第7回遺伝子治療臨床試験トレーニングコース
～DNA/RNAワクチン開発に向けて～

ワクチン開発オーバービュー

久米晃啓、2022年7月16日（福岡市）

おことわり

利益相反（COI）開示など

本発表は演者の個人的意見を示すものであり、所属する（していた）組織の公式な見解ではありません。

発表内容に関して、開示すべき利益相反関係にある企業などはありません。

はじめに

一口に「ワクチン」と言っても色々ありますが・・・

感染症予防ワクチン

　能動免疫（active immunization）

　　生ワクチン、不活化ワクチン、成分ワクチン、

　　DNA/RNAワクチン、ウイルスベクター

　受動免疫（passive immunization）

　　抗血清、免疫グロブリン製剤

癌ワクチン（主に治療目的）

本日の主な話題

ワクチンの歴史

厚生労働省予防接種基礎講座第3回講習会（国立成育医療研究センター・木下典子　2017）
www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/0000167045.pdf

生ワクチン	全細胞死菌ワクチン	蛋白or多糖体	遺伝子組換え
18世紀			
天然痘(1798)			
19世紀			
狂犬病(1885)	チフス(1896) コレラ(1896) ペスト(1897)		
20世紀前半			
BCG(1927) 黄熱(1935)	百日咳(1926) インフルエンザ(1936) チフス(1938)	ジフテリアトキソイド（1923 ） 破傷風トキソイド(1926)	
20世紀後半			
経口ポリオ(1963) 麻疹（1963） ムンプス(1967) 風疹(1969) アデノウイルス(1980) 腸チフス(1989) *Salmonella Ty21a* 水痘(1995) ロタウイルス(1999) コレラ(1994)	ポリオ注射(1955) 狂犬病(1980) 日本脳炎(1992) ダニ媒介脳炎(1981) A型肝炎(1996) コレラ(WC-rBS)(1991)	肺炎球菌多糖体(1977) 髄膜炎菌多糖体(1974) Hib(1985) 髄膜炎菌結合型(C型)(1999) Hib結合型(1987) B型肝炎(1981) 腸チフス多糖体(1994) 無細胞型百日咳(1996) 炭疽菌(1970)	HBV(1986) ライム病(1998) コレラ(1993)
21世紀			
インフルエンザ(2003) ロタウイルス(2006) 帯状疱疹(2006)	日本脳炎Vero細胞(2009) コレラ(WCのみ)(2009)	肺炎球菌結合型(2000) 肺炎球菌結合型13型(2010) 髄膜炎菌結合型(2005)	HPV4価(2006) HPV2価(2009)

Vaccine 6th edition

Vaccine Preventable Diseases（WHO）

www.who.int/teams/immunization-vaccines-and-biologicals/diseases

コレラ、COVID-19、デング熱、ジフテリア、
肝炎（HA/HB）、Hib、HPV、インフルエンザ、
日本脳炎、マラリア、麻疹、髄膜炎菌、ムンプス、
百日咳、肺炎球菌、ポリオ、狂犬病、ロタ、風疹、
破傷風、tick-borne encephalitis、結核、
チフス、水痘、黄熱

日本で接種可能なワクチン（2021年8月現在）

www.niid.go.jp/niid/ja/vaccine-j/249-vaccine/589-atpcs003.html

	生ワクチン	不活化ワクチン・トキソイド	mRNAワクチン・ ウイルスベクターワクチン
定期 臨時	BCG 麻疹・不審混合（MR） 麻疹 風疹 水痘 ロタウイルス：1価、5価	百日咳・ジフテリア・破傷風・不活化ポリオ混合（DPT-IPV） 百日咳・ジフテリア・破傷風・混合（DPT） 不活化ポリオ（IPV） ジフテリア・破傷風トキソイド（DT） 日本脳炎 肺炎球菌（13価結合型） インフルエンザ菌b型（Hib） B型肝炎 ヒトパピローマウイルス（HPV）：2価、4価 インフルエンザ 肺炎球菌（23価莢膜ポリサッカライド）	COVID-19
任意	ムンプス 黄熱 帯状疱疹（＝水痘ワクチン）	破傷風トキソイド 成人用ジフテリアトキソイド A型肝炎 狂犬病 髄膜炎菌：4価 帯状疱疹 ヒトパピローマウイルス（HPV）：9価	

ワクチンプラットフォーム

従来のワクチンを大別すると生ワクチンと不活化ワクチン

生ワクチン

　従来型の弱毒性ワクチン（麻疹、風疹、ムンプス、水痘、黄熱、ロタ）

　ヒト以外の動物に感染する近縁の病原体を用いる（BCG、ロタ）

　組換えウイルス　非増殖型（SeV、AdV、LentiV）

　　増殖型　既存のワクチン株を利用（ワクシニア、麻疹）

不活化ワクチン

　全粒子不活化（日本脳炎、不活化ポリオ、A型肝炎、狂犬病）

　サブユニット・精製蛋白（インフルエンザ、Hib、肺炎球菌、髄膜炎菌、帯状疱疹）

　Virus like particle（VLP）（B型肝炎、HPV）

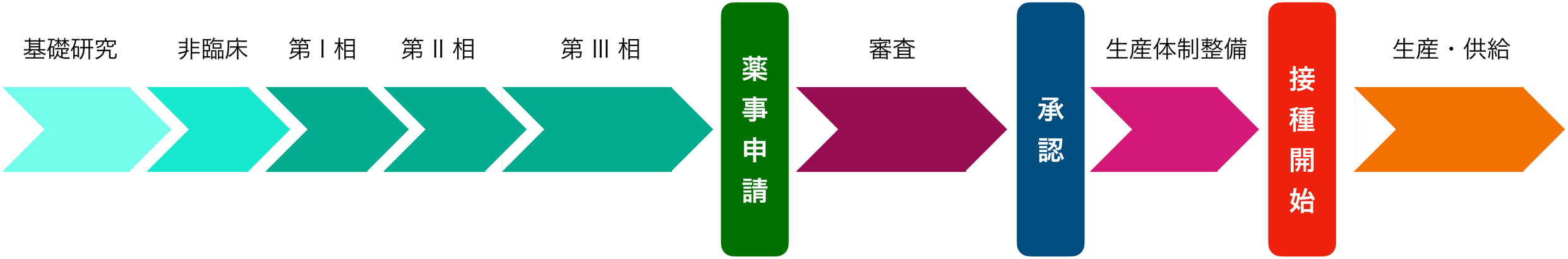
　トキソイド（破傷風、ジフテリア、百日咳）

核酸ワクチン

　DNAワクチン、mRNAワクチン

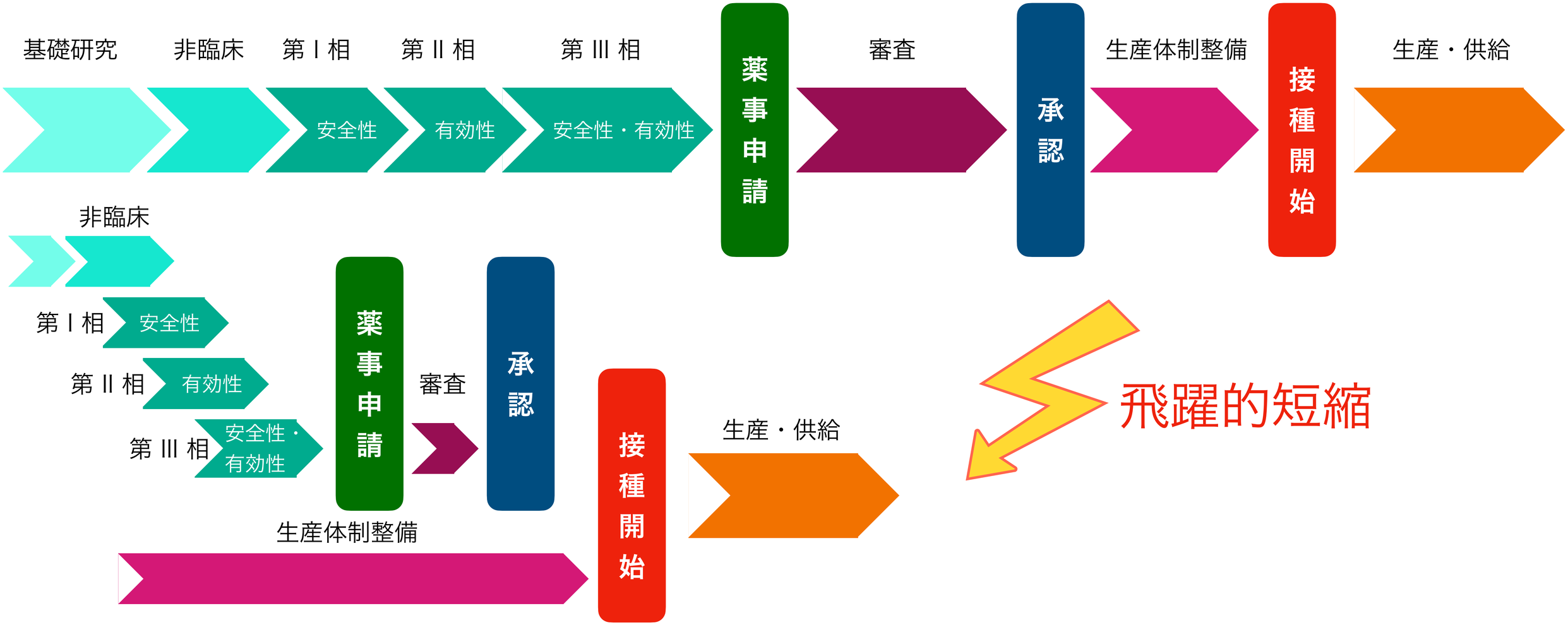
従来のワクチン開発：10年以上必要

日置仰、石井健（実験医学 39: 263, 2021）より引用



欧米のコロナワクチン開発：1年以内

日置仰、石井健（実験医学 39: 263, 2021）より引用



ワクチンプラットフォーム

新たなモダリティ：核酸ワクチン、組換えウイルスベクター

生ワクチン

従来型の弱毒性ワクチン（麻疹、風疹、ムンプス、水痘、黄熱、ロタ）

ヒト以外の動物に感染する近縁の病原体を用いる（BCG、ロタ）

組換えウイルス　非増殖型（SeV、AdV、LentiV）

増殖型　既存のワクチン株を利用（ワクシニア、麻疹）

不活化ワクチン

全粒子不活化（日本脳炎、不活化ポリオ、A型肝炎、狂犬病）

サブユニット・精製蛋白（インフルエンザ、Hib、肺炎球菌、髄膜炎菌、帯状疱疹）

Virus like particle（VLP）（B型肝炎、HPV）

トキソイド（破傷風、ジフテリア、百日咳）

核酸ワクチン

DNAワクチン、mRNAワクチン

DNAワクチン開発の歴史

研究開発のマイルストーン

マイルストーン	文献
1990　プラスミド・mRNA 直接筋注（マウス）	Science 247: 1465, 1990
1995　初の臨床試験（HIV）	J Inf Dis 178: 92, 1998
1997　遺伝子銃 Ph 1（HBV）	Vaccine 17: 2826, 1999
2005　日本企業関連Ph 1（HPV）	NCT00264732
2008　リポソーム Ph 1（Flu H5）	Vaccine 28: 2565, 2010
エレクトロポレーション Ph 1（HPV）	NCT00685412
2016　エレクトロポレーション Ph 1（ZikaV）	NCT02809443
2020　COVID-19 国内 Ph 1/2	NCT0443472, 04527081
国内 Ph 2/3	NCT04655625
2021　COVID-19 国内 Ph 1/2	NCT04993586

Direct Gene Transfer into Mouse Muscle in Vivo

JON A. WOLFF,* ROBERT W. MALONE, PHILLIP WILLIAMS, WANG CHONG, GYULA ACSADI, AGNES JANI, PHILIP L. FELGNER

RNA and DNA expression vectors containing genes for chloramphenicol acetyltransferase, luciferase, and β-galactosidase were separately injected into mouse skeletal muscle in vivo. Protein expression was readily detected in all cases, and no special delivery system was required for these effects. The extent of expression from both the RNA and DNA constructs was comparable to that obtained from fibroblasts transfected in vitro under optimal conditions. In situ cytochemical staining for β-galactosidase activity was localized to muscle cells following injection of the β-galactosidase DNA vector. After injection of the DNA luciferase expression vector, luciferase activity was present in the muscle for at least 2 months.

MOST EFFORTS TOWARD POSTNATAL gene therapy have relied on indirect means of introducing new genetic information into tissues: target cells are removed from the body, infected with viral vectors carrying the new genetic information, and then reimplanted into the body (*1*). For some applications, direct introduction of genes into tissues in vivo, without the use of viral vectors, would be useful. Direct in vivo gene transfer into postnatal animals has been achieved with formulations of DNA encapsulated in liposomes, DNA entrapped in proteoliposomes containing viral envelope receptor proteins (*2*), calcium phosphate–coprecipitated DNA (*3*), and DNA coupled to a polylysine-glycoprotein carrier complex (*4*). In vivo infectivity of cloned viral DNA sequences after direct intrahepatic injection with or without formation of calcium phosphate coprecipitates has also been described (*5*). With the use of cationic lipid vesicles (*6*), mRNA sequences containing elements that enhance stability can be efficiently translated in tissue culture cells (*7*) and in *Xenopus laevis* embryos (*8*). We now show that injection of pure RNA or DNA directly into mouse skeletal muscle results in significant expression of reporter genes within the muscle cells.

The quadricep muscles of mice were injected (*9*) with either 100 μg of pRSVCAT DNA plasmid (*10*) or 100 μg of βgCATβ-gA$_n$ RNA (*7, 11, 12*). The RNA consists of the chloramphenicol acetyl transferase (CAT) coding sequences flanked by β-globin 5′ and 3′ untranslated sequences and a 3′ polyadenylate tract. CAT activity was readily detected in all four RNA injection sites 18 hours after injection and in all six DNA injection sites 48 hours after injection (Fig. 1). Extracts from two of the four RNA injection sites (Fig. 1, lanes 6 and 8) and from two of the six DNA injection sites (Fig. 1, lanes 11 and 20) contained amounts of CAT activity comparable to those obtained from fibroblasts transiently transfected with the corresponding constructs in vitro under optimal conditions (Fig. 1, lanes 9 and 10 and 21 to 24, respectively). The average total amount of CAT activity expressed in muscle was 960 pg for the RNA injections and 116 pg for the DNA injections. The variability in CAT activity recovered from different muscle sites probably represents variability inherent in the injection and extraction technique, because significant variability was observed when pure CAT protein or pRSVCAT-transfected fibroblasts were injected into the muscle sites and immediately excised for measurement of CAT activity. CAT activity was also recovered from abdominal muscle injected with the RNA or DNA CAT vectors (*13*), indicating that other muscles can take up and express polynucleotides.

The site of gene expression was determined for the pRSV*lac-Z* DNA vector (*14*) expressing the *Escherichia coli* β-galactosidase gene (Fig. 2). Seven days after a single injection of 100 μg of pRSV*lac-Z* DNA into individual quadricep muscles, the entire muscles were removed, and every fifth 15-μm cross section was histochemically stained for β-galactosidase activity. Approximately 60 (1.5%) of the ~4000 muscle cells that comprise the entire quadriceps and ~10 to 30% of the cells within the injection area were stained blue (Fig. 2, A and B). Positive β-galactosidase staining within some individual muscle cells was at least 1.2 mm deep on serial cross sections (Fig. 2, D to F), which may be the result of either transfection into multiple nuclei or the ability of cytoplasmic proteins expressed from one nucleus to be distributed widely within the muscle cell (*15*). Longitudinal sectioning also revealed β-galactosidase staining within muscle cells for at least 400 μm (Fig. 2C). Fainter blue staining often appeared in the bordering areas of cells adjacent to intensely stained cells. This most likely represents an artifact of the histochemical β-galactosidase

J. A. Wolff, P. Williams, W. Chong, G. Acsadi, A. Jani, Departments of Pediatrics and Genetics, Waisman Center, University of Wisconsin, Madison, WI 53706.
R. W. Malone and P. L. Felgner, Vical Inc., San Diego, CA 92121.

*To whom correspondence should be addressed.

DNAワクチンの利点

Kim JJ, Weiner DB. Springer Semin Immunopathol 19: 175, 1999

Efficacy

Induce both humoral and cellular immune responses

Safety

Little risk for reversion to a disease-causing form
No risk for secondary infection

Better design

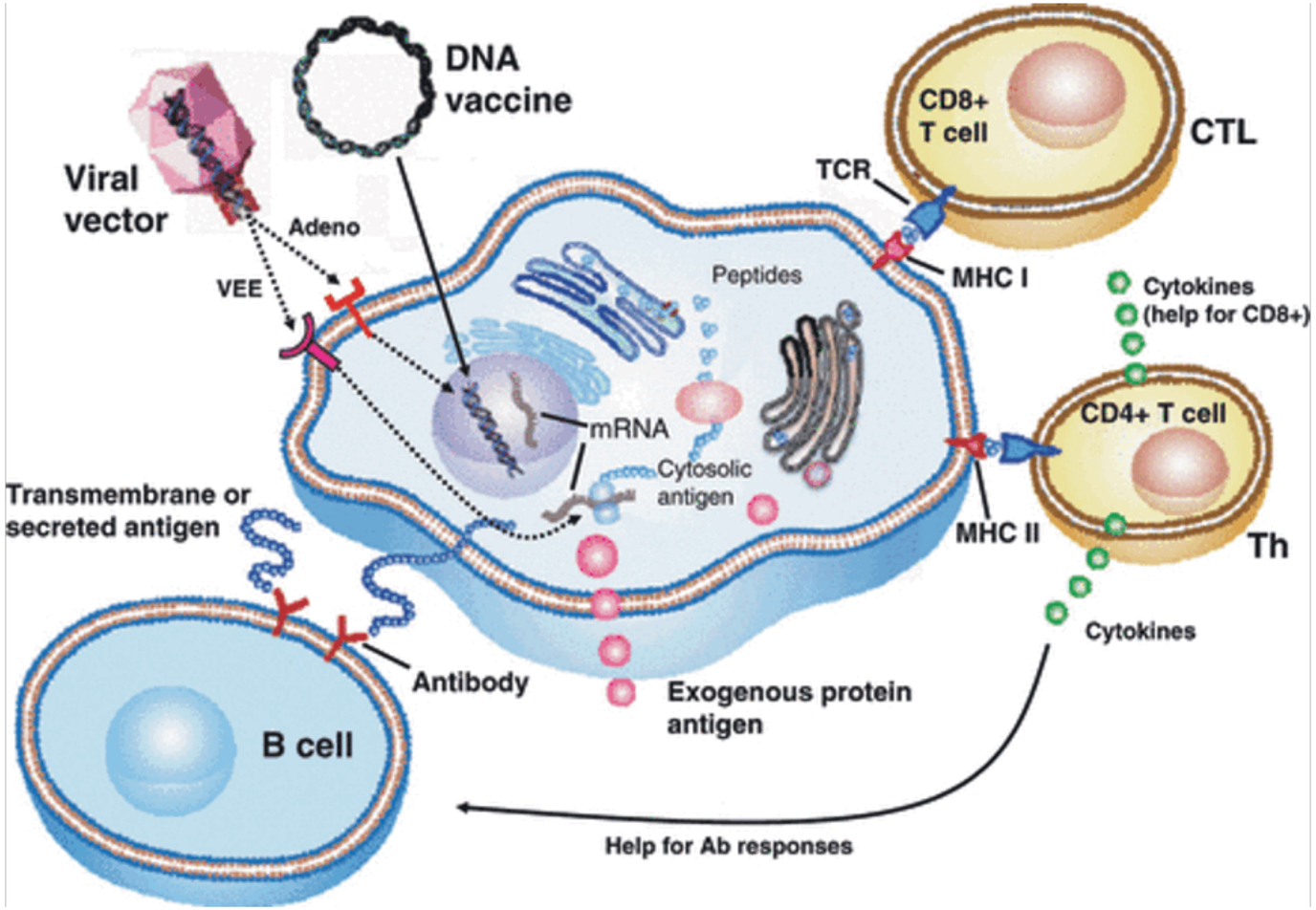
DNA vaccines can be manipulated to present all or part of the genome
Genes which lead to undesired immunological inhibition may be removed

Enhanced manufacturing and storage capability

Better stability and storage capability compared to traditional vaccine formulations
Simpler and less expensive manufacturing process

DNA Vaccine vs Viral Vector

Liu MA. Immunol Rev 239: 62, 2010



mRNAワクチン開発の歴史

研究開発のマイルストーン

マイルストーン		文献
1989	LNP/mRNAトランスフェクション（培養細胞）	PNAS 86: 6077, 1989
1990	プラスミド・mRNA 直接筋注（マウス）	Science 247: 1465, 1990
1993	LNP/mRNAでCTL誘導（マウス）	Eur J Immunol 23: 1719, 1993
2005	RNA修飾による自然免疫抑制	Immunity 23: 165, 2005
	・	
	・	
	・	
2017	初のmRNAワクチン臨床試験（黒色腫）	Nature 585:107, 2020
2020	COVID-19 mRNAワクチン英米緊急使用許可	investors.biontech.de/news-releases/news-release-details/pfizer-and-biontech-achieve-first-authorization-world-vaccine
2021	COVID-19 mRNAワクチン国内承認	www.pmda.go.jp/drugs/2021/P20210212001/672212000_30300AMX00231_A100_6.pdf www.pmda.go.jp/drugs/2021/P20210519003/400256000_30300AMX00266_A100_4.pdf

European Journal of Immunology
Basic·Clinical·Translational

Short Paper

Induction of virus-specific cytotoxic T lymphocytes *in vivo* by liposome-entrapped mRNA

Frédéric Martinon, Sivadasan Krishnan, Gerlinde Lenzen, Rémy Magné, Elisabeth Gomard, Jean-Gérard Guillet, Jean-Paul Lévy, Pierre Meulien

First published: July 1993 | https://doi.org/10.1002/eji.1830230749 | Citations: 228

Abstract

The induction of anti-influenza cytotoxic T lymphocytes (CTL) *in vivo* by immunizing mice with liposomes containing messenger RNA (mRNA) encoding the influenza virus nucleoprotein (NP) is described. NP mRNA, obtained by *in vitro* transcription, was encapsulated into simple cholesterol/phosphatidylcholine/phosphatidylserine liposomes by the detergent removal technique. The dependance of the route of mRNA-liposomes delivery on CTL induction was studied. The CTL induced were identical to those obtained *in vivo* with infectious virus in terms of specificity, lysing both peptide-sensitized and virus-infected targets. Furthermore, with the same mRNA-liposome preparation, virus-specific CTL responses could be also elicited in mice of three different haplotypes each of them known to present a distinct NP peptide in an MHC-restricted fashion. The relevance of these results in the context of vaccine development is discussed.

CpG RNA: Identification of Novel Single-Stranded RNA That Stimulates Human CD14$^+$CD11c$^+$ Monocytes

Takahiro Sugiyama,*† Mayda Gursel,* Fumihiko Takeshita,‡ Cevayir Coban,§¶ Jacqueline Conover,* Tsuneyasu Kaisho,† Shizuo Akira,§¶ Dennis M. Klinman,* and Ken J. Ishii[1]*§¶

Synthetic immunostimulatory nucleic acids such as CpG DNA are being harnessed therapeutically as vaccine adjuvants, anticancer or antiallergic agents. Efforts to identify nucleic acid-based agents capable of more specifically modulating the immune system are being developed. The current study identifies a novel class of single-stranded oligoribonucleotides (ORN) containing unmethylated CpG motifs and a poly(G) run at the 3′ end (CpG ORN) that directly stimulate human CD14$^+$CD11c$^+$ monocytes but not dendritic cells or B cells. CpG ORN activate NF-κB and p38 MAPK, resulting in IL-6 and IL-12 production and costimulatory molecule up-regulation but not IFNα. Methylation of cytosine at the 5′ portion in core CpG motif abrogates such activation. TLR3, 7, 8, or 9 alone did not confer response to CpG ORN, in contrast to previously reported respective nucleic acid ligands. These data suggest that CpG ORN represent a novel class of synthetic immunostimulatory nucleic acids with distinct target cells, receptors, and functions from that of previously known immunomodulatory nucleic acids. *The Journal of Immunology,* **2005, 174: 2273–2279.**

The innate immune system recognizes specific sequence motifs expressed in the DNA and/or RNA of various pathogens (1–4). For example, recognition of CpG motifs in DNA or dsRNA molecules triggers a strong proinflammatory response via TLRs (5, 6). The immunostimulatory activity of CpG DNA is being harnessed therapeutically to prevent or treat infectious diseases, allergy, cancer, and/or autoimmune diseases (7, 8).

Unmethylated CpG motifs are recognized via TLR9 expressed on certain immune cells (such as B cells, plasmacytoid dendritic cells (pDC),[2] and macrophages) (4, 7). CpG DNA is taken up via an endo/phagocytosis pathway controlled by PI3K (9) and interact with intracellular TLR9 (10). The interaction of CpG DNA with TLR9 triggers the recruitment of the MyD88 adaptor molecule, followed by the activation of IL-1R-associated kinase, TNFR-associated factor 6, and IκB kinase, culminating in the nuclear translocation of NF-κB (5, 11, 12). Such activation by CpG DNA results in the production of proinflammatory cytokines (such as IL-6, IL-12, and IFNα/γ), chemokines (such as MCP-1, IFN-γ-inducible protein 10, MIP-1α/β), and Igs (reviewed in Refs. 4 and 7). Recent studies suggest that CpG oligodeoxynucleotides (ODN) are categorized at least into two groups in both a structural and functional manner (7, 8): one is K (also known as B type) CpG ODN consisted of all phosphorothioate backbone with multiple TCG motifs that preferentially stimulate B cells to proliferate and secrete IL-6 and IgM, and the other is type D CpG ODN (also known as A type) with mixed backbone with one palindromic CpG motifs plus poly(G) run at the 3′ end that stimulates pDC to secrete large amount of IFNα, followed by myeloid DC maturation and NK cell activation. These CpG ODN optimized for humans, in contrast to those for mice, are not able to induce a robust amount of IL-12. Since IL-12 is considered to play a critical role in CpG-mediated efficacy in vivo, there is room to improve and/or modify such humanized CpG-based immunotherapeutic nucleic acids.

dsRNA, such as that found during intermediate stages of viral infection, is recognized by the innate immune system through TLR3. dsRNA is a potent stimulator of human monocytes and myeloid DC, inducing the secretion of both IL-12 and IFNα (6, 13). More recently, ssRNA was shown to activate pDC in mice and myeloid DC in humans to produce IFNα and IL-12, respectively (14–17). However, whether the immunomodulatory activity of RNA is sequence specific has not been fully investigated.

The present work examines whether ssRNA have sequence-specific immunomodulatory activity. Results indicate that ssRNA oligonucleotides expressing unmethylated CpG motifs and a poly(G) tail (CpG oligoribonucleotides (ORN)) stimulate CD14$^+$CD11c$^+$ monocytes to produce large amounts of IL-12. This activity is distinct from that of DNA-based CpG ODN. CpG ORN also stimulates human PBMC to activate NF-κB and p38 MAPK and to secrete IL-6, similar to the effect of K-type CpG ODN. Activation of cells by CpG ORN was not mediated through either TLR3, 7/8, or 9, the known receptors for dsRNA, ssRNA, or dsRNA, respectively. The activity of CpG ORN was blocked by inhibitors of PI3K. These data suggest that CpG ORN represent novel immunostimulatory agents stimulating human monocytes to produce IL-12 that is distinct from previously known immunostimulatory nucleic acids.

*Section of Retroviral Immunology, Division of Viral Products, Center for Biologics, Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892; †Laboratory for Host Defense, RIKEN Research Center for Allergy and Immunology, Yokahama, Japan; ‡Department of Molecular Biodefense Research, School of Medicine, Yokohama City University, Yokohama, Japan; §Exploratory Research for Advanced Technology, Japan Science and Technology Agency, Osaka University, Osaka, Japan; and ¶Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Received for publication August 4, 2004. Accepted for publication December 2, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

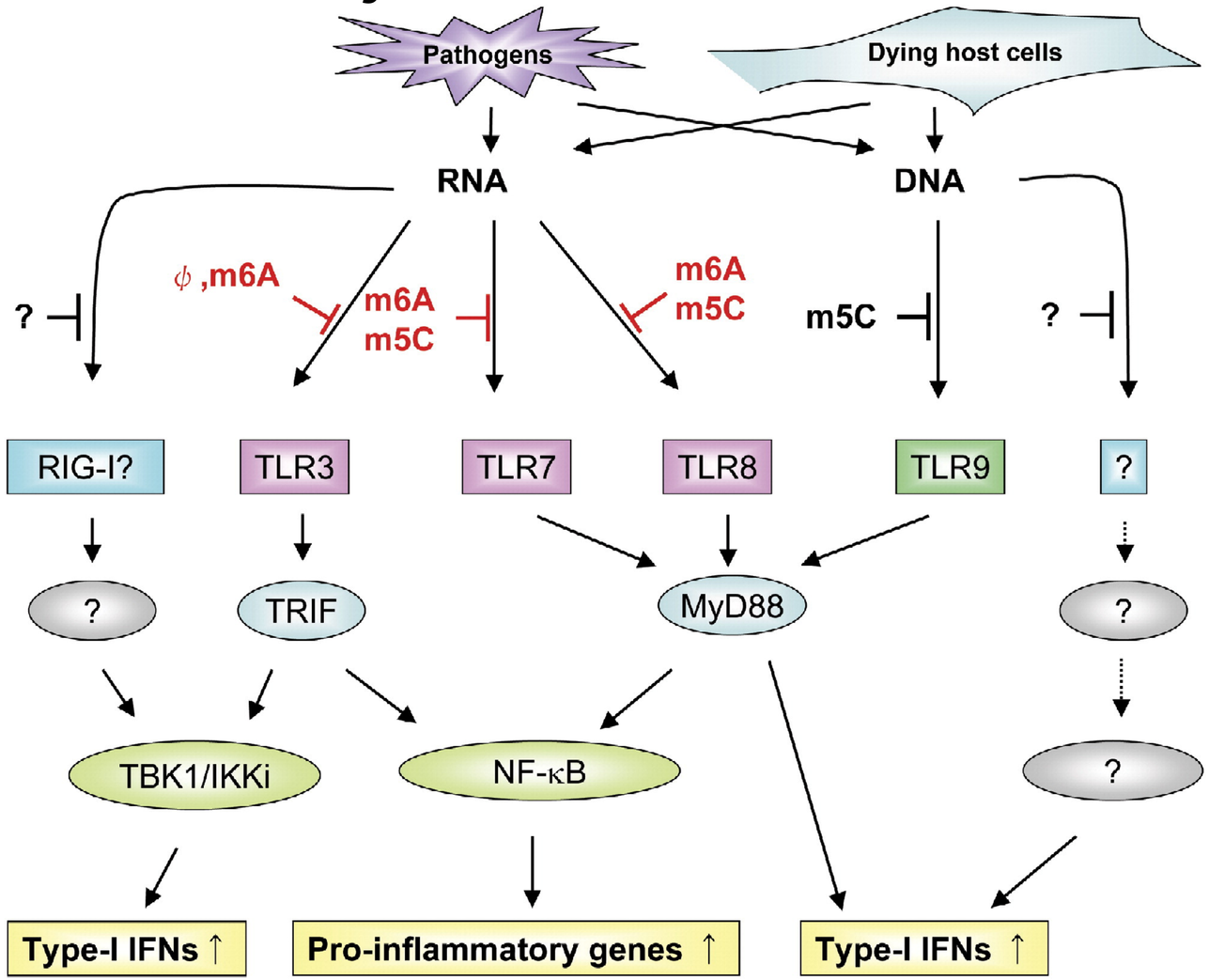
[1] Address correspondence and reprint requests to Dr. Ken J. Ishii, Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail address: kenishii@biken.osaka-u.ac.jp

[2] Abbreviations used in this paper: pDC, plasmacytoid dendritic cell; ODN, oligodeoxynucleotide; ORN, oligoribonucleotide; 2-OME, 2′-*O*-methyl.

0022-1767/05/$02.00

TLR ignores methylated RNA?

Ishii KJ, Akira S. Immunity 23: 111, 2005



Immunity, Vol. 23, 165–175, August, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.06.008

Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA

Katalin Karikó,[1,*] Michael Buckstein,[2] Houping Ni,[2] and Drew Weissman[2]
[1]Department of Neurosurgery
[2]Department of Medicine
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

Summary

DNA and RNA stimulate the mammalian innate immune system through activation of Toll-like receptors (TLRs). DNA containing methylated CpG motifs, however, is not stimulatory. Selected nucleosides in naturally occurring RNA are also methylated or otherwise modified, but the immunomodulatory effects of these alterations remain untested. We show that RNA signals through human TLR3, TLR7, and TLR8, but incorporation of modified nucleosides m5C, m6A, m5U, s2U, or pseudouridine ablates activity. Dendritic cells (DCs) exposed to such modified RNA express significantly less cytokines and activation markers than those treated with unmodified RNA. DCs and TLR-expressing cells are potently activated by bacterial and mitochondrial RNA, but not by mammalian total RNA, which is abundant in modified nucleosides. We conclude that nucleoside modifications suppress the potential of RNA to activate DCs. The innate immune system may therefore detect RNA lacking nucleoside modification as a means of selectively responding to bacteria or necrotic tissue.

Introduction

The innate immune system is the first line of defense against invading pathogens (Medzhitov, 2001). This system utilizes TLRs to recognize conserved pathogen-associated molecular patterns and orchestrate the initiation of immune responses. TLRs are germ line-encoded signaling receptors with extracellular leucine-rich repeats and intracellular signaling domains. In humans, ten distinct TLR family members have been identified, and corresponding microbial ligands for most have been identified. Several TLRs recognize and respond to nucleic acids. DNA containing unmethylated CpG motifs, characteristic of bacterial and viral DNA, activate TLR9 (Hemmi et al., 2000). Double-stranded (ds)RNA, a frequent viral constituent, has been shown to activate TLR3 (Alexopoulou et al., 2001; Wang et al., 2004), single-stranded (ss)RNA activates mouse TLR7 (Diebold et al., 2004), and RNA oligonucleotides with phosphorothioate internucleotide linkages are ligands of human TLR8 (Heil et al., 2004). Based on structural and sequence similarities, TLR7, TLR8, and TLR9 form a subfamily. Activation of these receptors depends upon endosomal acidification and leads to interferon production. Human TLR7 and TLR8 are stimulated by the synthetic antiviral compound R-848 (Jurk et al., 2002), but a natural ligand has not been identified.

It has been known for decades that selected DNA and RNA molecules have the unique property to activate the immune system. It was discovered only recently that secretion of interferon in response to DNA is mediated by unmethylated CpG motifs acting upon TLR9 present on immune cells (Hemmi et al., 2000). For years, bacterial and mammalian DNA were portrayed as having the same chemical structure, which hampered the understanding of why only bacterial, but not mammalian, DNA is immunogenic. Recently, however, the sequence and structural microheterogeneity of DNA has come to be appreciated. For example, methylated cytidine in CpG motifs of DNA has proven to be the structural basis of recognition for the innate immune system. In light of this finding and given that multiple TLRs respond to RNA, a question emerges as to whether the immunogenicity of RNA is under the control of similar types of modification. This possibility is not unreasonable given that RNA undergoes nearly one hundred different nucleoside modifications (Rozenski et al., 1999). Importantly, the extent and quality of RNA modifications depend on the RNA subtype and correlate directly with the evolutionary level of the organism from which the RNA is isolated. Ribosomal RNA, the major constituent (~80%) of cellular RNA, contains significantly more nucleoside modifications when obtained from mammalian cells versus bacteria. Human rRNA, for example, has ten times more pseudouridine (Ψ) and 25 times more 2′-O-methylated nucleosides than bacterial rRNA, whereas rRNA from mitochondria, an organelle that is a remnant of eubacteria (Margulis and Chapman, 1998), has very few modifications (Bachellerie and Cavaille, 1998). Transfer RNA is the most heavily modified subgroup of RNA. In mammalian tRNAs, up to 25% of the nucleosides are modified, whereas there are significantly less modifications in prokaryotic tRNAs. Bacterial mRNA contains no nucleoside modifications, whereas mammalian mRNAs have modified nucleosides such as 5-methylcytidine (m5C), N6-methyladenosine (m6A), inosine and many 2′-O-methylated nucleosides in addition to N7-methylguanosine (m7G), which is part of the 5′-terminal cap (Bokar and Rottman, 1998). The presence of modified nucleosides was also demonstrated in the internal regions of many viral RNAs including influenza, adeno, and herpes simplex; surprisingly, modified nucleosides were more frequent in viral than in cellular mRNAs (Bokar and Rottman, 1998). A substantial number of nucleoside modifications are uniquely present in either bacterial or mammalian RNA, thus providing an additional molecular feature for immune cells to discriminate between microbial and host RNA. Considering that cells usually contain five to ten times more RNA than DNA, presence of such distinctive characteristics on RNA could make them a rich molecular source for sampling by the immune system, a notion becoming evident by the identification of multiple TLRs signaling in response to RNA. The role

*Correspondence: kariko@mail.med.upenn.edu

LNP：mRNAワクチンのもう一つの鍵

1970年代から研究されてきた

www.nanomedicines.ca/2020/11/12/canadian-technology-enables-covid-19-vaccine/

DDSとして

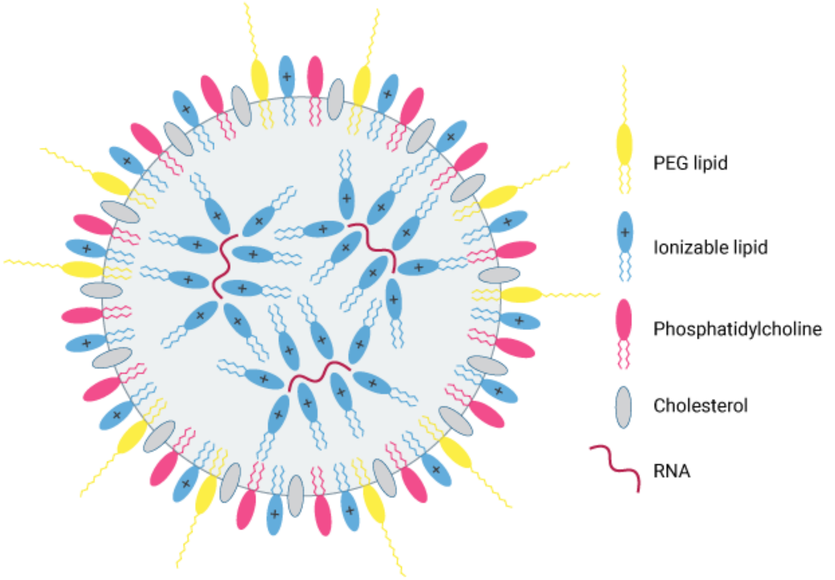
ヌクレアーゼからのmRNA保護

mRNAの負電荷中和

標的細胞指向性、細胞内トラフィッキング

アジュバントとして

副反応の違いにも影響

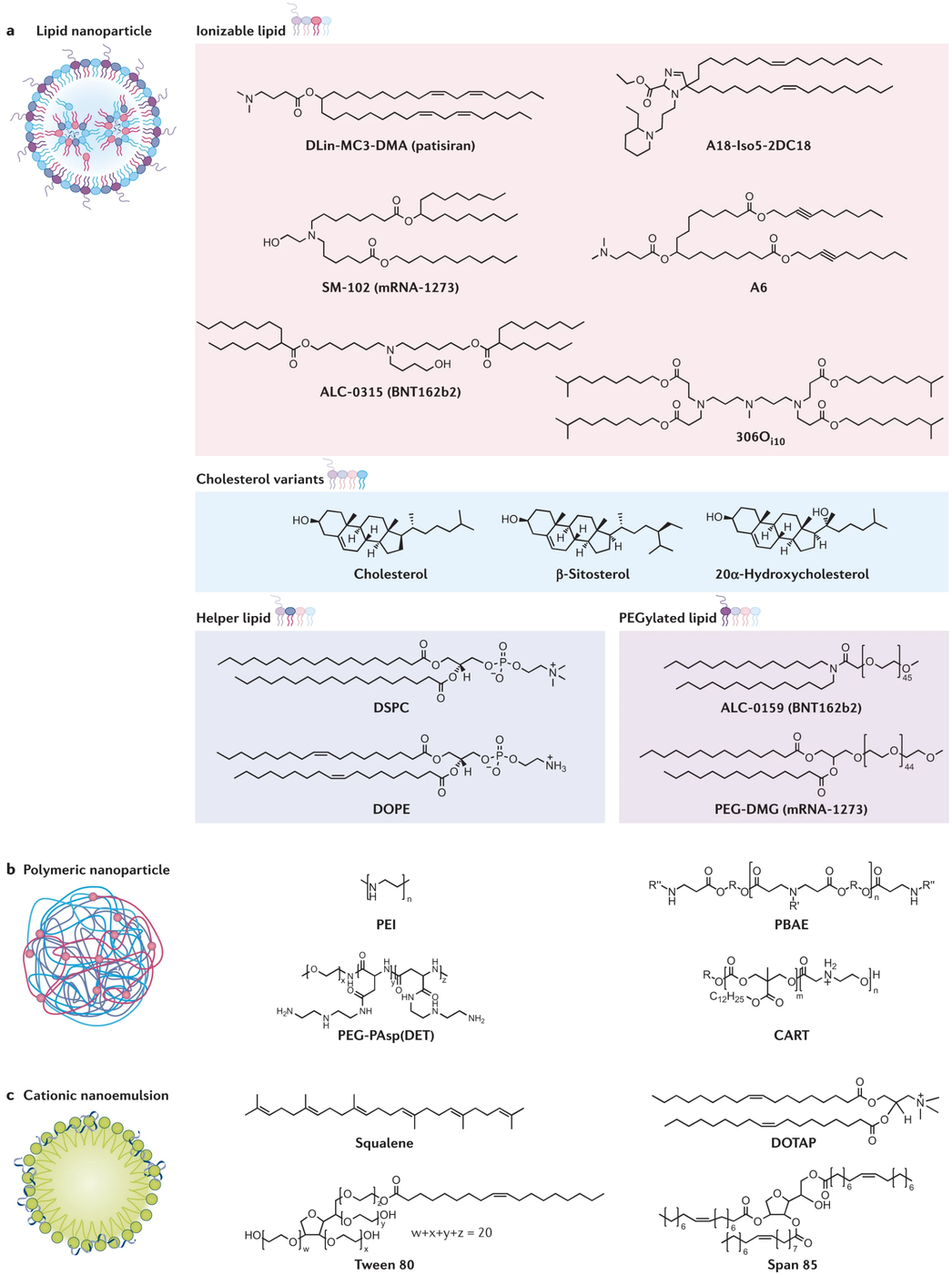


www.cosmobio.co.jp/product/detail/lipid-nanoparticles-for-rna-delivery-ecl.asp?entry_id=43081

Chaudhary N, Weissman D, Whitehead KA.

Nat Rev Drug Discov 20: 817, 2021

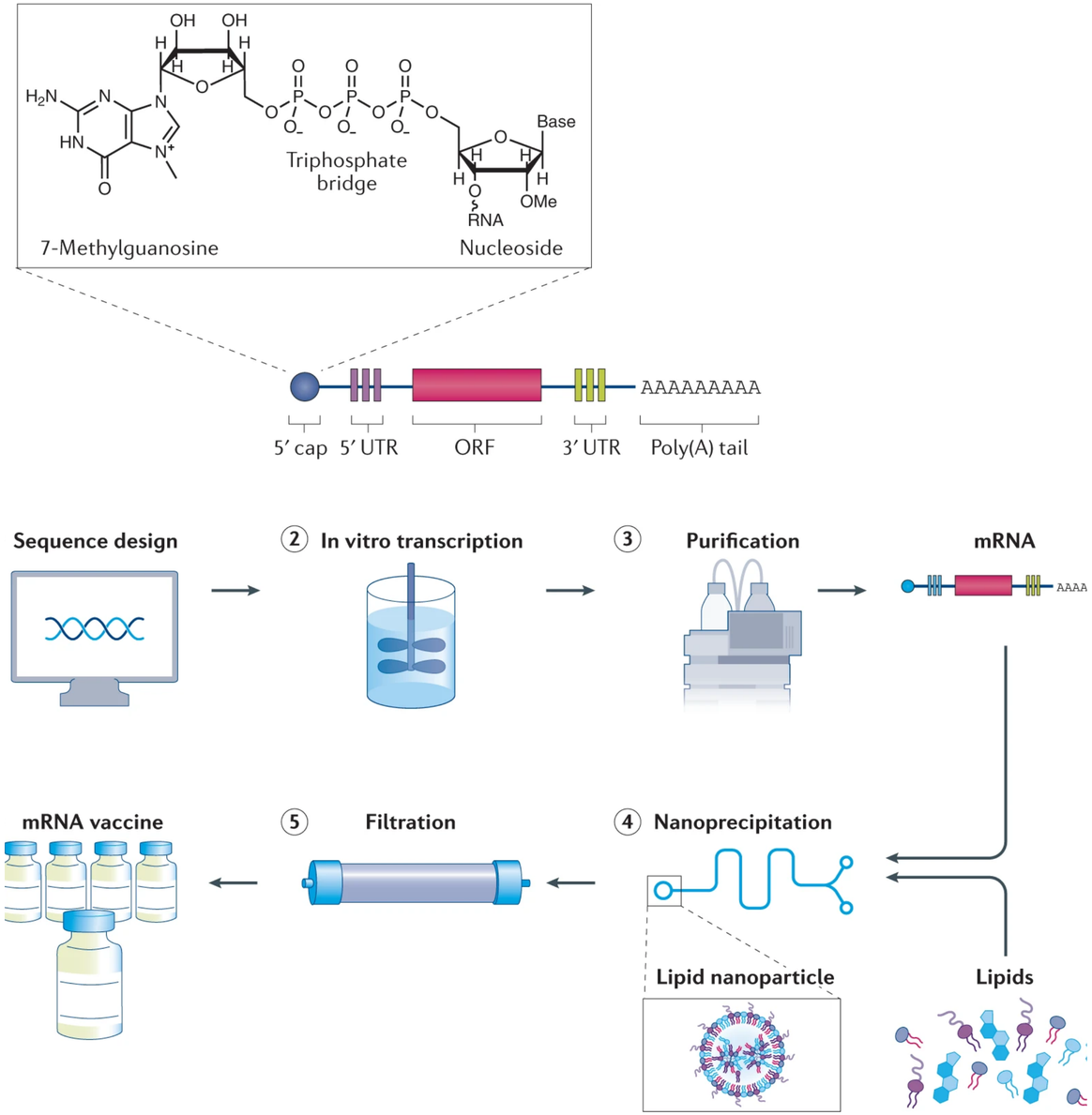
All mRNA delivery vehicles contain cationic or ionizable molecules.



Chaudhary N, Weissman D, Whitehead KA.

Nat Rev Drug Discov 20: 817, 2021

IVT mRNA is formulated into lipid nanoparticle vaccines using a cell-free production pipeline.



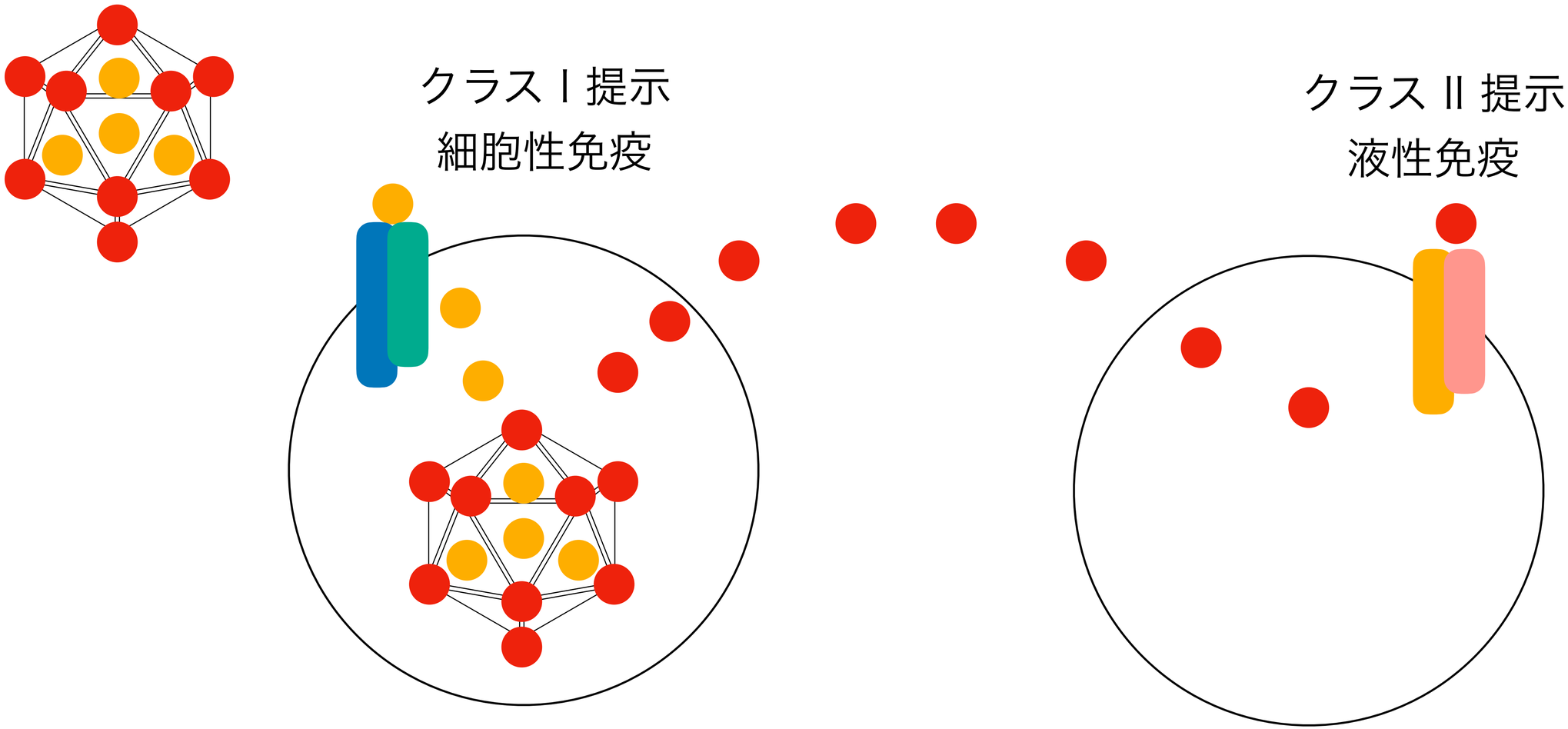
ワクチンの種類と誘導される免疫

生、不活化、成分、核酸ワクチン、ウイルスベクター

ワクチンの種類	例	細胞性免疫	液性免疫
生	麻疹	+	+
不活化	日本脳炎	-	+
成分	インフルエンザ	-	+
核酸	COVID-19	+	+
ウイルスベクター	COVID-19	+	+

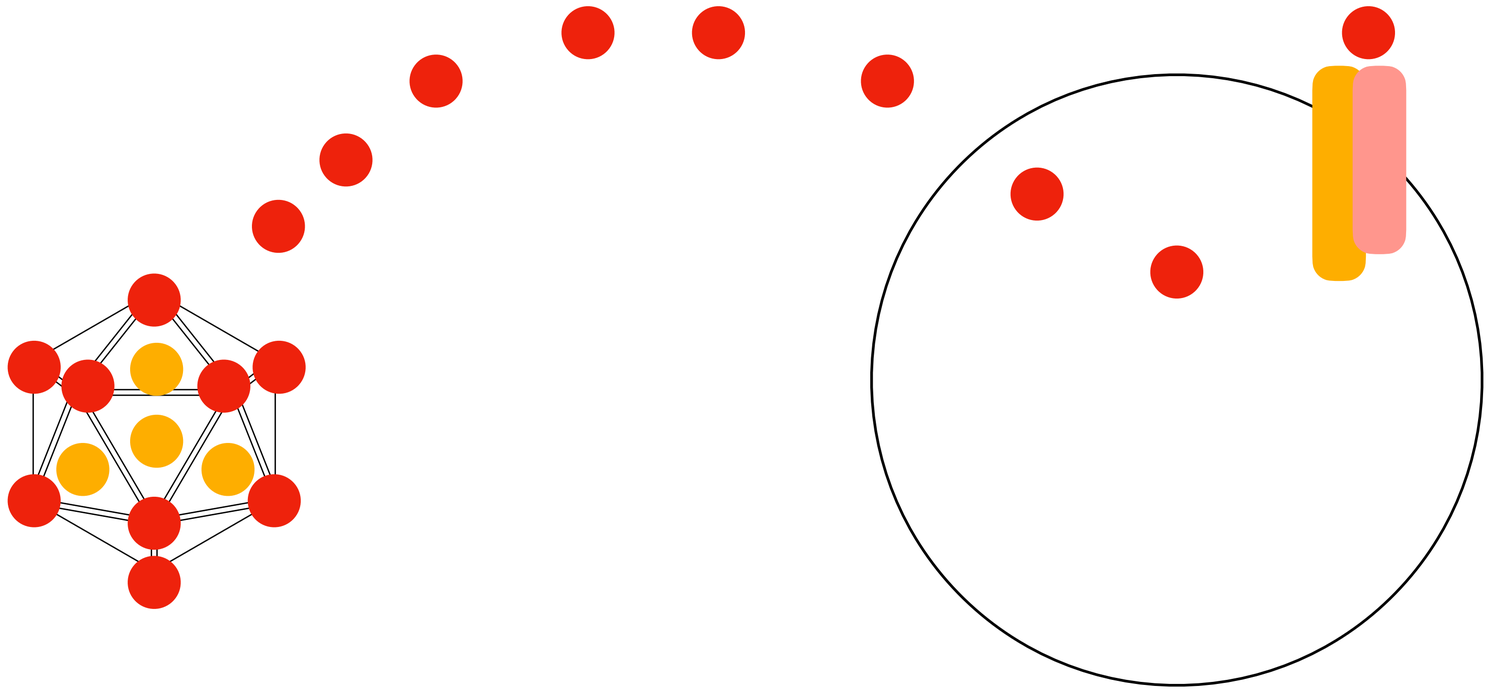
従来のワクチン

生ワクチン：自然感染と相似



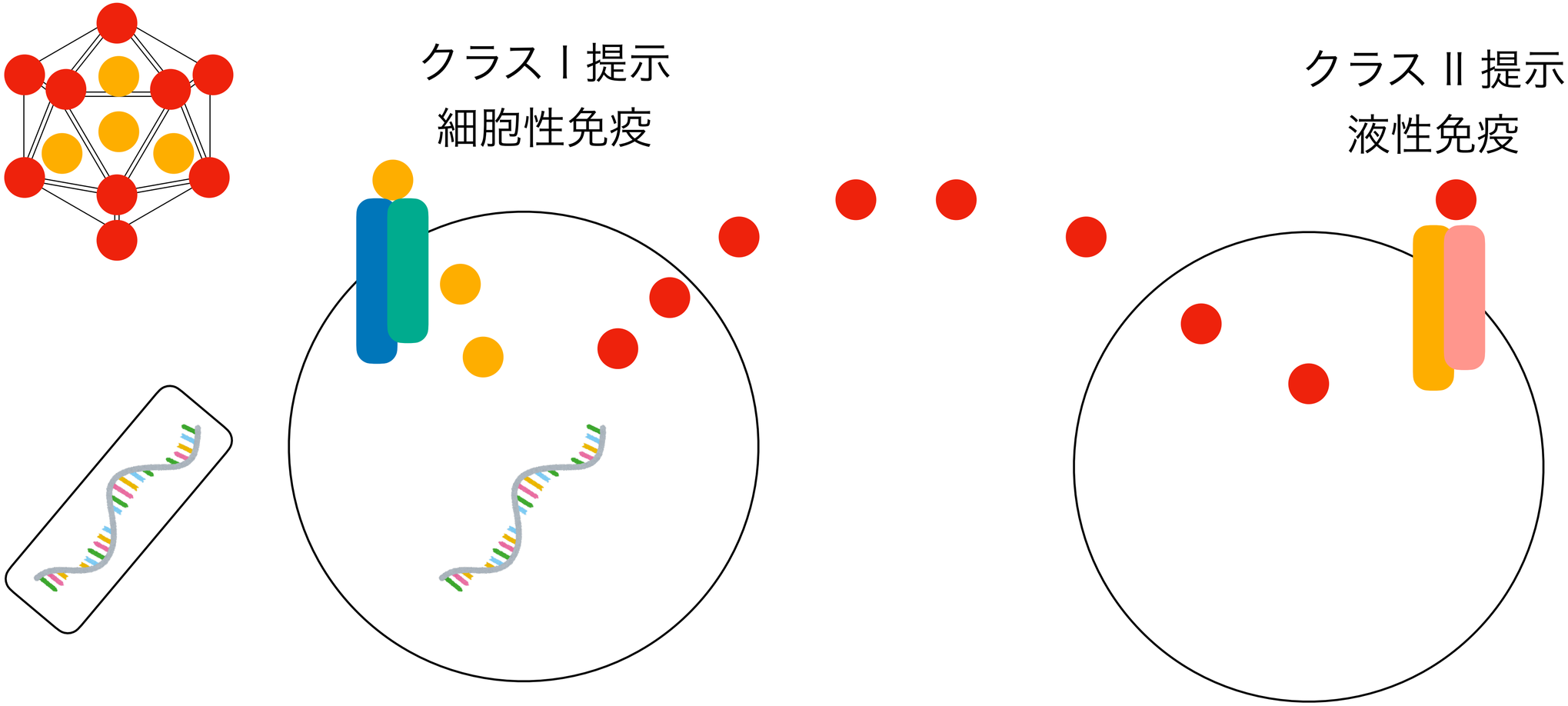
従来のワクチン

不活化／成分ワクチン、トキソイド



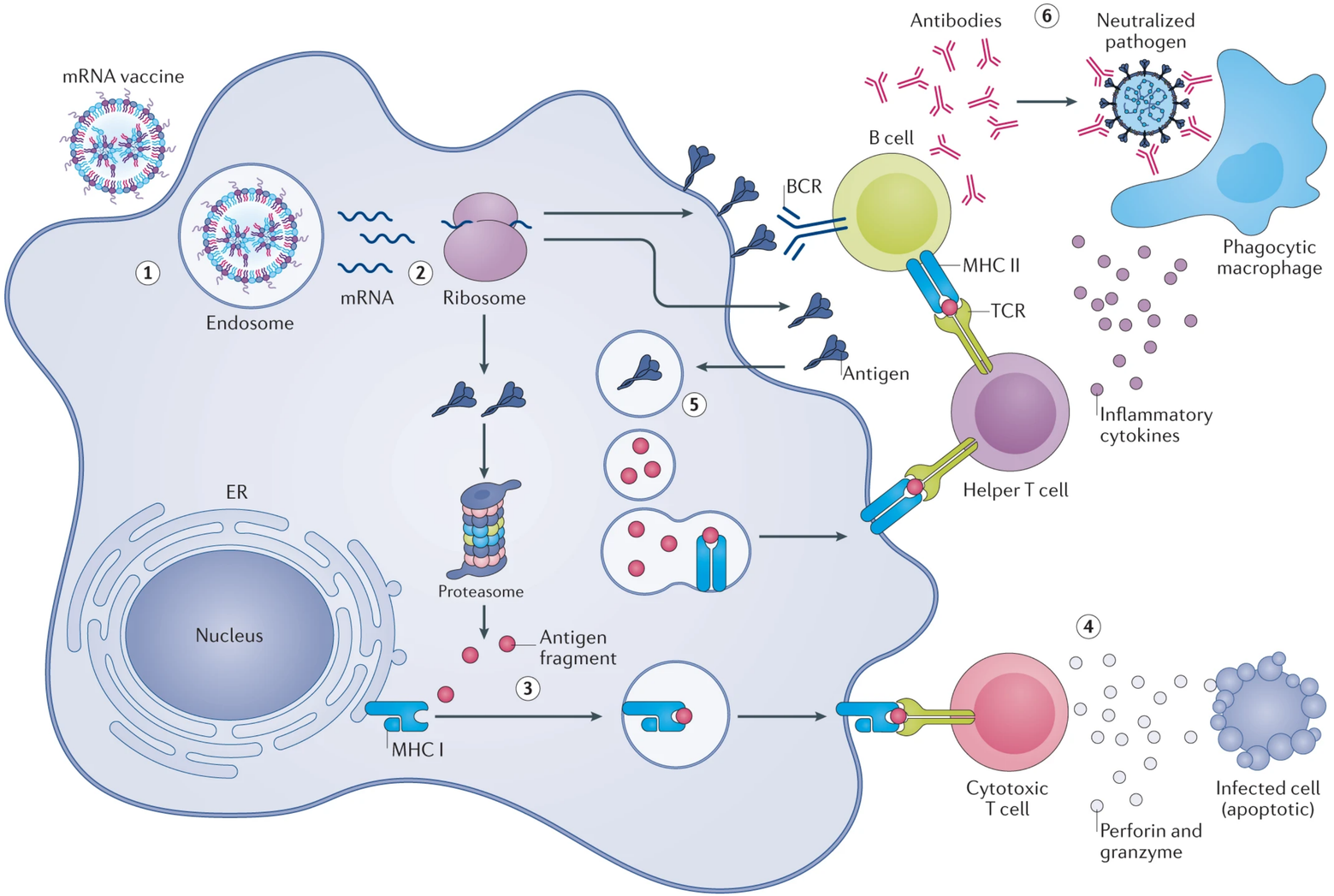
新しいワクチン

核酸ワクチン

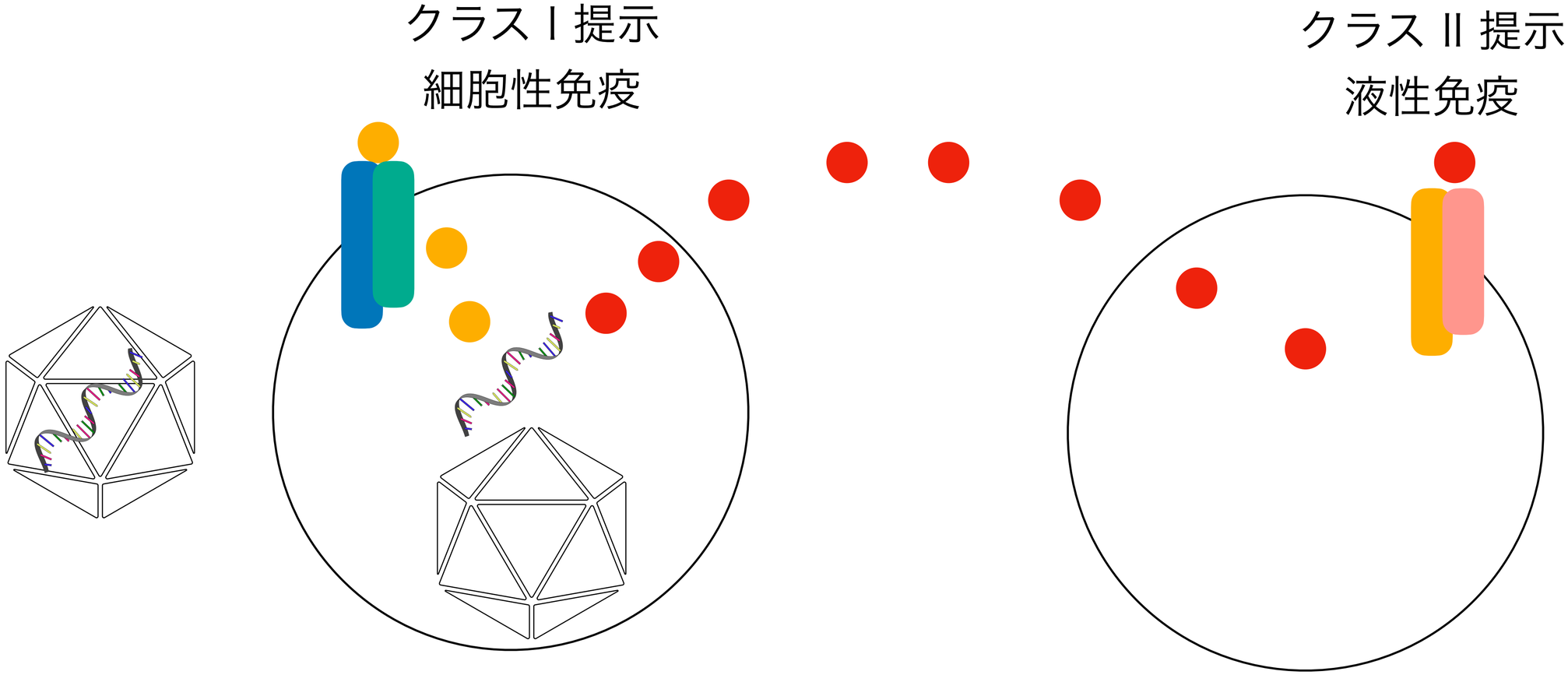


Chaudhary N, Weissman D, Whitehead KA.
Nat Rev Drug Discov 20: 817, 2021

Messenger RNA vaccines elicit immunity through transfection of antigen-presenting cells.



新しいワクチン
ウイルスベクター

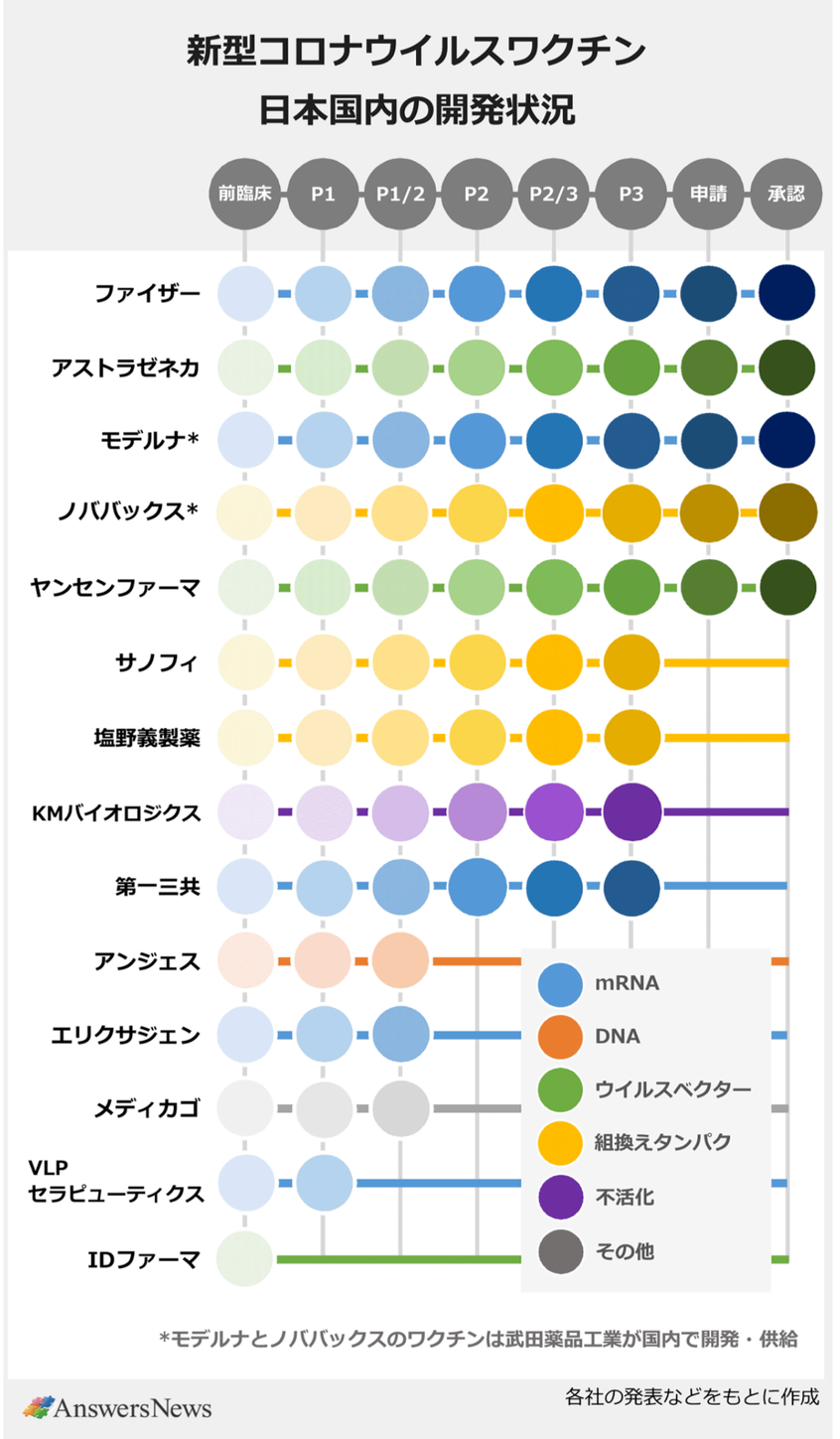


新型コロナウイルスワクチン
日本国内の開発状況（7/13）

answers.ten-navi.com/pharmanews/20139/

承認済

- mRNA
  - コミナティ
  - スパイクバックス
- 組換えウイルス
  - バキスゼブリア
  - ジェコビデン
- 組換え蛋白
  - ヌバキソビッド



Chaudhary N, Weissman D, Whitehead KA.

Nat Rev Drug Discov 20: 817, 2021

mRNA vaccines in development protect against an array of common pathogens using disease-specific targeting strategies.