the search for antiviral compounds. Antiviral potential of several natural compounds was assessed *in vitro* against two clinical coronavirus isolates (FFM-1 and FFM-2) from patients with SARS. Of all the compounds tested, GL was the most active in inhibiting SARS-CoV replication [36].

V. NEW HIV-1 ANTI-PROTEASE FORMULATIONS FOR FIGHTING LIPODYSTROPHY

5.1. Glucose Cargo Function. Proteasome Activity

The superior lipid profile of atazanavir (ATV) was recently described [37]. This potent azapeptide PI displays a lower incidence on lipid metabolism than other PIs. For the six PIs tested, the rank order and concentration dependency for disturbing effects on lipogenesis were associated with the degree of inhibition of proteasome activity *in vitro*. ATV has little effect on glucose transporters (GLUTs) compared to other PIs that inhibit GLUTs. A hypothesis was then proposed to explain the mechanism of eventual disturbances: when the proteasome function is inhibited in hepatocytes and adipocytes, the turnover of lipogenic transcription factors such as SREBP (sterol regulatory element binding protein) could be affected [37].

5.2. New Formula Presentation. Effect on Triglyceride and Cholesterol Plasma Levels

Significant reductions in cholesterol and triglyceride plasma levels were observed in the Trizal study. In a 48 wk comparative randomized study, subjects were switched from a successful HAART to Trizivir, a combination tablet of one anti-protease (abacavir) and two nucleoside RT inhibitors (lamivudine and zidovudine) [38].

DISCUSSION

HAART (highly active anti-retroviral therapy), including anti-RT nucleoside and/or non-nucleoside analogs (NRTIs and/or NNRTIs), and anti-proteases (PIs), dramatically improves rates of morbidity and mortality due to HIV infection. In the long term, HAART induces dyslipemia, insulin resistance, diabetes and triglyceridemia. Moreover, peripheral lipoatrophy and central fat accumulation, referred to as lipodystrophy (LD syndrome), complicate HIV therapy [2, review] with the occurrence of viral variants. Various other compounds are currently tested. New PI formulations [37,38], triterpene derivatives acting on the viral life cycle (HIV entry into host cells, HIV integrase, virion assembly and budding) [33-35], and lipid prodrugs [30], significantly reduce these side effects.

The quest for anti-viral compounds takes a different direction, i.e., HIV-1 lipopeptide therapeutic vaccination, which leads to reinforced immunologic defence mechanisms after interrupting HAART [3-13]. Lipopeptides are internalized and degraded by proteasome, a pathway

restricted to professional DCs (APCs) [6,7]. Moreover, HIV-1 protease dimerization inhibitory effect was recognized in lipopeptides, and this may provide a way of overcoming the drug resistance observed with antiproteases that bind to the active site [14-16]. On the other hand, anti-melanoma Mp- and Dp-MART 27-35 lipopeptide vaccines, constructed with 1 or 2 palmitic acid chains, were strongly recognized *in vivo* by transgenic mice, and by human MART 27-35 cytolytic T cells, derived from tumor-infiltrating lymphocytes [29]. These findings appear significant for the development of synthetic lipopeptide anti-cancer vaccines and for clinical trials [29].

An anti-HIV lipopeptide vaccine [3] injected in uninfected volunteers was well tolerated [8]. After two years, a sustained CD8(+) T cell response was observed with no significant difference between the two groups, with or without QS21 saponin adjuvant [9]. Consequently, vaccinotherapy with the lipopeptide preparation [3] was proposed to HIV seropositive individuals (ANRS 093, Lipo-6T). The vaccinated patients showed better control of HIV replication after interrupting HAART [10]. This evaluation was also conducted on blood samples from HIV infected Ivorian and French patients. Strong and multiepitopic B memory and T cell responses were obtained from simple lipopeptide vaccines, and immunogenicity of peptides was enhanced by the lipid tail. Attention is specially called to the fact that, in Africa, most vaccine trials are conducted with the clade B, while the majority of individuals are infected with the HIV subtype CRF02-AG [40]. However, the ANRS 1220 PRIMOCI study shows that individuals infected with this subtype can generate cross-reactive CD8(+) T cell responses against HIV-1 subtype B, indicating that there is no major variation in immunodominant regions corresponding to the currently tested lipopeptide vaccines [40]. In rhesus macaques, immunized with a mixture of seven SIV (simian immunodeficiency virus)-derived lipopeptides, intradermal vaccination elicited multispecific CD8(+) T lymphocytes better than intramuscular vaccination [39]. Moreover, mouse mucosal immunization by monopalmitoylated peptides represents a step forward, compared to previous attempts using larger constructs [13].

The role that synthetic cationic lipids have in gene transfection is already well-known, although they are sadly less efficient than viral vectors [17]. BGTC constructs described by Lehn's group [18-22; 24-27] improve and facilitate gene transfection, given that they can be used as micellar solutions. Directly suspended in an aqueous medium, BGTC is useful *in vitro* for gene transfer into mammalian cell lines, and *in vivo* for gene delivery to the mouse airway epithelium [20]. This may combine the membrane-compatible features of the cholesterol subunit and the favorable features of the guanidinium group for DNA binding [18,19]. Further, DNA-complexed BGTC/DOPE colloidal stabilization by PEG of these lipoplexes enhances efficiency [25].

The therapeutic potential of BGTC nonviral gene therapy, evaluated in a pancreatic peritoneal carcinomatosis mouse model, demonstrates that BGTC/DOPE liposomes can mediate plasmid transfection into peritoneal tumor nodules. Most interestingly, carcinomatosis progression was reduced in mice receiving the treatment [28]. Other lipoplexes proved well-adapted for gene transfection, e.g., the TGKC multimodular aminoglycoside-based system [27], and BGDA containing diacetylene units instead of cholesterol, which offer potential for polymerized domains to immobilize targeting ligands on gene delivery systems [26]. These vectors are sophisticated modular systems where each component helps the lipid/DNA complex to perform a critical step: endosomal escape, nuclear entry and *in vivo* targetability. Such supramolecular ''virus-like'' vectors constitute an alternative to recombinant viruses for gene therapy applications. DNA/molecular conjugate complexes enter cells by receptor-mediated endocytosis in much the same way as nonenveloped adenoviruses [21].

Given their favorable properties, the above synthetic vectors "may permit the transfer of nucleic acid sequences (chimeric RNA/DNA oligonucleotides, DNA fragments or triplex-forming oligonucleotides) allowing long-term genomic modification" [41], and cationic lipid-mediated mRNA delivery to the central nervous system [42]. It was reported [43] that the HIV-1 RNA sequence leader consists of a complex series of stem loop structures that are critical for viral replication. The packaging SL1 stem loop signal region, revealed a highly conserved purine rich loop. This loop binds HIV-1 Rev and has a second Rev-binding site that may have an additional role in the viral life cycle [43]. BGTC, BGDA and TGKC possess attractive properties, which might eventually participate in this additional role.

According to Quenelle *et al*. [32], orthopoxvirus diseases continue to pose challenges. Rapid diagnosis of smallpox and development of effective antiviral chemotherapies are essential. Oral HDP-CDV and ODE-CDV are, at least, equivalent to i.p. CDV and persist for relatively long periods of time in animal tissues. These lipid-drug complexes should be effective when used for prophylaxis, postexposure prophylaxis, or treatment for smallpox and other orthopoxvirus infections, including monkeypox infections, which have become a problem due to unexpected outbreaks and the rising incidence of natural transmission.

ACKNOWLEDGEMENTS

J. Raulin wishes to express her gratitude to Dr. Pierre Lehn, Director of U 458 Inserm and Dr. Joyce Beare Rogers, Dept. of Health and Welfare, Ottawa, Ontario, Canada, for their generous support, Dr. Dominique Frommel and Dr. Marthe Gautier, Inserm, for helpful discussions, and Georgina Caspari for clever improvements to the manuscript.

ABBREVIATIONS

APC	=	Antigen presenting cell
ANRS	=	Agence Nationale de Recherches sur le SIDA, France
BGDA	=	Bis-guanidinium diacetylene
BGTC	=	Bis-guanidinium-tren-[tris(2-aminoethyl)amine]-cholesterol
CD4, CD8	=	T cell receptors
CEA	=	Carcinoembryonic antigen
CTL	=	Cytotoxic T lymphocyte

CV = Cowpox virus

DC = Dendritic cell

DOPE = Dioleoylphosphatidylethanolamine

GL = Glycyrrhizic acid

GLUT = Glucose transporter

HAART = Highly active anti-retroviral therapy

HEDA = Hydroxyethylenediacetylene

HeLa cells = Human epithelial cells

HIV = Human immunodeficiency virus

HIV-1 Nef,Gag, Env,Rev = HIV-1 structural and regulatory proteins

HLA = Human leukocyte antigen

HTL = Helper T lymphocyte

IgA, IgG = Immunoglobin A, Immunoglobin G

IL = Interleukin

KanaChol = 3β-(6'-kanamycin-carbamoyl)-cholesterol

LD = Lipodystrophy syndrome

MART = Melanoma antigen transmembrane protein

MHC = Major histocompatibility complex

NRTI and NNRTI = Nucleoside and non-nucleoside RT inhibitors

PBMC = Peripheral blood mononuclear cell

PI = Protease inhibitor

QS21 = Natural saponin adjuvant (from *Quillaja Saponaria* tree)

RT = Reverse transcriptase

SARS-CoV = Severe acute respiratory syndrome associated coronavirus

SIV = Simian immunodeficiency virus

SREBP = Sterol regulatory element binding protein

TCR = T-cell receptor

TGKC = 3β-[6'-(1,3,3''-triguanidino)-kanamycin-carb-amoyl]-cholesterol

Th1,Th2 = T helper cells

VV = Vaccinia virus

REFERENCES

[1] Pomerantz, R.J.; Horn, D.L. *Nature Med*., **2003**, *9*(7), 867-873.

[2] Raulin, J. *Prog. Lipid Res.*, **2002**, *41*(1), 27-65, (review).

[3] Klinguer, C.; David, D.; Kouach, M.; Wieruszeski, J.-M.; Tartar, A.; Marzin, D.; Levy, J.-P.; Gras-Masse, H. *Vaccine*,**1999**, *18*(3), 259-267.

[4] Gahery-Ségard, H.; Pialoux, G.; Charmeteau, B.; Sermet, S.; Poncelet, H.; Raux, M.; Tartar, A.; Lévy, J.-P.; Gras-Masse, H.; Guillet, J.G. *J. Virol.* **2000**, *74*(4), 1694-1703.

[5] Gras-Masse, H. *Molec. Immunol.*, **2001**, *38*, 423-431.

[6] Hosmalin, A.; Andrieu, M.; Loing, E.; Desoutter, J.-F.; Hanau, H.; Gras-Masse, H.; Dautry-Varsat, A.; Guillet, J.-G. *Immunol. Lett.*, **2001**, *79*, 97-100.

[7] Andrieu, M.; Desoutter, J.-F.; Loing, E.; Gaston, J.; Hanau, D.; Guillet, J.-G.; Hosmalin, A. *J. Virol*., **2003**, *77*(2), 1564-1570.

[8] Pialoux, G.; Gahery-Ségard, H.; Sermet, S.; Poncelet, H.; Fournier, S.; Gérard, L.; Tartar, A.; Gras-Masse, H.; Lévy, J.-P.; Guillet, J.-G. *AIDS*, **2001**, *15*, 1239-1249.

[9] Gahéry-Ségard, H.; Pialoux, G.; Figueiredo, S.; Igéa, C.; Surenaud, M.; Gaston, J.; Gras-Masse, H.; Lévy, J.-P.; Guillet, J.-G. *J. Virol*., **2003**, *77*(20),11220-11231.

[10] Lévy, Y.; Gahéry-Ségard, H.; Durier, C.; Lascaux, A.-S.; Meiffrédy, V.; Goujard, C.; Cassuto, J.-P.; Rouzioux, C.; Elhabib, R.; Guillet, J.-G.; Aboulker, J.-P.; Delfraissy, J.-F. and ANRS 093, *10th Conf. on Retroviruses and Opportunistic Infection, Immune-Based Therapy*, Section14 **2003**, Boston (USA).

[11] Katlama, C. *Presse Med*, **2003**, *32*(21), 964.

[12] Autran, B.; Debré, P.; Walker, B.; Katlama, C. *Nature Rev. Immunol.*, **2003**, *3*, 503-508.

[13] BenMohamed, L.; Belkaid, Y.; Loing, E.; Brahimi, K.; Gras-Masse, H.; Druilhe, P. *Eur. J. Immunol.*, **2002,** *32*(8), 2274-2281.

[14] Schramm, H.J.; de Rosny, E.; Reboux-Ravaux, M.; Buttner, J.; Dick, A.; Schramm, W. *Biol. Chem.*, **1999,** *380*(5), 593-596.

[15] Dumond, J.; Boggetto, N.; Schramm, H.J.; Schramm, W.; Takahashi, M.; Reboud-Ravaux, M. *Biochem. Pharmacol.*, **2003**, *65*(7), 1097-1102.

[16] Boggetto, N.; Reboud-Ravaux, M. *Biol. Chem*., **2002**, *383*(9), 1321-1324.

[17] Hirko, A;. Tang, F.; Hughes, J.-A. *Curr. Med. Chem.*, **2003**, *10*(14), 1185-1193.

[18] Vigneron, J.-P.; Oudrhiri, N.; Fauquet, M.; Vergely, L.; Bradley, J.-C.; Basseville, M.; Lehn, P.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.*, **1996**, *93*, 9682-9686.

[19] Aissaoui, A.; Oudrhiri, N.; Petit, L.; Hauchecorne, M.; Kan, E.; Sainlos, M.; Julia, S.; Navarro, J.; Vigneron, J.-P.; Lehn, J.-M.; Lehn, P. *Curr. Drug Targets*, **2002,** *3*, 1-16.

[20] Oudrhiri, N.; Vigneron, J.-P.; Peuchmaur, M.; Leclerc, T.; Lehn, J.-M.; Lehn, P., *Proc. Natl. Acad. Sci. U.S.A.*, **1997**, *94*, 1651-1656.

[21] Lehn, P.; Fabrega, S.; Oudrhiri, N.; Navarro, J. *Adv. Drug Deliv. Rev*., **1998**, *30*, 5-11.

[22] Behr, J.-P. *Bioconjug. Chem*., **1994**, *9*, 382-389.

[23] Zuhorm, I.S.; Hoekstra, D. *J. Membr. Biol*., **2002**, *189*, 167-179.

[24] Pitard, B.; Oudrhiri, N.; Vigneron, J.-P.; Hauchecorne, M. Aguerre, O.; Toury, R.; Airiau, M.; Ramasawmy, R.; Scherman, D.; Crouzet, J.; Lehn, J.-M.; Lehn, P. *Proc. Natl. Acad. Sci. U.S.A.*, **1999**, *96*, 2621-2626.

[25] Pitard, B.; Oudrhiri, N.; Lambert, O.; Vivien, E.; Masson, C.; Wertzer, B.; Hauchecorne, M.; Scherman, D.; Rigaud, J.-L.; Vigneron, J.-P.; Lehn, J.-M.; Lehn, P. *J. Gene. Med.*, **2001**, *3*, 478-487.

[26] Patel, M.; Vivien, E.; Hauchecorne, M.; Oudrhiri, N.; Ramasawmy, R.; Vigneron, J.-P.; Lehn, P.; Lehn, J.-M. *Biochem. Biophys. Res. Comm.*, **2001**, *281*, 536-543.

[27] Belmont, P.; Aissaoui, A.; Hauchecorne, M.; Oudrhiri, N.; Petit, L.; Vigneron, J.-P.; Lehn, J.-M.; Lehn, P. *J. Gene. Med.*, **2002**, *4*, 517-526.

[28] Hajri, A.; Wack, S.; Lehn, P.; Vigneron, J.-P.; Lehn, J.-M.; Marescaux, J.; Aprahamian, M. *Cancer Gene. Ther.*, **2004,** *11*(1), 16-27.

[29] Le Gal, F.-A.; Prévost-Blondel, A.; Lengagne, R.; Bossus, M.; Farace, F.; Charboissier, A.; Gras-Masse, H.; Engelhard, V.H.; Guillet, J.-G.; Gahéry-Ségard, H. *Int. J. Cancer*, **2002**, *98*, 221-227.

[30] Morris-Natschke, S.L.; Ishaq, K.S.; Kucera, L.S. *Curr. Pharm. Design.*, **2003**, *9*(18), 1441-1451.

[31] Kinman, L.; Brodie, S.J.; Tsai, C.C.; Bui, T.; Larsen, K.; Schmidt, A.; Anderson, D.; Morton, W.R.; Hu, S.L.; Ho, R.J. *J. Acquir. Immune Defic. Syndr.*, **2003**, *34*(4), 387-397.

[32] Quenelle, D.C.; Collins, D.J.; Brad Wan, W.B.; Beadle, J.R.; Hostetler, K.Y.; Kern, E.R. *Antimicrob. Agents Chemother.*, **2004**, *48*(2), 404-412.

[33] Akihisa, T.; Ogihara, J.; Kato, J.; Yasukawa, K.; Ukiya, M.; Yamanouchi, S.; Oishi, K. *Lipids*, **2001**, *36*(5), 507-512.

[34] Baltina, L.A. *Curr. Med. Chem.*, **2003**, *10*(2), 155-171.

[35] Kanamoto, T.; Kashiwada, Y.; Kanbara, K.; Gotoh, K.; Yoshimori, M.; Goto, T.; Sano, K.; Nakashima, H. *Antimicrob. Agents Chemother*., **2001**, *45*(4), 1225-1230.

[36] Cinatl, J.; Morgenstern, B.; Bauer, G.; Chandra, P.; Rabenau, H.; Doerr, H. *Lancet*, **2003**, *361*(9374), 2045-2046.

[37] Parker, R.A.; Wang, S.; Mulvey, R.; Fenderson, W.; Elosua, C.; Laing, N.; Yang, W.P.; Flint,O.P. *2nd IAS Conf. on HIV Pathogenesis and Treatment, Paris* (F), July **2003**, Abstract # 708.

[38] Katlama, C.; Fenske, S.; Gazzard, B.; Lazzarin, A.; Clumeck, N.; Mallolas, J.; Lafeuillade, A.; Mamet, J.-P.; Beauvais, L. *HIV Med*., **2003**, *4*(2), 79.

[39] Coutsinos, Z.; Villefroy, P.; Gras-Masse, H.; Guillet, J.-G.; Bourgault-Villada, I. *2nd IAS Conf. on HIV Pathogenesis and Treatment*, Paris (F), July **2003**, Abstract # 499.

[40] Inwoley, A.; Recordon-Pinson, P.; Dupuis, M.; Gaston, J.; Minga, J.-G.; Masquelier, B.; Choppin, J.; Rouet, F.; Fleury, H.; Guillet, J.-G.; Andrieu, M.; and ANRS 1220 PRIMOCI group. *2nd IAS Conf. on HIV Pathogenesis and Treatment*, Paris (F), July **2003**, Abstract # 498.

[41] Yoon K. in: *Progress in Gene Therapy: basic and clinical frontiers*., Bertolotti R, Parvez SH, Nagatsu T. eds, VSP BV, AH Zeist, The Netherlands, **2000**; pp. 475-512.

[42] Anderson, D.M.; Hall, L.L.; Ayyalapu, A.R.; Irion, V.R.; Nantz, M.H.; Hecker, J.G. *Hum. Gene. Ther.*, **2003**, *14*(3), 191-202.

[43] Gallego, J.; Greatorex, J.; Zhang, H.; Yang, B.; Arunachalam, S.; Fang, J.; Sceamons, J.; Lea, S.; Pomerantz, R.J.; Lever, A.M. *J. Biol. Chem*., **2003**, *278*(41), 40385-40391.

APPENDIX - PREPARATION OF LIPOPEPTIDES

a - Bonnet, D.; Rommens, C.; Gras-Masse, H.; Melnyk, O. *Tetrahedron Lett.*, **2000**, *41*, 45-48.

b - Bonnet, D.; Bourel, L.; Gras-Masse, H.; Melnyk, O. *Tetrahedron Lett.*, **2000**, *41*, 10003-10007.

c - Bonnet, D.; Ollivier, N.; Gras-Masse, H.; Melnyk, O. *J. Org. Chem.*, **2001**, *66*, 443-449.

d - Bonnet, D.; Thiam, K.; Loing, E.; Melnyk, O.; Gras-Masse, H. *J. Med. Chem.*, **2001,** *44*, 468-471.

e - Bourel-Bonnet, L.; Bonnet, D.; Malingue, F.; Gras-Masse, H.; Melnyk, O. *Bioconjugate Chem.*, **2003**, *14*(2), 494-499.