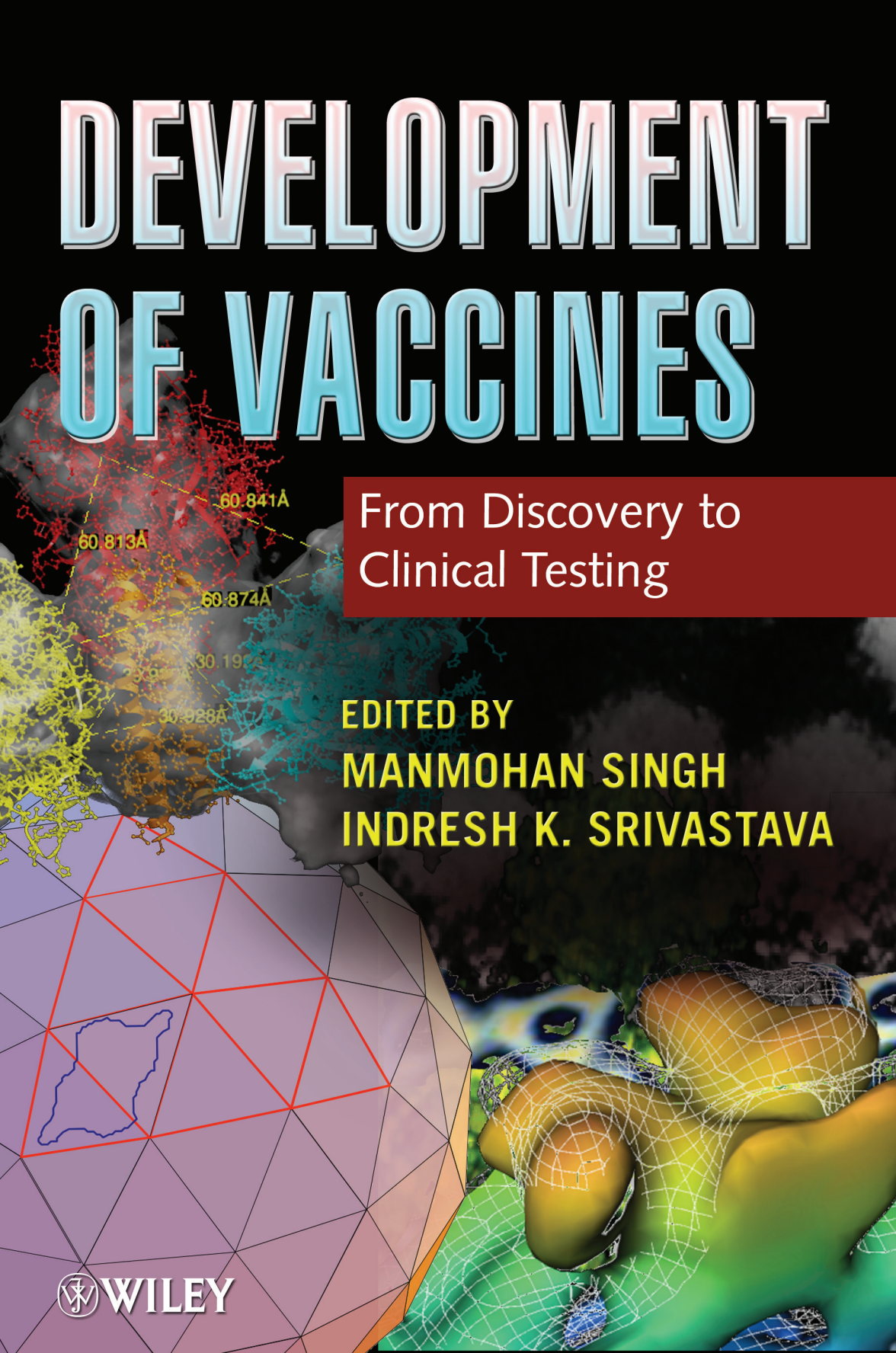
DEVELOPMENT OF VACCINES

From Discovery to Clinical Testing



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DESIGN PLATFORMS OF NANOCAPSULES FOR HUMAN THERAPEUTICS OR VACCINES

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5.1 APPLICATION OF VIRUS-LIKE PARTICLES FOR VACCINATION

The mammalian immune system is highly attuned to recognizing and eliminating viral particles following infection. The use of particle-based immunogens, often delivered as live-attenuated viruses, has been an effective vaccination strategy for a variety

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of viruses [1]. A number of virus-like particles (VLPs) for vaccination have been approved for clinical use. Clinical trials involving hepatitis E virus (HEV), influenza, hepatitis C virus (HCV), poliovirus, human immunodeficiency virus (HIV), Ebola virus, Norwalk virus, rotavirus, and severe acute respiratory syndrome (SARS) coronavirus are underway (Table 5.1). Some of those currently in preclinical testing are the hepatitis B virus (HBV) and the human papillomavirus (HPV). The structural capsid proteins of many viruses have the ability to self-assemble into VLPs. Such VLPs are 20–150 nm in diameter, and their exact size and morphology depend greatly on the particular viral proteins. These VLPs resemble intact virions but are noninfectious because they assemble without incorporating the viral genome (Fig. 5.1). Several expression systems for the production of VLPs have been reported. These include various mammalian cell lines (either transiently or stably transfected or transduced with viral expression vectors), baculovirus expression systems, and various yeast and *Escherichia coli* expression systems.

The HPV and HBV vaccines are the first VLP-derived vaccines approved by the Food and Drug Administration (FDA). Expression of the small envelope protein of HBV in yeast or mammalian cells leads to the formation of 22-nm-wide VLPs that are essentially identical to that of a natural product of HBV infection provided as the first-generation HBV vaccines. Similarly, expression of the L1 protein of HPV leads to the assembly of VLPs that are somewhat similar to the empty virus particles formed during HPV replication. These VLPs can induce strong immune responses after administration due to high-density display of repetitive epitopes on the surface of the capsid. This is further enhanced by the particulate nature of VLPs, especially in the size range of around 40 nm, which appears to be optimal for uptake by dendritic cells (DCs) [2].

5.2 INNATE AND ADAPTIVE CELLULAR IMMUNE RESPONSES AGAINST VIRUS-LIKE PARTICLES

HPV–VLPs comprised of the viral capsid protein L1 are immunogenic in mice and humans when injected intradermally or applied to mucosal surfaces in the absence of adjuvant [3–6]. Recently, the molecular mechanism of the immune response against HPV–VLP has been revealed (Fig. 5.2). Innate immune response of DC is triggered by the Toll-like receptors (TLRs)–MyD88 signaling pathway and promotes adaptive immune responses [7]. HPV–VLPs can rapidly induce specific immune responses via TLR4-mediated signaling through MyD88 adaptor molecule [8]. MyD88 then activates NF-κB and activating protein 1 transcription factors for proinflammatory responses [9]. Innate immune response also induces expression of DC maturation markers. DC maturation is essential for stimulation of both innate and adaptive immune responses [2, 10]. HPV–VLP is taken up by DCs for antigen processing and presentation by the major histocompatibility complex (MHC) class I and II to activate CD8+ and naïve CD4+ T cells, respectively. Mature DCs induce the polarization of naïve CD4+ T-helper 1 (Th1) and 2 (Th2) cells through antigen presentation of MHC class II against T-cell receptor of Th1/2 cells and co-stimulatory signals between CD80/86 and CD28

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• Q2

TABLE 5.1. Capsids Used for Vaccines and Vaccine Platforms

Capsid Platforms	Capsid Composition	References
Hepatitis B virus	Small envelope protein (HBsAg): Licensed	(McAleer, W.J. et al. 1984, and Andre, F.E. et al. 1987)
	Small envelope protein (HBsAg): Preclinical	(Kong, Q. et al. 2001)
	PreS1+2 and HBsAg: Licensed	(Yap, I. et al. 1992, Shouval, D. et al. 1994, Madalinski et al. 2001, and Yap, I. and Chan, S.H. 1996)
	HBsAg: Licensed	(Krugman, S. et al. 1971)
	GFP; malaria epitopes; HBV preS1; immunodominant epitopes of numerous viral pathogens, including bacterial and protozoan epitopes on hepatitis B virus core	(Pumpens, P. and Grens, E. 2001, Stahl, S.J. et al. 1989, Kratz, P.A. et al. 1999, Birkett, A.J. et al. 2002, Nardin, E.H. et al. 2004, Chen, X. et al. 2004, and Jegerlehner, A. et al. 2002)
	Various model epitopes on woodchuck hepatitis B virus core	(Billaud, J.N. et al. 2005)
	HCV HVR1; plant signal petides; Dengue virus envelope protein; HIV gp41 2F5 epitope on hepatits B virus S antigen	(Netter, H.J. et al. 2001, Sojikul, P. et al. 2003, Bisht, H. et al. 2001, Bisht, H. et al. 2002, Schlienger, K. et al. 1992, and Eckhart, L. et al. 1996)
Hepatitis C virus	Core, E1, E2: Preclinical	(Baumert, T.F. et al. 1998, Jeong, S.H. et al. 2004, Lechmann, M. et al. 2001, and Murata, K. et al. 2003)
Hepatitis E virus	Truncated major capsid protein (ORF2)	(Li, T.C. et al. 1997, Li, T.C. et al. 2005, Purcell, R.H. et al. 2003 and Emerson, S.U. and Purcell, R.H. 2001)
	HEV B cell epitope on hepatitis E virus	(Niikura, M. et al. 2002)
Human papilloma virus	L1, major capsid protein: Licensed	(Zhou, J. et al. 1991, Kirnbauer, R. et al. 1992, Koutsky, L.A. et al. 2002, and Villa, L.L. et al. 2005)
	SHIV (HIV tat, rev; SIV gag); HPV E6/E7 on human papillomavirus	(Frazer, I.H. et al. 2004 and Dale, C.J. et al. 2002)
	CTL epitopes of HPV and HIV on bovine papillomavirus	(Liu, W.J. et al. 2000)
Human immunodeficiency virus	Pr55gag, envelope: Preclinical	(Sakuragi, S. et al. 2002, Gheysen, D. et al. 1989, Shioda, T. and Shibuta, H. 1990, Deml, L. et al. 2005, and Doan, L.X. et al. 2005)

(*continued overleaf*)

TABLE 5.1. (*Continued*)

Capsid Platforms	Capsid Composition	References
	Various HIV env epitopes on HIV; simian HIV chimera	(Deml, L. et al. 2005 and Doan, L.X. et al. 2005)
Influenza	HA, NA, matrix: Preclinical	(Pushko, P. et al. 2005, Galarza, J.M. et al. 2005, and Latham, T. and Galarza, J. 2001)
Rotavirus	VP2, VP6, VP7: Preclinical	(Vieira, H.L. et al. 2005, Bertolotti-Ciarlet, A. et al. 2003, and Crawford, S.E. et al. 1994)
Norwalk virus	Capsid: Phase 1	(Ball, J.M. et al. 1999, Mason, H.S. et al 1996, and Tacket, C.O. et al. 2003)
Poliovius	Capsid (VP0, 1, 3)	(Brautigam, S. et al. 1993)
Ebola virus; Marburg virus	Glycoprotein (GP) and matrix (VP40): Preclinical	(Swenson, D.L. et al. 2005, Warfield, K.L. et al. 2003, and Warfield, K.L. et al. 2005)
SARS (severe acute respiratory syndrome) coronavirus	S, E, and M: Preclinical	(Mortola, E. and Roy, P. 2004)
Polyomavirus	VP1 and VP2 fused with 1–683 amino acid region of the extracellular and transmembrane domain of HER-2/*neu*	(Tegerstedt, K. et al. 2005; 2007)
Yeast Ty	HIV V3 loop; HIV p24; malaria epitopes	(Griffiths, J.C. et al. 1991, Weber, J. et al. 1995, and Gilbert, S.C. et al. 1997)
Phage Qbeta	Nicotine	(Maurer, P. et al. 2005)

[11–13]. Th1 cells produce cytokines such as IFN-g and TNF-a that direct B cells to secrete antigen-specific IgG2a, whereas Th2 cells express cytokines such as IL-4, IL-5, IL-9, and IL-13 to promote IgG1 and IgE class switch. Secretion of various cytokines by matue DCs further stimulates differentiation into B and T cells, resulting in antibody release and cytotoxic T-cell responses, respectively.

5.3 TAILORING VIRUS-LIKE PARTICLES BY ALTERING THE CAPSID SURFACE FOR VACCINE DEVELOPMENT

Through genetic fusion, VLPs can also be used to deliver immunogenic epitopes of other pathogens [14, 15]. Introduction of the immunogenic amino acid sequence to the surface region of the capsid allows the display of the immunogenic epitope with high density on the capsid surface. This technological innovation has greatly broadened the

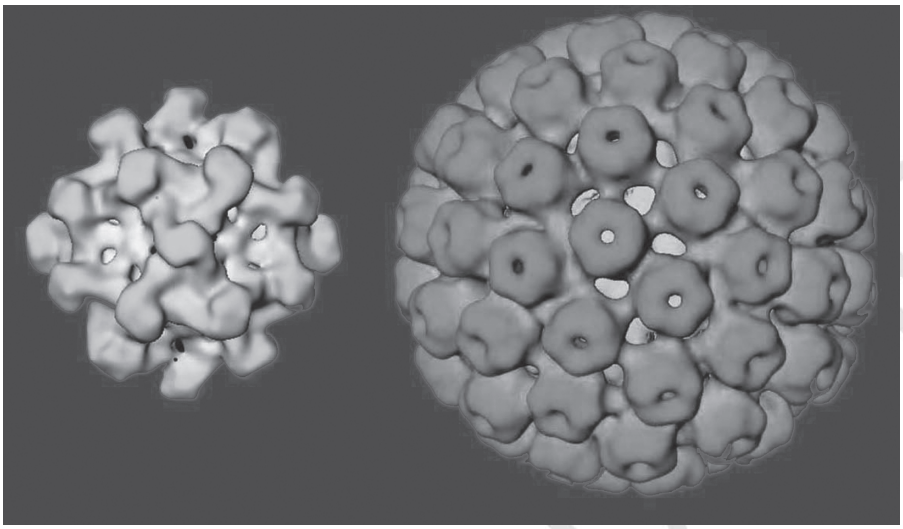

Figure 5.1. Surface morphology of VLPs. Left, T = 1 VLP of HEV. Light, T = 7d VLP of human BK polyomavirus. (*See insert for color representation of this figure*.)

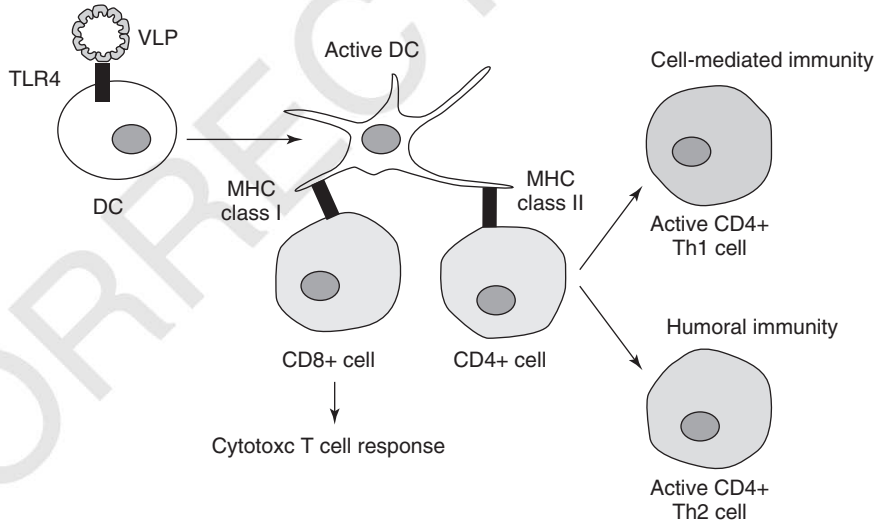

Figure 5.2. Immunoreaction against VLP. Natural and adaptive responces are induced by the association of VLP to TLR4 followed by the cell signalings of dendritic cells.

scope of their use, from immunizing against microbial pathogens to immunotherapy for chronic diseases [16]. For example, several VLPs have been developed as platforms to expose immunogens for vaccine development based on HBV, human and bovine papillomaviruses, yeast Ty, HIV/simian HIV gag, and HEV cores (Table 5.1). These capsid platforms are capable of inducing an immunogenic response against surface-exposed epitope. However, a major limitation to these VLP platforms is the small size of foreign epitopes that can be accommodated within the specific surface region of the capsid, which can preclude the presentation of large antigens such as HIV envelope or influenza hemagglutinin proteins. In order to overcome such spatial limitations, it is possible to immobilize peptide epitopes through the reactive site on the capsid platform by using chemical crosslinking. This has been done by coupling cysteine-containing peptides to the lysine residue situated in the immunodominant exposed region on HBV core particles [17]. By utilizing chemical crosslinking, it is also possible to conjugate nonprotein antigens such as nicotine on the surface of the capsid platform [18], which can induce an immunoresponse to nicotine.

Another approach to utilize the inner void space of VLPs is to encapsulate immunogens for the use of vaccination, expecting that the encapsulated immunogens would be processed and presented by MHC class I and II after the uptake into DCs. Murine polyomavirus (MPyV) VP1 is sufficient to form a spherical outer capsid structure. MPyV VP2/3 localize inside the assembled capsid. A fusion protein between MPyV VP2 and the 1-683 amino acid region of the extracellular and transmembrane domain of HER-2/neu (Her2) were encapsulated into MPyV VLP when it co-expressed with MPyV VP1 [19]. Vaccination against these Her2 containing VLPs inhibited Her2-expressing tumor growth in in vivo mouse models. The results show the feasibility of using MPyV-VLPs carrying Her2 fusion proteins as safe and efficient vaccines against Her2-expressing tumors.

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5.4 USE OF FLUORESCENT-LABELED VIRUS-LIKE PARTICLES TO ISOLATE ROTAVIRUS-SPECIFIC B-CELL CLONES FOR HUMAN MONOCLONAL ANTIBODY PRODUCTION

Green fluorescent protein (GFP)–VLP of rotavirus (RV) has been used as antigen probe to select RV-specific B-cell clones from peripheral CD19+ cells obtained from RV-infected patients [20]. In this chapter, GFP fusions of the rotavirus VP2 protein were co-expressed with VP6 and VP7. VP2 forms the innermost core layer of the capsid and binds the ribonucleic acid (RNA) genome [21] VP6 and VP7 are major inner capsid protein and glycoproteins of the outer surface of the capsid, respectively [22]. Using a fluorescence-activated cell sorter, B-cell clones that bind to both GFP–VLP and anti-CD19-PE conjugate were detected and sorted into 96-well plates (one cell per well) with feeder cells. Wells with VP6- or VP7-specific immunoglobulins were identified with ELISA, and the genes encoding the antibody VH and VL regions from those B-cell clones were subcloned into a Fab expression vector. This technology allows for rapid enrichment of B-cell clones that produce RV-specific antibodies.

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5.5 VLP APPLICATION AS A DELIVERY CARRIER

There have been several attempts to use VLPs as a drug delivery system (DDS) (Fig. 5.3). Development of therapeutic molecules includes small chemical compounds, polymers such as DNA and RNA, and proteins. The carrier for DDS has to be functionalized in the nanoscale space in which the carrier has to hold drugs tightly but at the same time enable delivery and release of the drug at the designated target cell types. In addition, the carrier should be biodegradable following drug targeting and delivery. This is important since nonbiodegradable carriers may have toxic side effects. A variety of materials has been made from chemical-based materials to construct nanocarriers including ceramics [23], polymers [24], dendrimers [25, 26], micelles [26], nanospheres and nanocapsules [27], fullerenes and nanotubes [25], liposomes [28], and metals [29, 30]. In these carriers, therapeutic drugs are incorporated into the carriers by entrapment, adsorption, or encapsulation with both hydrophobic and hydrophilic surfaces. For active targeting to specific cell types, antibodies or ligands directed against specific tumor epitopes or receptors can be conjugated to the surface of the nanocarrier. To facilitate the release of drugs, the liposome carriers may be comprised of pH-sensitive components that degrade in a low pH environment, such as in areas of tumor hypoxia [31]. Alternatively, one may use thermo-labile liposomes, which can release encapsulated agents in target tumor tissue by local hyperthermia [32]. Liposomal doxorubicin (Doxil) and paclitaxel-loaded

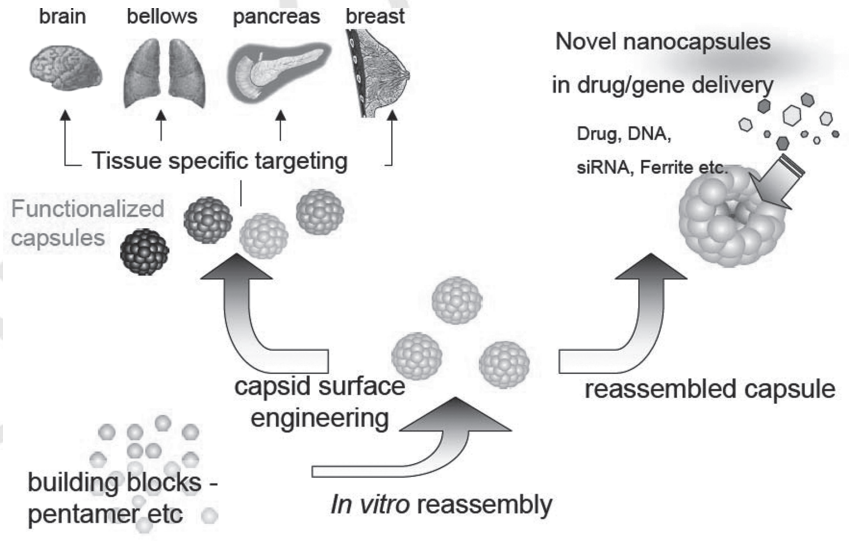

Figure 5.3. Overview of application of VLPs for therapeutic purpose of human. VLPs would be applicable to deliver the encapsulated agents to the target cells for cell therapy. (*See insert for color representation of this figure*.)

• Q12

human serum albumin nanoaggregate (Abraxane) are the two nanotherapeutics that have been approved by the FDA for cancer treatment.

Viruses have been used as carriers for human gene therapy because of their intrinsic cell-specific targeting properties and high transduction activity. Currently, such viruses include adenovirus, adeno-associated virus, herpes simplex virus, measles virus, Sendai virus, retro- and lenti-viruses, poxvirus, alphavirus, rhabdovirus, simian virus 40 (SV40), parvovirus, and Epstein–Barr virus (Table 5.2). However, there is great concern regarding the use of live viruses for this application. For example, recombinant viral genome may recover native viral activity and integrate into the host genome to disrupt and alter its expression, resulting in cancer formation and development of other diseases. To overcome this problem, methodologies have been developed to use the viral capsid alone, rather than live viruses, as protein-based nanocarriers. VLPs resemble intact virions, and hence they retain active specific targeting activity. The intrinsic capacity of VLPs to encapsulate nucleic acids, small molecules, and proteins make them ideal for gene and drug delivery (Table 5.2). Osmotic shock [33] and in vitro self-assembly of capsid subunits into VLPs [34–37] have been developed to encapsidate foreign deoxyribonucleic acid (DNA) into polyomavirus VLPs (Fig. 5.4). In HBV VLPs, foreign DNA and fluorescent dye were incorporated by the electroporation system for gene and drug delivery [38]. They showed that encapsidated DNA and fluorescence dye were selectively delivered into hepatocytes, which suggests that recombinant HBV VLPs still possess active targeting and high transduction activity similar to wild-type HBV virion. As for drug delivery by papillomavirus and polyomavirus VLPs, carboxyfluorescein diacetate and propidium iodide were incorporated into these VLPs [39–41]. They were able to show that hormones, vitamins, and peptides can be delivered into cells, thus suggesting that protein can potentially be delivered inside mammalian cells via these viral capsid nanocarriers. Unlike DNA transduction with viral or nonviral nanocarriers, protein delivery by VLPs avoids unexpected integration events into the genome but still enable transient and dose-controlled delivery of proteins in vivo. For example, it has been shown that pseudotyped lentivirions containing linamarase can deliver the enzyme to target cancer cells. As a result, the cancer cells become sensitized to linamarin, a cyanogenic flucoside substrate [42]. Similarly, in a polyomavirus system, heterologous proteins were genetically fused to VP1 proteins or fragments of minor coat proteins and successfully sequestered into VLPs [43–46]. Using this system, yeast cytosine deaminase (yCD), a prodrug-modifying enzyme that converts 5-fluorocytosine to 5-fluorouracil, was encapsulated into SV40 VLP. Tumor cells challenged by the yCD-encapsulating VLP became sensitive to 5-fluorocytosine-induced cell death [47] (Fig. 5.4b).

There have been several attempts to change or to broaden the cell-targeting activity of the viral nanocarriers by incorporating or chemically ligating cell surface-targeting peptide sequences to the viral capsid protein (Fig. 5.5). (Table 5.3). Integrin receptors are heterodimers composed of α and β subunits that play essential roles in cell–cell and cell–extracellular matrix interactions. Many investigators have reported the use of short RGD peptide (arginine–glycine–asparate, an integrin-binding motif) to alter the cell tropism of the virus capsids [48–52]. For example, the incorporation of the

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• Q13

TABLE 5.2. Capsids Used as Carriers of Therapeutic Agents

Capsid Platforms	Encapsidated Agents	References
Adenovirus	DNA	(Douglas, J.T. 2007, and Majhen, D. and Ambriovic-Ristov, A. 2006)
Adeno-associated virus	DNA	(Daya, S. and Berns, K.I. 2008, and Grieger, J.C. and Samulski, R.J. 2005)
Herpes simplex virus	Foreign gene	(Smith, K.D. et al. 2007, and Srinivasan, R. et al. 2008)
Hepatitis B virus	Foreign gene and small molecule	(Yamada, T. et al. 2003)
Measles virus	DNA	(Blechacz, B. and Russell, S.J. 2008)
Sendai virus	DNA	(Yonemitsu, Y. et al. 2008)
Retro and lenti viruses	DNA	(Lim, K.I. and Schaffer, D.V. 2008, Loewen, N. and Poeschla, E.M. 2005, and Sauter, S.L. and Gasmi, M. 2001)
	Protein	(Link, N. et al. 2006)
Poxvirus	DNA	(Arlen, P.M. et al. 2007, and Moroziewicz, D. and Kaufman, H.L. 2005)
Alphavirus	DNA	(Lundstrom, K. 2005, and Yamanaka, R. 2004)
Rhabdovirus	DNA	(Finke, S. and Conzelmann, K.K. 2005)
Human papillomavirus	Small molecule	(Drobni, P. et al. 2003, and Bergsdorf, C. et al. 2003)
Polyomavirus	DNA	(Barr, S.M. et al. 1979, Braun, H. et al. 1999, Stokrova, J. et al. 1999, Henke,S. et al. 2000)
	Protein	(Schmidt, U. et al. 2001, Abbing, et al. 2004, and Boura, E. et al. 2005)
Human JC polyomavirus	Small molecule	(Goldmann, C. et al. 2000)
Simian virus 40	DNA	(Strayer, D.S. et al. 2002, and Vera, M. and Fortes, P. 2004, Kimchi-Sarfaty, C. and Gottesman, M.M. 2004, and Tsukamoto, H. et al. 2007)
	RNA	(Kimchi-Sarfaty, C. et al. 2005)
	Protein	(Inoue, T. et al. 2008)
Parvovirus	DNA	(Maxwell, I.H. et al. 2002, and Srivastava, A. 2001)
Epstein–Barr virus	DNA	(Komaki, S. and Vos, J.M. 2000, and Sclimenti, C.R. and Calos, M.P. 1998)

RGD motif into the maloney murine leukemia virus capsid results in the expansion of its tropism from mouse NIH 3T3 cells to human melanoma cells [51]. As an alternative approach to changing cell tropism of viral capsids, folate was conjugated to the antiadenovirus fiber monoclonal antibody [53]. Binding of this antibody on the adenovirus capsid inhibited adenovirus infection through the adenovirus receptor, but allowed folate receptor mediated entry. In order to immobilize the anti-CD4 mono-

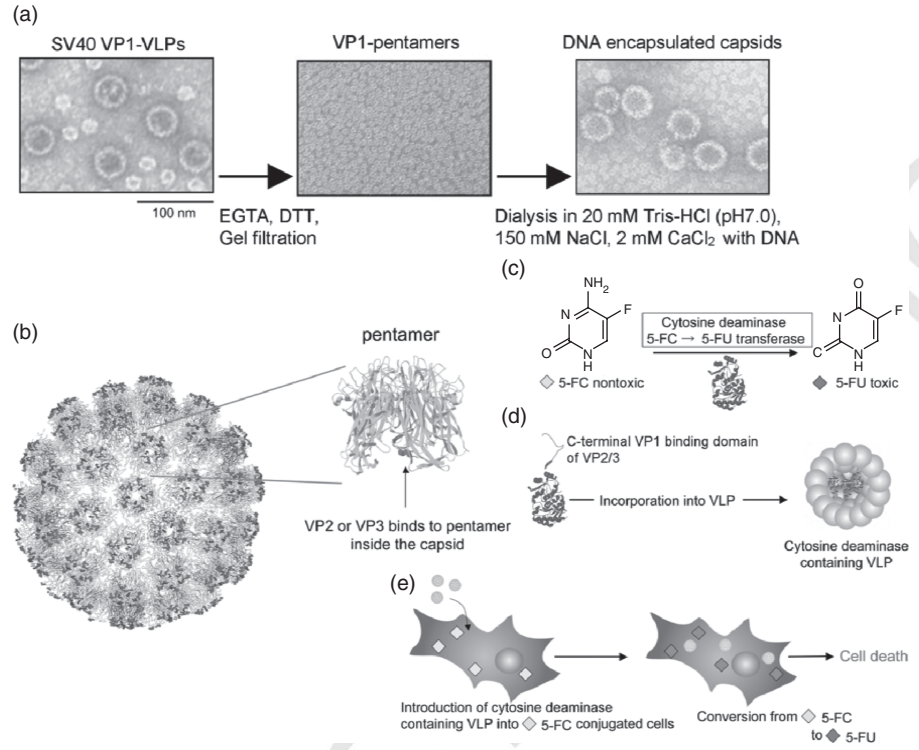

Figure 5.4. Examples of encapsulation inside the VLPs. (a) DNA-selective encapsulation into the VLP. SV40 VP1–VLPs are dissociated with calcium chelating agent (EGTA) and reducing agent (DTT), and then gel-filtrated to prepare pentamers, the building block of the VLP. In the presence of DNA, pentamer reassembled into the VLP to selectively encapsulate the DNA in physiological condition [20 mM Tris-HCl (pH7.0), 150 mM NaCl, and 2 mM CaC_{l2}]. (b) to (e) Protein selective encapsulation into the VLP. (b) C-terminus of VP2/3 can interact with pentamer from inside the capsid. (d) Using this fragment, cytosine deaminases were selectively encapsulated into the VLPs by fusing the C-terminal VP2/3 fragment at the N-terminus. (c) Cytosine deaminase converts the prodrug into the active form. (e) Using cytosine deaminase encapsulated VLP, prodrug administration succeeded to kill the cells only when the cells were incubated with cytosine deaminase encapsulated VLPs. (*See insert for color representation of this figure.*)

• Q14

clonal antibody on the capsid, the IgG-binding domain of protein A was inserted on the Sindbis virus capsid [54]. Binding of anti-CD4 antibody through protein A binding domain allowed viral entry into CD4-positive HeLa cells. It would also be possible to target specific cell types such as liver cells by introducing the preS sequence of HBV on the capsid using chemical crosslinking. Similarly, the many cancer targeting ligands that have been identified by combinatorial chemistry [6, 55–57] can be chemically synthesized, covalently linked to the viral capsid through site-specific ligation

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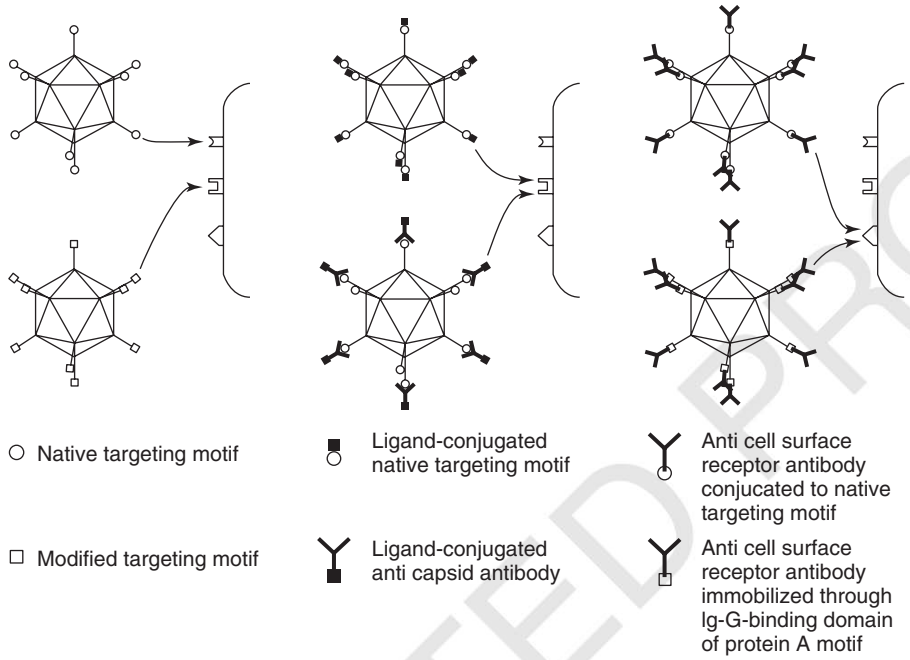

Figure 5.5. Tropism alteration using peptide sequence, ligand, and antibody. Tropism can be changed with peptide insertion at the surface residue of the capsid, ligand immobilization on the capsid by chemical crosslinking or through anticapsid antibody, and anti-cell-surface receptor antibody immobilization on the capsid by chemical crosslinking or through IgG-binding domain of protein A motif inserted at the surface residue of the capsid.

• Q17

reactions, and the resulting targeting VLPs can be used as an efficient drug delivery system for cancer therapy.

5.6 CONCLUSION

Viral capsid was originally applied to the vaccine development using its high immunogenicity. In recent years, viral capsids have been recognized as potential nanocarriers for efficient delivery of biologically active materials to specific cell types. For this latter application, the VLPs can be genetically and chemically modified so that their targeting property can be optimized and their immunogenicity minimized.

Acknowledgments

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• Q19

TABLE 5.3. Foreign Epitope Insertion to Change Capsid Tropism

Capsid Platforms	Foreign Epitopes	References
Adenovirus	(GS)5-ACDCRGDCFCG and (GS)5-KKKKKKK	(Wickham, T. et al. 1997, and Vigne et al. 1999)
Adeno-associated virus	RGD motif	(Shi, X. et al. 2006)
Polyomavirus	protein Z	(Gleiter, S. and Lilie, H. 2001)
	dihydrofolate reductase	(Gleiter, S. et al. 1999)
	eight glutamic acid residues and one cysteine	(Stubenrauch, K. et al. 2000, and May, T. et al. 2002)
	WW domain Yes-kinase associated protein	(Schmidt, U. et al. 2001)
	urokinase activator domain	(Shin, Y.C. and Folk, W.R. 2003)
Simian Virus 40	RGD motif	(Takahashi, R.U. et al. 2008)
Sindbis virus	IgG-binding domain of protein A	(Ohno, K. et al. 1997)
Retro and Lenti viruses	RGD motif, gastrin-releasing protein	(Gollan, T. and Green, M.R. 2002; 2002)

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Queries in Chapter 5

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