the TLR-7/9-MyD88-dependent signaling pathway (Kawai and Akira 2006) (Fig. 12.1).

Notably, TLR activation upon recognition by nucleic acids occurs mainly in the endosome. Conceivably, TLR localization in the endosome is necessary to prevent contact with "self" nucleic acids, which are not taken into the endosome without additional components, as described below.

12.3.1.2 Cytoplasmic Sensors for Exogenous Nucleic Acids

Although endosomal TLRs that recognize nucleic acids are expressed mainly in the specialized immune cells, such as B cells and DCs, there are receptors in the cytoplasm that are also capable of sensing nucleic acids, namely RLRs and NLRs. In addition, several molecules have been proposed as capable of recognizing double-stranded B-form DNA (Takeshita and Ishii 2008; Vilaysane and Muruve 2009).

RIG-I and Melanoma Differentiation-Associated gene 5 (MDA5) belong to RLRs (Yoneyama et al. 2004; Takeuchi and Akira. 2008). Both of these cytoplasmic proteins contain helicase domains, including ATP-binding domains, C-terminal regulatory domains capable of binding tRNA, and Caspase Activation and Recruitment Domains (CARDs) to interact with adaptor molecule Interferon-β Promoter Stimulator 1 (IPS-1, also known as MAVS, Cardif and VISA). RIG-I discerns viral RNA by detecting the 5′-triphosphates of ssRNA and its short double-stranded form, while MDA5 recognizes long double-stranded RNA (Takeuchi and Akira 2008; Saito and Gale 2008). However, the exact element in viral RNA identified by RIG-I and MDA5 is currently unknown. It has been shown so far that genomic RNA of influenza viruses, paramyxoviruses, and HCV and short ($\approx$1 kb) dsRNAs are detected by RIG-I, while picornaviruses such as Encephalomyocarditis Virus (EMCV) and longer (> 2 kb) dsRNA such as poly I:C are detected by MDA5 (Kato et al. 2008; Saito et al. 2008). Both RIG-I and MDA5 signal through IPS-1, which lies on the outer membrane of mitochondria and is associated with Stimulator of IFN Genes (STING; also known as MITA) (Ishikawa and Barber 2008; Zhong et al. 2008) and Fas-Associated Death Domain (FADD) (Balachandran et al. 2004). This complex activates NF-κB and IRF3 via IκK complex and TBK1/IκKi, respectively, initiating pathways that culminate in proinflammatory cytokines and IFN-β production (Fig. 12.1). LGP2 is also an RLR and shares homology with RIG-I and MDA5 in the helicase domain but lacks a CARD domain. It was shown in vitro to be a negative regulator of RIG-I and MDA5; however, results derived from knockout mice suggest that it is actually a positive regulator (Venkataraman et al. 2007; Satoh et al. 2010).

Cytoplasmic DNA recognition is quite distinct from RLR-mediated cytoplasmic RNA recognition. As initially shown by Isaacs et al. (1963) and rediscovered by Suzuki et al. (1999), DNA, especially double-stranded DNA, has been shown to be immunomodulatory. Ishii et al. refined their findings that transfection by natural DNA, or by synthetic polynucleotides that form double-stranded structures,

stimulates cells to produce type-I IFNs and induces cell-autonomous protection from viral replication, independently of TLR9. Unlike the CpG motifs needed for TLR9 activation, methylation of such dsDNA has no effect on activity. Rather, poly (dA-dT) • poly(dT-dA) induces higher levels of type-I IFNs compared with poly (dG-dC) • poly (dC-dG), suggesting that the right-handed helical structure of B-form DNA (B-DNA) is essential for cellular activation of type-I IFNs production; this process is mediated through a TLR-independent, TBK1-dependent means (Ishii et al. 2006). Therefore, TLR-independent, TBK1-dependent cytoplasmic DNA recognition plays an important role in immune responses during viral and bacterial infections (Yasuda et al. 2005; Ishii et al. 2006; Stetson and Medzhitov 2006; Cortez-Gonzalez et al 2006; Martin and Elkon 2006), and in controlling the ensuing adaptive immune responses (Ishii et al. 2008a; Baccala et al. 2007; Babiuk et al. 2004; Spies et al. 2003).

DNA derived from dying host cells can reportedly accumulate when nuclease functions are obstructed, including DNase-I, II, and -III (also known as TREX) (Okabe et al. 2005; Yoshida et al. 2005; Yasutomo et al. 2001; Morita et al. 2004; Napirei et al. 2000). The resultant activation of immune responses through TLR-independent DNA recognition by as-yet undefined receptors can lead to immunological disorders, such as autoimmune diseases (Kawane et al. 2006; Stetson et al. 2008).

Many receptors have been proposed for this TLR-independent, TBK1-dependent type-I IFN production by ds B-form DNA. The first candidate DNA sensor was reported to be DAI (DNA-dependent activator of IFN-regulatory factors), previously called DLM-1 and Z-DNA binding protein 1 (ZBP1) (Takaoka et al. 2007). However, mice lacking DAI (ZBP-1) did not show any expected phenotypes, in vitro or in vivo, suggesting that DAI is not essential for DNA-induced, TBK1-dependent type-I IFN production, or for DNA vaccine immunogenicity (Ishii et al. 2008a). More recent reports suggest that RNA polymerase-III can recognize AT-rich dsDNA, to generate 5′-triphosphate RNA, activating RIG-I in human cells (Ablasser et al. 2009), or in transformed cells (Chiu et al. 2009). Moreover, RIG-I (Choi et al. 2009), HMGB proteins (Yanai et al. 2009), and histone H2B (Kobiyama et al. 2010) were shown to recognize ds B-form DNA and respond by promoting TBK1-dependent type-I IFN production. Although it may take time to resolve how these distinct proteins recognize ds B-form DNA, it will be an exciting field of research.

Recent reports indicate that both type-I IFN (through RLRs) and IL-1β/18 (through inflammasome activation) are engaged in immune response to cytoplasmic nucleic acids (Muruve et al. 2008; Kanneganti et al. 2006a, b). Initially, microbial recognition by innate immune receptors like TLRs induces pro-IL-1β/18 accumulation in the cytosol through MAPK or NF-κB activation. Pro-IL-1β/18 is then cleaved with caspase-1, a major component of all types of inflammasomes, and released as mature form, IL-1β/18. Currently, four types of inflammasome complexes, NLRP1, NLRC4, NLRP3, and AIM2 inflammasome, have been partially characterized. Among them, only NLRP3 and AIM2 appear to be involved in nucleic acid sensing (Vilaysane and Muruve 2009; Franchi et al. 2009).

The NLRP3 inflammasome consists of NLRP3 (also known as NALP3 or cryopyrin), which is an NLR – an Apoptosis-Associated Speck-Like Protein Containing a CARD (ASC) and caspase-1. NLRs are a large family of cytoplasmic sensors, containing 23 members in human and 34 members in mice; their ligands have been only partially elucidated. NLRP3 consists of three domains: C-terminal LRR, central Nucleotide-Binding Oligomerization (NOD) domain and N-terminal ligand-sensing domain, and Pyrin Domain (PYD). A variety of ligands, including nucleic acids and their metabolites, such as RNA, RNA analogs, uric acids crystals, and ATP, are known to trigger NLRP3 inflammasomes (Martinon et al. 2006; Mariathasan et al. 2006; Kanneganti et al. 2006a), inducing formation of inflammasome complexes. These complexes include NLRP3 multimers, the adaptor molecule ASC, and pro-caspase-1 recruited via the ASC CARD domain, leading to autocleavage of caspase-1 (Fig. 12.1). However, it is unclear how NLRP3 detects its nucleic acid ligands.

In contrast to the NLRP3 inflammasome, which is mainly engaged in the recognition of RNAs, such as bacterial and viral RNA, synthetic dsRNA (poly I:C), and ssRNA (imidazoquinoline), the AIM2 inflammasome is activated by ds B-form DNA derived from bacteria, viruses, and host. AIM2 was recently identified as a member of HIN200 protein family; it consists of two domains: HIN200 domain, which binds to cytoplasmic dsDNA , and PYD, which recruits ASC (Roberts et al. 2009; Burckstummer et al. 2009; Fernandes-Alnemri et al. 2009; Hornung et al. 2009). The AIM2 inflammasome leads to activation of caspase-1 in the same manner as the NLRP3 inflammasome (Fig. 12.1).

12.3.2 The Mechanism to Avoid the False Recognition of the Endogenous Nucleic Acids: The Case of Tissue Damage

The innate immune system protects the host against invading infectious agents; however, the same system can also respond to endogenous stimuli (Gallucci et al. 1999; Ishii et al. 2001; Tsan and Gao 2004; Kono and Rock 2008). These stimuli include molecules released from damaged or dying host cells; some are endogenous cytokines and chemokines, including HMGB proteins, IL-1α, and IL-33; the others are heat-shock proteins, hyaluronan degradation fragments, oxidized lipids, nucleic acids, etc. RNA and DNA are normally sequestered tightly in the cells but can be released from host cells in the case of tissue damage, such as necrosis and apoptosis (Matzinger 2002; Ishii and Akira 2005). Nevertheless, nucleic acids are barely recognized by host immune system as there are safety mechanisms. One of these is the limited accessibility to the endosomal compartments where nucleic acid-sensing TLRs are expressed. Another is the immediate elimination of nucleic acids by RNases or DNases within the phagosome, extracellular matrix, or in the serum. A third is the sequential or molecular modification of nucleic acids (Kariko et al 2005). The presence of so many safety mechanisms suggests that any nucleic acid

species can be immunostimulatory if not in the right place in the cells or tissues. In fact, endogenous RNA and DNA are quite immunostimulatory if these safety mechanisms are broken – for example, if immune complexes of RNA or DNA with anti-RNA/DNA antibodies or RNPs are exogenously introduced into cells by transfection reagents, or if nucleotides are subjected to nuclease-resistant modification , or host nucleotides are removed or naturally modified. Otherwise, host RNA and DNA are normally inert to our immune system.

12.3.3 The Mechanism to Recognize Endogenous Nucleic Acids and Their Metabolites as Danger Signal: Another Case of Tissue Damage

Normally, extracellular concentrations of nucleic acids are very low. However, several kinds of molecules, including nucleic acids and their metabolites, which are mainly stored inside the host cell, are released from damaged or dying host cells in the case of tissue injury. Aberrant high concentration of extracellular nucleotides, such as ATP and its metabolic end products, uric acids, can be detected by innate immune sensors as danger signals (Mariathasan et al. 2006; Martinon et al. 2006) (Fig. 12.2). These responses culminate in IL-1β/18 production via NLRP3 inflammasome activation. Although several kinds of mechanisms that activate inflammasomes appear to be involved, the ATP-gated P2X7 receptor contained in the P2 receptor, a class of ubiquitous plasma membrane receptors, plays an important role in the upstream signals that trigger inflammasome activation (Kahlenberg et al. 2005; Ferrari et al. 2006; Di Virgilio 2007). Extracellular high concentration of ATP, a predominantly intracellular molecule, stimulates the P2X7 receptor and induces the activation of a cation channel that mediates potassium efflux. It also induces high concentrations of end-product uric acids and generates uric acids crystals, both of which can activate NLRP3 inflammasomes in gout (Martinon et al. 2006). Extracellular ATP is also engaged in the pathogenesis of bronchial asthma (Idzko et al. 2007). Uric acids, ATP, and adenosine – which is degraded from ATP through a cascade of ectonucleotidases, including nucleoside triphosphate diphosphorylase (NTPDase, also called CD39) and 5′-ectonucleotidase (Ecto50NTase, also called CD73) – induce immune responses (Hasko and Cronstein 2004). Extracellular adenosine binds to and activates four G protein-coupled cell surface receptors, A1, A2A, A2B, and A3, contained in P1 receptors, which signal through alterations in intracellular cyclic AMP and Ca^{2+} concentrations.

Nucleotides and their metabolites can both activate immune responses as danger signals and control or suppress immune reactions (Di et al. 2009). Adenosine's interaction with A2A receptors, which are the predominant subtype in immune cells, may inhibit inflammation by cAMP induction. For instance, hypoxia-inducible factor-1α (HIF-1α) mediates 5′-nucleotidase induction following accumulation of extracellular adenosine; therefore, hypoxic conditions can also

induce immunosuppressive effects via the A2A receptor (Ohta and Sitkovsky 2009). Thus, the recovery process from tissue damage is built upon a strict immunomodulatory mechanism.

12.3.4 *Endogenous Nucleic Acids Recognition: The Case of Autoimmune Diseases*

Despite the mechanisms to prevent false recognition of "self" nucleic acids and their metabolites described above, innate immune recognition of endogenous nucleic acids can induce detrimental adaptive immune response to "self" antigen – the so-called autoimmune diseases (Marshak-Rothstein and Rifkin 2007). Two major mechanisms of "self" nucleic acids have been identified to trigger autoimmune diseases: delayed clearance of damaged host cells, and unfavorable reactions to immune complexes (Fig. 12.3).

Damaged host cells generated from microbial infections and tissue injuries are immediately eliminated by phagocytes in normal conditions; however, necrotic cells always lose membrane integrity and unavoidably release intracellular contents,

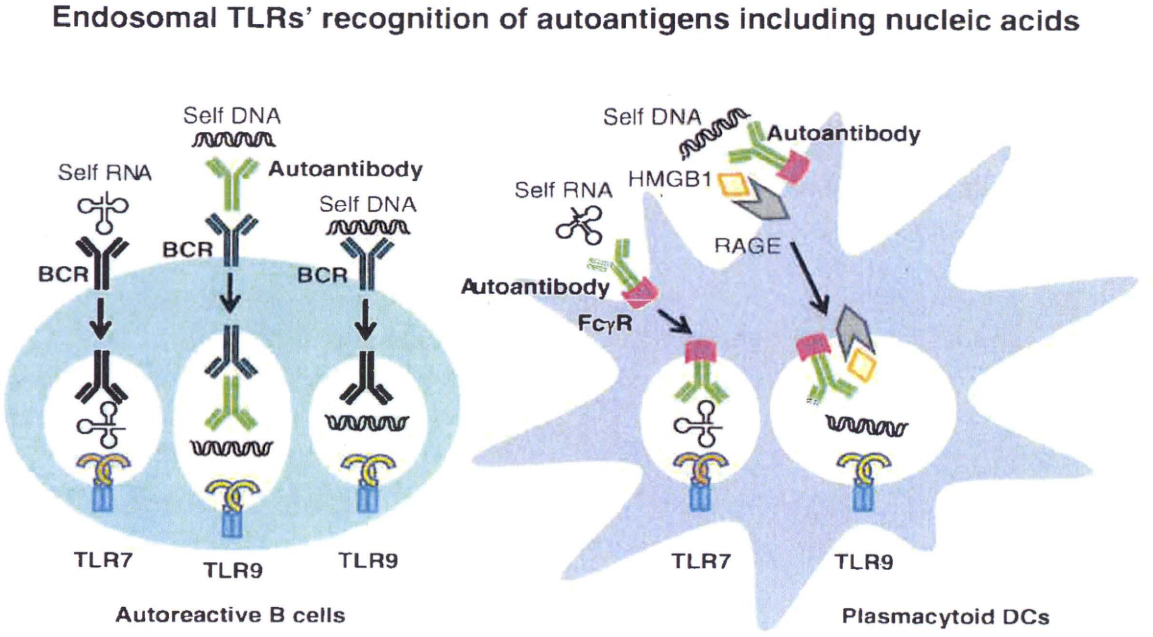

Fig. 12.3 B-cell receptors (BCRs) expressed on the surface of autoreactive B cells can bind autoantigen (DNA or RNA) directly or immune complexes including DNA or RNA and transport both BCRs and autoantigen or immune complexes to the cytoplasmic endosomal compartment containing TLR7/9. pDCs express receptors for the Fc portion of IgG (FcγRs). Immune complexes including DNA or RNA bind FcγRs on pDCs and are transported to cytoplasm. The interaction between HMGB1 (High-Mobility Group Box 1) and RAGE (receptor for advanced glycation end products) also contributes to the recognition of self-DNA-containing immune complexes

inducing immune responses via danger signals (Matzinger 2002). In contrast to necrotic cells, apoptotic cells are strictly cleared through interactions between opsonins and their receptors, such as scavenger receptors, complement receptors on phagocyte cell surfaces (Savill et al. 2002; Erwig and Henson 2008), and the recently identified peroxisome proliferator-activated receptor-δ (PPAR-δ) sensing system (Mukundan et al. 2009). Recent studies also suggest that impaired clearance of nucleic acids in apoptotic cells plays an important role in autoimmunity pathogenesis (Kawane et al. 2006; Napirei et al. 2000; Stetson et al. 2008).

Autoreactive B cells can bind autoantigens released from dying host cells via specific B-Cell Receptors (BCR), which are shown to exist in 5–20% of healthy individuals (Wardemann et al. 2003), and in a greater percentage of autoimmune-disease patients, due to defective early B-cell tolerance (Yurasov et al. 2005). Once these autoantigens are endocytosed by BCR, nucleic acids contained in the cells can stimulate endosomal TLR-7 and -9. These immune system activations, especially type-I IFNs responses via TLRs, play a key role in autoantibody production from proliferated and differentiated autoreactive B cells (Le Bon and Tough 2002; Theofilopoulos et al. 2005; Marshak-Rothstein and Rifkin 2007). B cells also express TLR-9 and the antigen receptor for self-immunoglobulin-gamma (IgG). Both are engaged in the recognition of IgG2a-chromatin immune complexes and induce production of a class of autoantibodies known as Rheumatoid Factors (RF) (Leadbetter et al. 2002). Both B cells and pDCs are involved in recognition of immune complexes – including self-RNA and DNA. PDCs express FcγRs (also known as CD32) to detect the Fc portions of autoantibodies, and Receptor for Advanced Glycation End Products (RAGE), to detect extracellular High-Mobility Group Box-1 (HMGB1) derived from necrotic cells (Tian et al. 2007). These interactions induce engulfment of immune complexes and stimulate TLR-7/8 and -9 in endosomal components, as with B cells.

Microbial infections can also trigger or accelerate a detrimental cascade mediated through increased proinflammatory cytokines (Munz et al. 2009) and damaged host cell products; interestingly, the gene dosage of TLR-7 directly contributes to the risk of autoimmune diseases (Pisitkun et al. 2006; Subramanian et al. 2006).

12.4 Therapeutic Applications of Nucleic Acids as Innate Immune Activators: Vaccine and Vaccine Adjuvants (Table 12.2)

As described above, extracellular nucleic acids and their metabolites can induce both immune activation and suppression via immune receptors, suggesting that nucleic acids and their analogs are candidates for therapeutic agents against infectious diseases, such as vaccines and vaccine adjuvants (Table 12.2) as well as therapies for autoimmune diseases and allergies. Here, we discuss vaccines and vaccine adjuvants that utilize the immunostimulatory effect of nucleic acids: DNA

Table 12.2 Nucleic acids based vaccines and vaccine adjuvants

Vaccine/Vaccine adjuvants (Formation of nucleic acids)	Receptors and adaptors involved in the recognition
Inactivated influenza whole virus vaccine (ssRNA)	TLR7/8-MyD88, RIG-I-IPS-1, NLRP3-ASC
Imidazoquinolines (ssRNA)	TLR7/8-MyD88, NLRP3-ASC
Poly I:C, Poly I:C[12]U (dsRNA)	TLR3-TRIF, MDA5-IPS-1, NLRP3-ASC
CpG oligonucleotides (ssDNA)	TLR9-MyD88
Poly dA:dT (dsDNA)	Unknown-STING-TBK1
DNA vaccine (dsDNA)	Unknown-STING-TBK1

Underlined innate immune signalings are essential for their immunogenicity
Poly I:C: polyinosinic:polycytidylic acid, STING: stimulator of interferon genes

vaccine (dsDNA), inactivated Whole Virus Influenza vaccine (influenza WV) (ssRNA) and poly I:C (dsRNA). CpG-ODNs (ssDNA), which are also very good candidates for therapeutic agents, are discussed in detail in the next section (Klinman 2004).

DNA vaccines are DNA plasmids encoding target antigen genes. Once these vaccines are administered, target antigens are expressed in host cells, inducing adaptive humoral and cellular immune responses. The immunogenicity of DNA vaccine was recently shown to depend on plasmid dsDNA (but not unmethylated CpG-motifs) and mediated through the DAI-independent, TBK1-dependent signaling pathway (the sensor for DNA vaccine has yet to be identified) (Ishii et al. 2008a). On the other hand, the immunogenicity including B cells and Th1-type CD4T cells activation of the influenza WV is dominantly controlled by a TLR7/MyD88-dependent signaling pathway, although TLR7/MyD88, RIG-I/IPS-1, and inflammasome activation are shown to be involved in live influenza virus infection (Allen et al. 2009; Koyama et al. 2007; Thomas et al. 2009; Ichinohe et al. 2009; Koyama et al. 2009). It suggested that genomic ssRNA remaining in influenza WV is essential for vaccine efficacy as an immune activator, detectable by only TLR but not RLR or NLR (Koyama et al. 2010).

A vaccine adjuvant is a compound that promotes and modulates vaccine immunogenicity. In theory, all PAMPs and DAMPs could be candidates for vaccine adjuvants (if safety were not an issue). Poly I:C, a synthetic dsRNA, is a vaccine adjuvant commonly used in animal models. Poly I:C can be detected by both endosomal TLR3 and cytoplasmic MDA5 (Miyake et al. 2009; McCartney et al. 2009); its adjuvanticity is mediated via both pathways (Kumar et al. 2008). The adjuvanticity of poly I:C (Longhi et al. 2009), DNA vaccines (Ishii et al. 2008a), and influenza WV (Koyama et al. 2010) requires dendritic cell activation and type-I IFNs production.

These studies demonstrate that specific signaling pathways in specific host cells play key roles in the efficacy of nucleic acids-based vaccines and vaccine adjuvants, though they can stimulate multiple immune sensors. Therefore, improved efficacy of vaccines and clinical applications require that target antigens and adjuvants be delivered to key immune cells, utilizing various manipulations of drug delivery systems, vaccine formation, routes of administration, and so on.

12.5 Conclusions

Nucleic acids and their metabolites are recognized by the specific host receptors such as TLRs, RIG-like receptors (RLRs), and NLRs, purinergic receptors such as P2X and P2Y receptors, and adenosine receptors such as A2A receptors. Resultant responses vary and may contribute to host defenses, aid homeostatic clearance of dying host cells, or even promote deleterious autoimmune diseases. As more questions emerge about nucleic acids and their relationships with the immune system, more efforts will be needed to elucidate the mechanism of immune recognition of, and regulation by, poly- and oligonucleotides such as RNA and DNA and their metabolites – including mononucleotides, nucleosides, bases, sugars, and uric acids – and the therapeutic potential of nucleic acids and their metabolites.

Acknowledgements The authors thank all members of Laboratory of Host Defense and Department of Molecular Protozoology in the Research Institute for Microbial Diseases, Osaka University. This work is supported by JSPS Research Fellowships for Young Scientists (S.K.), the Knowledge Cluster Initiative (K.J.I.), Grant-in-Aid for Scientific Research (KAKENHI) (K.J.I.), Target protein project (K.J.I. and S.A.), the Ministry of Education, Culture, Sports, Science and Technology in Japan and by CREST, JST (K.J.I.).

References

Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V (2009) RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat Immunol 10:1065–1072

Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499–511

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124:783–801

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413:732–738

Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, Guthrie EH, Pickles RJ, Ting JP (2009) The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity 30:556–565

Babiuk S, Mookherjee N, Pontarollo R, Griebel P, van Drunen Littel-van den Hurk S, Hecker R, Babiuk L (2004) TLR9-/- and TLR9+/+ mice display similar immune responses to a DNA vaccine. Immunology 113:114–120

Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN (2007) TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 13:543–551

Balachandran S, Thomas E, Barber GN (2004) A FADD-dependent innate immune mechanism in mammalian cells. Nature 432:401–405

Bauer M, Redecke V, Ellwart JW, Scherer B, Kremer JP, Wagner H, Lipford GB (2001) Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123+ dendritic cells. J Immunol 166:5000–5007

Burckstummer T, Baumann C, Bluml S, Dixit E, Durnberger G, Jahn H, Planyavsky M, Bilban M, Colinge J, Bennett KL, Superti-Furga G (2009) An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol 10:266–272

Chiu YH, Macmillan JB, Chen ZJ (2009) RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell 138:576–591

Choe J, Kelker MS, Wilson IA (2005) Crystal structure of human Toll-like receptor 3 (TLR3) ectodomain. Science 309:581–585

Choi MK, Wang Z, Ban T, Yanai H, Lu Y, Koshiba R, Nakaima Y, Hangai S, Savitsky D, Nakasato M, Negishi H, Takeuchi O, Honda K, Akira S, Tamura T, Taniguchi T (2009) A selective contribution of the RIG-I-like receptor pathway to type I interferon responses activated by cytosolic DNA. Proc Natl Acad Sci USA 106:17870–17875

Cortez-Gonzalez X, Pellicciotta I, Gerloni M, Wheeler MC, Castiglioni P, Lenert P, Zanetti M (2006) TLR9-independent activation of B lymphocytes by bacterial DNA. DNA Cell Biol 25:253–261

Di Virgilio F (2007) Liaisons dangereuses: P2X(7) and the inflammasome. Trends Pharmacol Sci 28:465–472

Di VF, Boeynaems JM, Robson SC (2009) Extracellular nucleotides as negative modulators of immunity. Curr Opin Pharmacol 9:507–513

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303:1529–1531

Erwig LP, Henson PM (2008) Clearance of apoptotic cells by phagocytes. Cell Death Differ 15:243–250

Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES (2009) AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 458:509–513

Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di VF (2006) The P2X7 receptor: a key player in IL-1 processing and release. J Immunol 176:3877–3883

Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G (2009) The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 10:241–247

Gallucci S, Lolkema M, Matzinger P (1999) Natural adjuvants: endogenous activators of dendritic cells. Nat Med 5:1249–1255

Haas T, Metzger J, Schmitz F, Heit A, Muller T, Latz E, Wagner H (2008) The DNA sugar backbone 2' deoxyribose determines Toll-like receptor 9 activation. Immunity 28:315–323

Hasko G, Cronstein BN (2004) Adenosine: an endogenous regulator of innate immunity. Trends Immunol 25:33–39

Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408:740–745

Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G (2002) Quantitative expression of Toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168:4531–4537

Hornung V, Barchet W, Schlee M, Hartmann G (2008) RNA recognition via TLR7 and TLR8. Handb Exp Pharmacol 183:71–86

Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA (2009) AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458:514–518

Hou B, Reizis B, DeFranco AL (2008) Toll-like receptors activate innate and adaptive immunity by using dendritic cell-intrinsic and -extrinsic mechanisms. Immunity 29:272–282

Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A (2009) Inflammasome recognition of influenza virus is essential for adaptive immune responses. J Exp Med 206:79–87

Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, Hoogsteden HC, Luttmann W, Ferrari D, Di Virgilio F, Virchow JC Jr, Lambrecht BN (2007) Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. Nat Med 13:913–919

Isaacs A, RA COX, Rotem Z (1963) Foreign nucleic acids as the stimulus to make interferon. Lancet 2:113–116

Ishii KJ, Akira S (2005) Innate immune recognition of nucleic acids: Beyond Toll-like receptors. Int J Cancer 117:517–523

Ishii KJ, Akira S (2008) Potential link between the immune system and metabolism of nucleic acids. Curr Opin Immunol 20:524–529

Ishii KJ, Suzuki K, Coban C, Takeshita F, Itoh Y, Matoba H, Kohn LD, Klinman DM (2001) Genomic DNA released by dying cells induces the maturation of APCs. J Immunol 167:2602–2607

Ishii KJ, Gursel I, Gursel M, Klinman DM (2004) Immunotherapeutic utility of stimulatory and suppressive oligodeoxynucleotides. Curr Opin Mol Ther 6:166–174

Ishii KJ, Coban C, Kato H, Takahashi K, Torii Y, Takeshita F, Ludwig H, Sutter G, Suzuki K, Hemmi H, Sato S, Yamamoto M, Uematsu S, Kawai T, Takeuchi O, Akira S (2006) A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat Immunol 7:40–48

Ishii KJ, Kawagoe T, Koyama S, Matsui K, Kumar H, Kawai T, Uematsu S, Takeuchi O, Takeshita F, Coban C, Akira S (2008a) TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature 451:725–729

Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S (2008b) Host innate immune receptors and beyond: making sense of microbial infections. Cell Host Microbe 3:352–363

Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455:674–678

Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H, Lipford G, Bauer S (2002) Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat Immunol 3:499

Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, Liu YJ (2001) Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. J Exp Med 194:863–869

Kahlenberg JM, Lundberg KC, Kertesy SB, Qu Y, Dubyak GR (2005) Potentiation of caspase-1 activation by the P2X7 receptor is dependent on TLR signals and requires NF-kappaB-driven protein synthesis. J Immunol 175:7611–7622

Kanneganti TD, Body-Malapel M, Amer A, Park JH, Whitfield J, Franchi L, Taraporewala ZF, Miller D, Patton JT, Inohara N, Nunez G (2006a) Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. J Biol Chem 281:36560–36568

Kanneganti TD, Ozoren N, Body-Malapel M, Amer A, Park JH, Franchi L, Whitfield J, Barchet W, Colonna M, Vandenabeele P, Bertin J, Coyle A, Grant EP, AkiraS NG (2006b) Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature 440:233–236

Kariko K, Buckstein M, Ni H, Weissman D (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 23:165–175

Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S (2008) Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene I and melanoma differentiation-associated gene 5. J Exp Med 205:1601–1610

Kawai T, Akira S (2006) Innate immune recognition of viral infection. Nat Immunol 7:131–137

Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, Yoshikawa H, Nagata S (2006) Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature 443:998–1002

Kerkmann M, Costa LT, Richter C, Rothenfusser S, Battiany J, Hornung V, Johnson J, Englert S, Ketterer T, Heckl W, Thalhammer S, Endres S, Hartmann G (2005) Spontaneous formation of nucleic acid-based nanoparticles is responsible for high interferon-alpha induction by CpG-A in plasmacytoid dendritic cells. J Biol Chem 280:8086–8093

Klinman DM (2004) Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat Rev Immunol 4:249–258

Kobiyama K, Takeshita F, Jounai N, Sakaue-Sawano A, Miyawaki A, Ishii KJ, Kawai T, Sasaki S, Hirano H, Ishii N, Okuda K, Suzuki K (2010) Extrachromosomal histone H2B mediates innate antiviral immune responses induced by intracellular double-stranded DNA. J Virol 84:822–832

Kono H, Rock KL (2008) How dying cells alert the immune system to danger. Nat Rev Immunol 8:279–289

Koyama S, Ishii KJ, Kumar H, Tanimoto T, Coban C, Uematsu S, Kawai T, Akira S (2007) Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. J Immunol 179:4711–4720

Koyama S, Coban C, Aoshi T, Horii T, Akira S, Ishii KJ (2009) Innate immune control of nucleic acid-based vaccine immunogenicity. Expert Rev Vaccines 8:1099–1107

Koyama S, Aoshi T, Tanimoto T, Kumagai Y, Kobiyama K, Tougan T, Sakurai K, Coban C, Horii T, Akira S, Ishii KJ (2010) Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. Sci Transl Med 2:25ra24

Krieg AM (2002) CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 20:709–760

Kumar H, Koyama S, Ishii KJ, Kawai T, Akira S (2008) Cutting edge: cooperation of IPS-1- and TRIF-dependent pathways in poly IC-enhanced antibody production and cytotoxic T cell responses. J Immunol 180:683–687

Latz E, Verma A, Visintin A, Gong M, Sirois CM, Klein DC, Monks BG, McKnight CJ, Lamphier MS, Duprex WP, Espevik T, Golenbock DT (2007) Ligand-induced conformational changes allosterically activate Toll-like receptor 9. Nat Immunol 8:772–779

Le Bon A, Tough DF (2002) Links between innate and adaptive immunity via type I interferon. Curr Opin Immunol 14:432–436

Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416:603–607

Leonard JN, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, Segal DM (2008) The TLR3 signaling complex forms by cooperative receptor dimerization. Proc Natl Acad Sci USA 105:258–263

Longhi MP, Trumpfheller C, Idoyaga J, Caskey M, Matos I, Kluger C, Salazar AM, Colonna M, Steinman RM (2009) Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. J Exp Med 206:1589–1602

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440:228–232

Marshak-Rothstein A, Rifkin IR (2007) Immunologically active autoantigens: the role of Toll-like receptors in the development of chronic inflammatory disease. Annu Rev Immunol 25:419–441

Martin DA, Elkon KB (2006) Intracellular mammalian DNA stimulates myeloid dendritic cells to produce type I interferons predominantly through a Toll-like receptor 9-independent pathway. Arthritis Rheum 54:951–962

Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440:237–241

Matzinger P (2002) The danger model: a renewed sense of self. Science 296:301–305

McCartney S, Vermi W, Gilfillan S, Cella M, Murphy TL, Schreiber RD, Murphy KM, Colonna M (2009) Distinct and complementary functions of MDA5 and TLR3 in poly(I:C)-mediated activation of mouse NK cells. J Exp Med 206:2967–2976

Miyake T, Kumagai Y, Kato H, Guo Z, Matsushita K, Satoh T, Kawagoe T, Kumar H, Jang MH, Kawai T, Tani T, Takeuchi O, Akira S (2009) Poly I:C-induced activation of NK cells by CD8 alpha + dendritic cells via the IPS-1 and TRIF-dependent pathways. J Immunol 183:2522–2528

Morita M, Stamp G, Robins P, Dulic A, Rosewell I, Hrivnak G, Daly G, Lindahl T, Barnes DE (2004) Gene-targeted mice lacking the Trex1 (DNase III) 3'– > 5' DNA exonuclease develop inflammatory myocarditis. Mol Cell Biol 24:6719–6727

Mukundan L, Odegaard JI, Morel CR, Heredia JE, Mwangi JW, Ricardo-Gonzalez RR, Goh YP, Eagle AR, Dunn SE, Awakuni JU, Nguyen KD, Steinman L, Michie SA, Chawla A (2009) PPAR-delta senses and orchestrates clearance of apoptotic cells to promote tolerance. Nat Med 15:1266–1272

Munz C, Lunemann JD, Getts MT, Miller SD (2009) Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol 9:246–258

Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, Parks RJ, Tschopp J (2008) The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature 452:103–107

Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, Moroy T (2000) Features of systemic lupus erythematosus in Dnase1-deficient mice. Nat Genet 25:177–181

Ohta A, Sitkovsky M (2009) The adenosinergic immunomodulatory drugs. Curr Opin Pharmacol 9:501–506

Okabe Y, Kawane K, Akira S, Taniguchi T, Nagata S (2005) Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation. J Exp Med 202:1333–1339

Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol 4:161–167

Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S (2006) Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 312:1669–1672

Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, Hardy LL, Garceau V, Sweet MJ, Ross IL, Hume DA, Stacey KJ (2009) HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science 323:1057–1060

Saito T, Gale M Jr (2008) Differential recognition of double-stranded RNA by RIG-I-like receptors in antiviral immunity. J Exp Med 205:1523–1527

Saito T, Owen DM, Jiang F, Marcotrigiano J, Gale M (2008) Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. Nature 454: 523–527

Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, Tsujimura T, Fujita T, Akira S, Takeuchi O (2010) LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. Proc Natl Acad Sci USA 107:1512–1517

Savill J, Dransfield I, Gregory C, Haslett C (2002) A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2:965–975

Schulz O, Diebold SS, Chen M, Naslund TI, Nolte MA, Alexopoulou L, Azuma YT, Flavell RA, Liljestrom P, Reis e Sousa C (2005) Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature 433:887–892

Spies B, Hochrein H, Vabulas M, Huster K, Busch DH, Schmitz F, Heit A, Wagner H (2003) Vaccination with plasmid DNA activates dendritic cells via Toll-like receptor 9 (TLR9) but functions in TLR9-deficient mice. J Immunol 171:5908–5912

Stetson DB, Medzhitov R (2006) Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity 24:93–103

Stetson DB, Ko JS, Heidmann T, Medzhitov R (2008) Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell 134:587–598

Subramanian S, Tus K, Li QZ, Wang A, Tian XH, Zhou J, Liang C, Bartov G, McDaniel LD, Zhou XJ, Schultz RA, Wakeland EK (2006) A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc Natl Acad Sci USA 103:9970–9975

Suzuki K, Mori A, Ishii KJ, Saito J, Singer DS, Klinman DM, Krause PR, Kohn LD (1999) Activation of target-tissue immune-recognition molecules by double- stranded polynucleotides. Proc Natl Acad Sci USA 96:2285–2290

Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, Ban T, Lu Y, Miyagishi M, Kodama Y, Honda K, Ohba Y, Taniguchi T (2007) DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 448:501–505

Takeda K, Akira S (2005) Toll-like receptors in innate immunity. Int Immunol 17:1–14
Takeshita F, Ishii KJ (2008) Intracellular DNA sensors in immunity. Curr Opin Immunol 20:383–388
Takeuchi O, Akira S (2008) MDA5/RIG-I and virus recognition. Curr Opin Immunol 20:17–22
Theofilopoulos AN, Baccala R, Beutler B, Kono DH (2005) Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol 23:307–336
Thomas PG, Dash P, Aldridge JR Jr, Ellebedy AH, Reynolds C, Funk AJ, Martin WJ, Lamkanfi M, Webby RJ, Boyd KL, Doherty PC, Kanneganti TD (2009) The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity 30:566–575
Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, Hua J, An LL, Audoly L, La Rosa G, Bierhaus A, Naworth P, Marshak-Rothstein A, Crow MK, Fitzgerald KA, Latz E, Kiener PA, Coyle AJ (2007) Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat Immunol 8:487–496
Tissari J, Siren J, Meri S, Julkunen I, Matikainen S (2005) IFN-{alpha} enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression. J Immunol 174:4289–4294
Tsan MF, Gao B (2004) Endogenous ligands of Toll-like receptors. J Leukoc Biol 76:514–519
Venkataraman T, Valdes M, Elsby R, Kakuta S, Caceres G, Saijo S, Iwakura Y, Barber GN (2007) Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. J Immunol 178:6444–6455
Vilaysane A, Muruve DA (2009) The innate immune response to DNA. Semin Immunol 21:208–214
Wagner H (2004) The immunobiology of the TLR9 subfamily. Trends Immunol 25:381–386
Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC (2003) Predominant autoantibody production by early human B cell precursors. Science 301: 1374–1377
Weyand S, Shimamura T, Yajima S, Suzuki S, Mirza O, Krusong K, Carpenter EP, Rutherford NG, Hadden JM, O'Reilly J, Ma P, Saidijam M, Patching SG, Hope RJ, Norbertczak HT, Roach PC, Iwata S, Henderson PJ, Cameron AD (2008) Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter. Science 322:709–713
Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S (2003) Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. Science 301:640–643
Yanai H, Ban T, Wang Z, Choi MK, Kawamura T, Negishi H, Nakasato M, Lu Y, Hangai S, Koshiba R, Savitsky D, Ronfani L, Akira S, Bianchi ME, Honda K, Tamura T, Kodama T, Taniguchi T (2009) HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature 462:99–103
Yasuda K, Yu P, Kirschning CJ, Schlatter B, Schmitz F, Heit A, Bauer S, Hochrein H, Wagner H (2005) Endosomal translocation of vertebrate DNA activates dendritic cells via TLR9-dependent and -independent pathways. J Immunol 174:6129–6136
Yasutomo K, Horiuchi T, Kagami S, Tsukamoto H, Hashimura C, Urushihara M, Kuroda Y (2001) Mutation of DNASE1 in people with systemic lupus erythematosus. Nat Genet 28:313–314
Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 5:730–737
Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S (2005) Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA. Nat Immunol 6:49–56
Yurasov S, Wardemann H, Hammersen J, Tsuiji M, Meffre E, Pascual V, Nussenzweig MC (2005) Defective B cell tolerance checkpoints in systemic lupus erythematosus. J Exp Med 201:703–711
Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, Lei C, He X, Zhang L, Tien P, Shu HB (2008) The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. Immunity 29:538–550

h

粘膜アジュバント

はじめに

粘膜は生体内において，外来生物，食べ物，代謝産物などの物理的・化学的ストレスに常にさらされながら第一線で防御の役割を担っている．さらにあるときは病原微生物の侵入を認識し，それらを排除する免疫を誘導する一方，常在菌に対して不必要な免疫応答は誘導しない，というように巧妙な仕組みによって免疫応答を使い分けている．そしてこのような繊細な仕組みの破綻が，アレルギー・自己免疫疾患などの疾患発症とも関連していることはすでに10章で概説した．このような意味で，ワクチンによって粘膜免疫をコントロールすることは，単に感染症の予防や治療にとどまらず，アレルギー・自己免疫疾患の病態解明や治療にも関連している．

アジュバントとは，ラテン語の“促進する”“増強する”という意味をもつ“adjuvare”に由来し，もともとは標的抗原とともに投与して，その抗原に対する免疫原性を増強する目的で使用された．

アジュバントに関する報告は，19世紀末にまでさかのぼるが，1920年代にRamonやGlennyらがaluminum hydroxide（alum）を用いてジフテリアや破傷風の類毒素の免疫原性を改善したことによって，アジュバントの重要性が認識されるようになった．これまでアジュバントの作用機序に関しては，非特異的に標的抗原を投与部分に長期間とどめる作用や抗原提示細胞の遊走を促進する作用などによって抗原提示の確率を高くして免疫原性を増強することが想定されていた．しかしながら，近年の自然免疫学の進歩に伴い，アジュバントの多くがToll-like receptors（TLRs），retinoic acid-inducible gene（RIG）-like receptors（RLRs），nucleotide-binding oligomerization domain protein（NOD）-like receptors（NLRs）などの自然免疫受容体に特異的に作用して樹状細胞（dendritic cell：DC）を中心とした抗原提示細胞を活性化し，その遊走や成熟，抗原提示能や補助シグナル分子の発現を促進し，T細胞やB細胞の抗原特異的な活性化を増強することが明らかになった（図1）[1,2]（4章a参照）．そしてアジュバントによる作用は単に免疫原性の増強によって，標的抗原の必要量を減少させたり，接種の回数を減少させたり，免疫力の弱い新生児や高齢者への効果を改善したりするだけにとどまらず，その種類や組み合わせによっては，主に抗体産生（B細胞活性）を誘導するもの，Th1型を誘導するもの，Th2型を誘導するもの，または細胞傷害性T細胞（cytotoxic T lymphocyte：CTL）の活性を誘導するものといったように獲得免疫の方向性をも制御することができる．つまり，ワクチンによって粘膜免疫をコントロールするためにはアジュバントの理解は必要不可欠のものと考えられる．

アジュバントの作用機序をよく理解し，メカニズムに基づいた利用が可能になれば，病原微生物や疾患の特性に応じて，たとえばウイルス感染に対する抗体誘導，悪性腫瘍に対する細胞傷害性T細胞の誘導，アレルギー疾患に対するTh2の抑制などのように，疾患に応じたアジュバントの組み合わせを選択することが可能になり，有効な免疫療法のツールになることが考えられる．

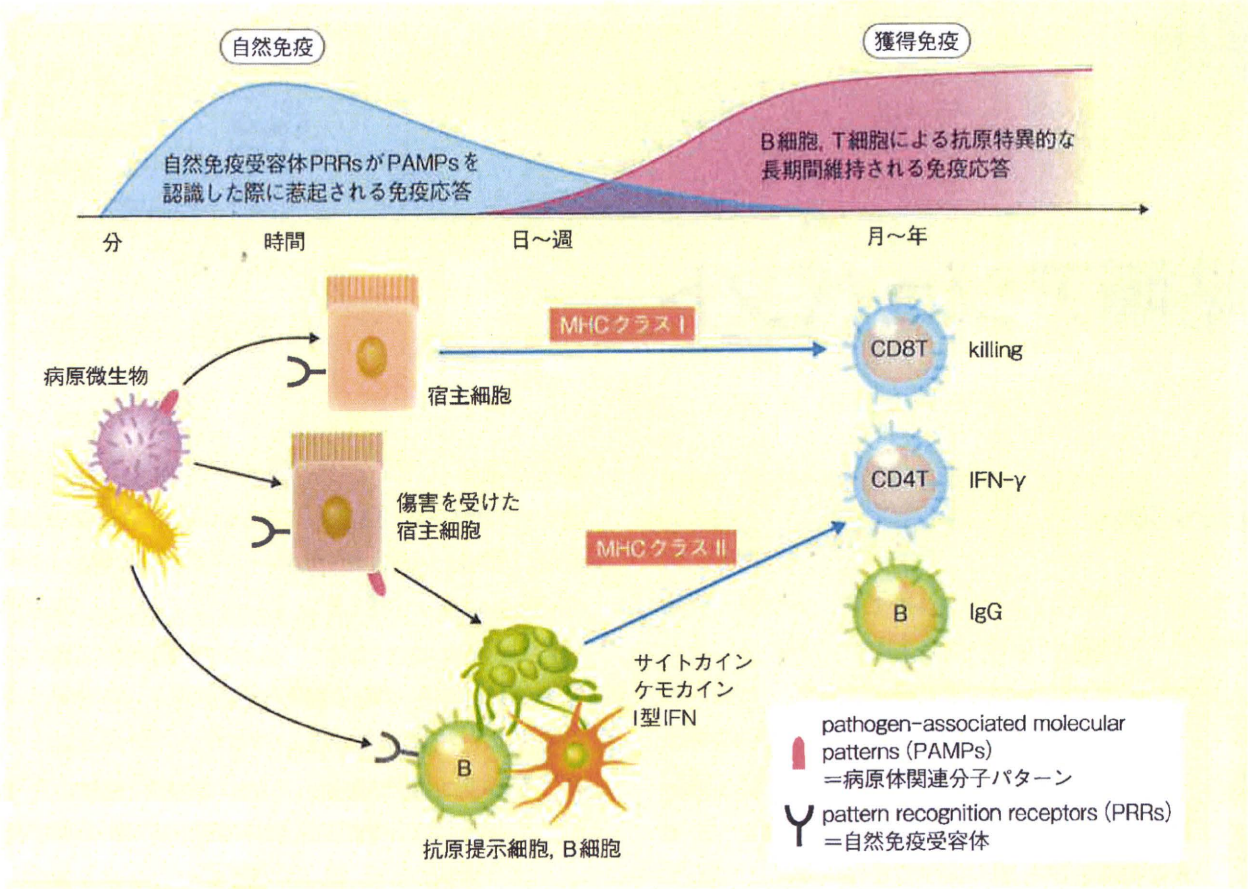

図1　自然免疫と獲得免疫

病原微生物の侵入は宿主細胞に発現した様々な種類の自然免疫受容体PRRsによって認識され，短時間に自然免疫応答が誘導される．その後に誘導される抗原特異的な獲得免疫の有効な活性化には自然免疫の誘導が必須である．

本項では，アジュバントによって誘導される自然免疫応答について解説した後，実際に使用されている粘膜アジュバントを例示し，その作用機序や獲得免疫誘導能について最近の知見を含めて概説する．

アジュバント効果にかかわる自然免疫受容体―リガンドとシグナル伝達経路

粘膜ワクチンは，経鼻，経口（舌下），経腟などさまざまな経路で投与され，獲得免疫を誘導することが可能である（12章a〜d参照）．組織や臓器の特異性によって，それぞれの投与経路で主役となる免疫担当細胞・所属リンパ節は異なると考えられるが，基本的にはワクチンの一部として投与されたアジュバントは，粘膜の上皮細胞や抗原提示細胞によって最初に認識される．その際，多くのアジュバントが自然免疫受容体によって認識されることでアジュバントとしての効果を発揮することが最近明らかになってきた．ここでは，アジュバントの認識にかかわる代表的な自然免疫受容体としてTLRs，RLRs，NLRsについてそのリガンド（自然免疫受容体のリガンドはpathogen-associated molecular patterns〈PAMPs〉とも呼ばれ，いずれもアジュバントとしてのポテンシャルを有する）およびシグナル伝達経路を解説し，さらにアジュバント認識にかかわるその他の自然免疫受容体について補足する（近年膨大な数の自然免疫受容体が明らかとなり，網羅的に解説することは困難なため，ここでは代表的なもののみを示す）（図2）．

Toll-like receptors（TLRs）

TLRsは現在までヒトおよびマウスにおいてTLR1から11までが機能性の受容体として知られ

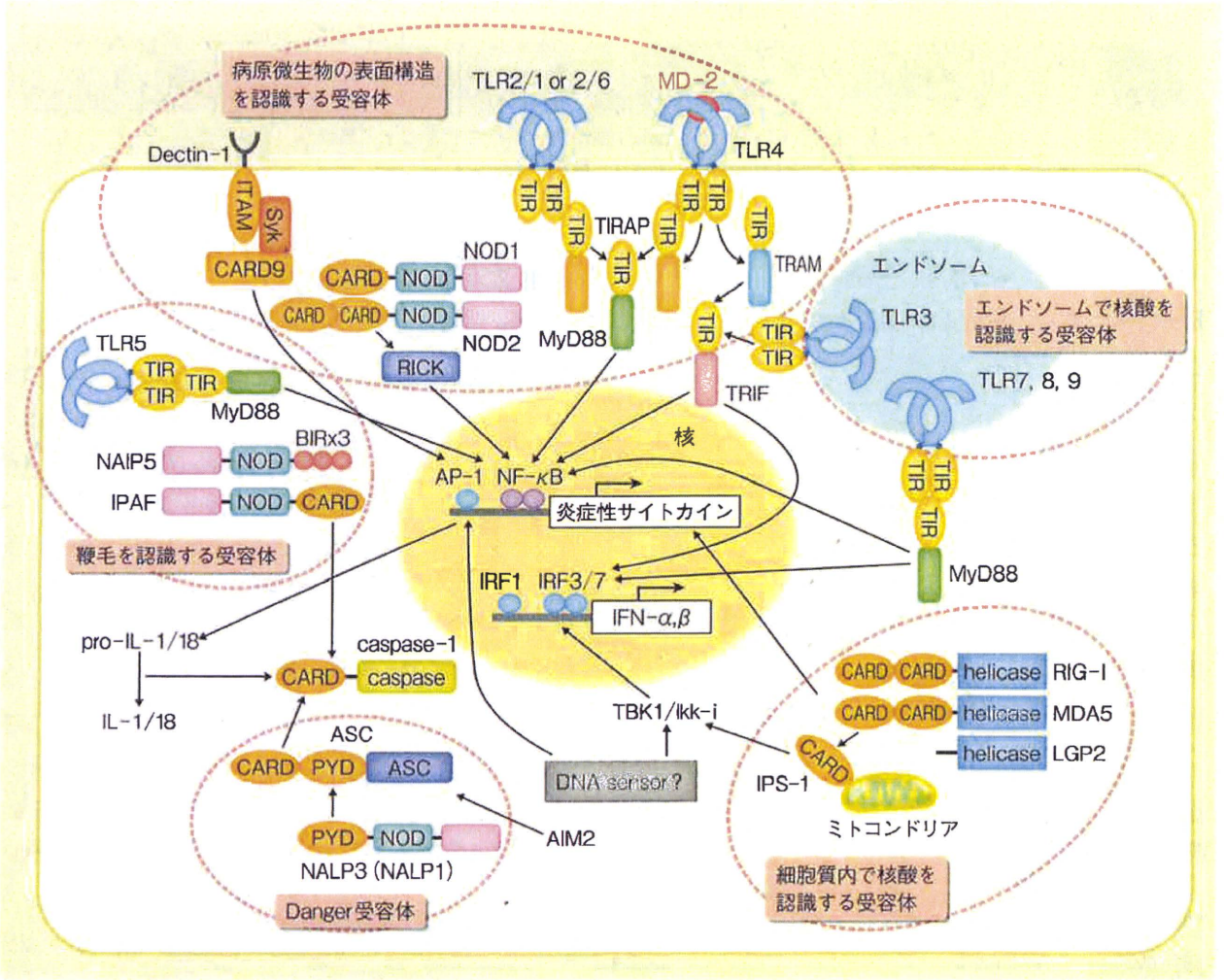

図2 自然免疫受容体とそのシグナル伝達経路

TLRは二量体を形成してPAMPsを認識し，MyD88かTRIFを介して炎症性サイトカイン・IFNの産生を誘導する．RLRsはいずれも細胞質内に存在しhelicaseドメインで核酸を認識し，IPS-1を介して免疫応答を誘導する．様々な細胞傷害ストレスの応答に関与していることが知られるようになったNLRsに属するNALP3はASCを介してcaspase-1の活性化を誘導し，IL-1，IL-18の産生を誘導する．

ている（4章a参照）．TLRsは，N末端のロイシンリッチリピートモチーフ，それに続く膜貫通領域とC末端のToll/IL-1R homology（TIR）ドメインから構成される．各TLRは，そのリガンドとしてさまざまな特異的構成成分PAMPsをN末端で認識し，C末端を介して下流にシグナルを伝えることにより免疫系を賦活化する．TLRsは細胞膜表面に発現するTLR2/1，TLR2/6，TLR4，TLR5と，エンドソームの膜に発現するTLR3，7，8，9に分けられる（図2）．それぞれが表1に示すようなリガンドをN末端で認識すると，TLR2/1，TLR2/6はアダプターとしてmyeloid differentiation primary response protein 88（MyD88）/TIR domain-containing adaptor protein（TIRAP），TLR5はMyD88を活性化する（図2）．TLR4は時間経過に応じて初めはMyD88/TIRAPを，エンドサイトーシス後はTIR domain-containing adaptor inducing IFN-β（TRIF）/Trif-related adaptor molecule（TRAM）を活性化する．また，TLR3はアダプターとしてTRIF，TLR7，8，9はMyD88を活性化する．さらにその下流では，TANK-binding kinase 1（TBK1），mitogen-activated protein kinases（MAPKs），IkB kinase（Ikk）複合体を通じてinterferon regulatory factor（IRF）3，IRF7，nuclear factor-kappa B（NF-κB）などの転写因子が活性化され，最終的にI型IFNや炎症性サイト

表1 自然免疫受容体とそのリガンド（非合成性）

自然免疫受容体	外来性リガンド	由来となる病原微生物	内因性リガンド
TLR2/1 TLR2/6	ペプチドグリカン，糖脂質，diacyl or triacyl lipopeptides，phospholipomannan	Gram陽性菌，マイコプラズマ，麻疹ウイルス，真菌	HSP70
TLR3	dsRNA，siRNA	ウエストナイルウイルス，マウスサイトメガロウイルス，脳心筋炎ウイルス	mRNA
TLR4	LPS，RSウイルス融合蛋白，phosphorylcholine，glycan，mannan	Gram陰性菌，RSウイルス，炭疽菌，蠕虫，真菌	HSP70，β-デフェンシン，fibrinogen，fibronectin，hyaluronic acids
TLR5	フラジェリン	鞭毛をもつ細菌	−
TLR7	ssRNA	RNAウイルス全般	autoantigens
TLR9	非メチル化CpG，hemozoine	細菌，DNAウイルス，マラリア	クロマチン複合体
TLR11	profilin-like molecule	トキソプラズマ	−
RIG-I	ssRNA 5′末端3リン酸，短い（～1 kb）dsRNA	センダイウイルス，VSV，インフルエンザウイルス	−
MDA5	長い（＞2 kb）dsRNA	脳心筋炎ウイルス，メンゴウイルス	−
NOD1 NOD2	NOD1：diaminophilic acid（iE-DAP） NOD2：muramyl dipeptides	NOD1：クラミジア，赤痢菌，カンピロバクター，ヘリコバクター・ピロリ NOD2：結核菌，サルモネラ，リステリア	− −
NLRP3（NALP3）	細菌RNA，リポ多糖，pore-forming toxins，muramyl dipeptides，アスベスト，シリカ	細菌，真菌，インフルエンザウイルス	uric acid/ATP，βアミロイド，ピロリン酸カルシウム
NLRC4	フラジェリンand？	レジオネラ，サルモネラ，緑膿菌，結核菌	−
NAIP5	フラジェリン	レジオネラ	−
Dectin-1	β-グルカン ザイモサン	真菌（カンジダ，アスペルギルス，ニューモシスティスなど）	−

リポ多糖：lipopolysaccharide：LPS

カインの産生が誘導される[3]．

RIG like receptors（RLRs）

TLRs以外に細胞質内に侵入した核酸を認識する受容体としてRLRsが知られている（図2）．RLRsは，C末端にRNA helicase domainを持ち，それが細胞質内に侵入してきた非自己のRNAを認識する．N末端には2つのcaspase activation and recruitment domain（CARD）が存在する．RLRsにはRIG-I，MDA5と呼ばれる2つの類似した分子のほか，RIG-Iのnegative regulatorと考えられているLGP2の合計3つが存在する．RLRsの発現は免疫担当細胞に限らず，ほとんどすべての細胞にユビキタスに発現している．

RLRsがいったん表1に示すようなリガンドを認識すると（LGP2に関しては依然として不明な点が多いため，ここではRIG-I，MDA5について示す），RIG-IとMDA5の共通のアダプターであるIFN-β-promoter stimulator 1（IPS-1）（MAVS，VISA，CARDIFとも呼ばれる）とCARDを介して結合しシグナル伝達を開始する．下流では，TBK1やIKK複合体を通じてIRF3，NF-κBなどの転写因子が活性化され，エフェクターとしてI型IFNや炎症性サイトカインの産生が誘導される[3]．

NOD like receptors（NLRs）

細胞内の自然免疫受容体には，核酸の認識に特化したRLRs以外にNLRsが存在する（図2）．NLRsは現在までにヒトでは23種類（蛋白レベル），マウスにおいては34種類（遺伝子レベル）が存在することが知られている．N末端には，CARDもしくはpyrin domain（PYD）もしくはbaculovirus inhibitor domain（BIR）を有し，NOD domainを挟んで，C末端にロイシンリッチリピートモチーフを持つ．大まかには，NLRsの原型ともいえるNOD1やNOD2のようにinflammasomeを活性化しないグループと，NLRP1，NLRP3やNLRC4などのようにinflammasomeの活性化を伴うグループに分類できる．それぞれのNLRsが表1のようなリガンドの刺激を受けると，前者ではアダプターとしてRICKが活性化し，後者ではASC，caspase-1から構成されるinflammasomeの活性化が生じる．RICKの下流では，mitogen-activated protein kinases（MAPKs），IkB kinase（Ikk）複合体を通じてnuclear factor-kappa B（NF-κB）などの転写因子が活性化され，炎症性サイトカインの産生が誘導される．inflammasomeの活性化の下流では，caspase-1によってpro IL-1β，pro IL-18から活性化型のIL-1β，IL-18の産生が誘導される[4]．

その他の自然免疫受容体（CLRsなど）

TLRs，RLRs，NLRs以外の自然免疫受容体のなかには，スカベンジャー受容体やFc受容体なども知られているが，なかでもC-type lectin receptors（CLRs）が大きなグループを占める（図2）．CLRsはその構造によってさらに17種類のグループに分けられる．ここでは詳細は割愛するが，CLRsの代表としてDectin-1を例にあげると，リガンドとして表1に示すとおり，C末端の細胞外ドメインでβ-グルカンを認識すると，細胞内のimmunoreceptor tyrosine-based activation（ITAM）-likeモチーフを介してspleen tyrosine kinase（Syk），さらにCARD9の活性化を経て炎症性サイトカインの産生が誘導される[5]．

粘膜アジュバントの現状と方向性

先述のとおり，以前からワクチンとともに使用されてきたアジュバントの多くが自然免疫受容体のリガンドとして作用していることが近年明らかになった．逆にいうと，純度，安全性やTh1，Th2バランスなどを度外視すれば，すべての自然免疫受容体リガンドは，その合成が可能であれば，粘膜アジュバントとしても使用可能であると考えられる．ただし，自然免疫受容体に作用することだけがアジュバント効果を生み出すわけではなく，たとえば標的抗原を投与部分に長時間とどめておく作用や，炎症性細胞浸潤を促進することなども大切なアジュバント効果の一役を担っている．さらにターゲットは自然免疫受容体でも，組織特異的な反応を誘導するために投与経路を変更したり，ナノテクノロジーなどを利用しドラッグデリバリーシステムを巧みにコントロールすることで，自然免疫応答自体の質と量を高めることも今後のワクチンおよびアジュバント開発にとって必須の戦略である．ここでは，以前から利用されてきた粘膜アジュバントや今後使用が注目されているアジュバントなどいくつかに分類して概説する．

代表的な粘膜アジュバント

古くから利用されている粘膜アジュバントとして，ここでは細菌から抽出した3種類の構成成分，①ADP-ribosylating enterotoxin（cholera toxin〈CT〉および大腸菌のheat-labile enterotoxin〈LT〉），②CpGモチーフを持つoligodeoxynucleotides（CpG ODN），③monophosphoryl lipid A（MPLA）について説明する（図3）．

●cholera toxin（CT），heat-labile enterotoxin（LT）

CTおよびLTはAサブユニットとBサブユニットからなる．

Aサブユニットは毒素の活性を持ち，Bサブユニットが粘膜上皮のGM1ガングリオシドに接着する．アジュバントとしての効果を維持しながら，元来有

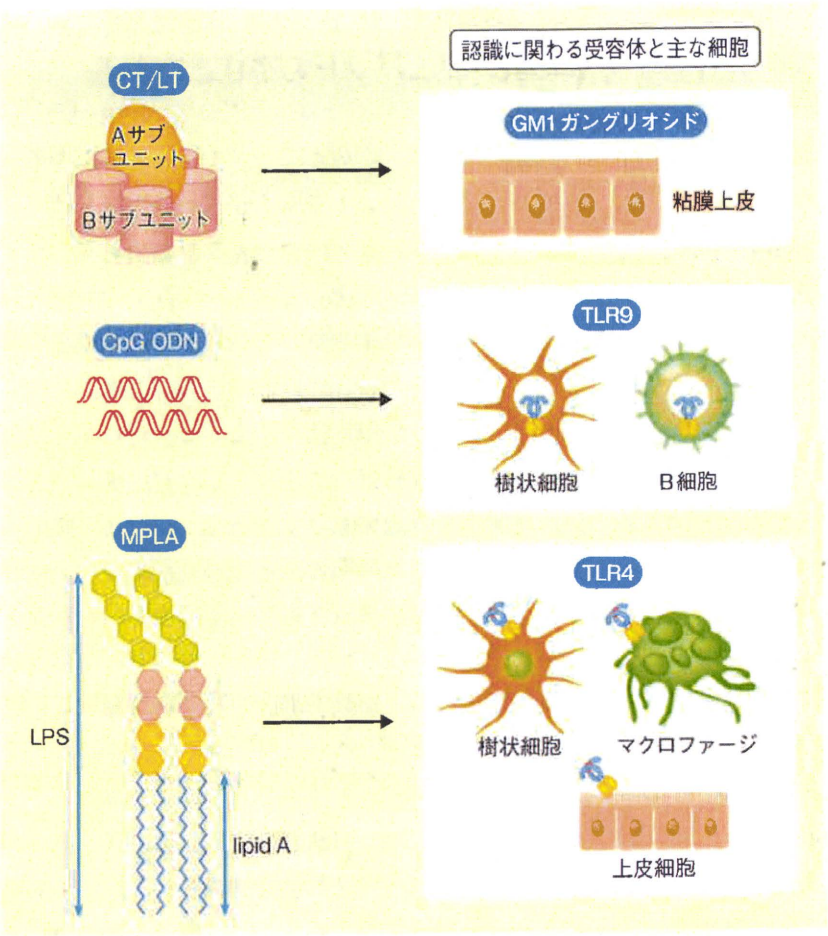

図3 代表的な粘膜アジュバント
cholera toxin (CT)・heat-labile enterotoxin (LT) は一つのAサブユニットと五量体のBサブユニットからなる．TLR9の発現はヒトでは形質細胞様樹状細胞とB細胞に限局している．

する腸管毒性を軽減するためAサブユニットの改変を作製するなどの方法が施行されている．改変としてはLTの場合，63番のリジンをセリンにしたもの（LT〈S63K〉）や72番のアルギニンをアラニンにしたもの（LT〈A72R〉）がよく知られている．CT・LTいずれもAサブユニットがADP-リボシルトランスフェラーゼ活性を有し，CT・LTが作用するとアデニル酸シクラーゼが常に活性化された状態になり，細胞内サイクリックAMP濃度が高まる．これを契機に粘膜上皮細胞や抗原提示細胞が活性化され，細胞透過性の亢進，炎症性サイトカインの産生，樹状細胞の成熟促進などが誘導される．類似の作用機序を有するが，アジュバントとしてはCTがTh2型の免疫反応（IL-4，IL-5分泌型のCD4$^+$T細胞の活性化やIgA，IgG1，IgEの産生）およびTh17を誘導するのに対して，LTはTh1，Th2型の両方の免疫反応（IFN-γ分泌型のCD4$^+$T細胞活性化およびIgG2の産生）を誘導する[6,7]．

しかしながら，これらのアジュバントは効果が高い反面，投与経路の制限，投与部位における強い炎症や組織の壊死，アレルギー反応の誘発などの副作用の問題を依然として有する．実際2000年にヨーロッパにおいてはLTをアジュバントとして用いたインフルエンザ経鼻ワクチンの臨床試験が実施されたが，顔面神経麻痺の副作用により毒素系アジュバントの臨床応用が難しい状況となっているのが事実である[8]．

● oligodeoxynucleotides（CpG ODN）

細菌やウイルスのDNAには，哺乳類のDNAと比べると約20倍ほど多く非メチル化CpGモチーフが存在する．近年の自然免疫学の進歩に伴い，CpGは現在ではTLR9のリガンドとして広く知られるようになった．免疫活性を持ったCpG ODNにはその配列，構造，免疫活性の違いから，少なくとも3種類（D/A型CpG，K/B型CpG，C型CpG）のタイプに分けられる．D/A型CpGは，主にpDC

の活性化，K/B 型 CpG は主に B 細胞の活性化を誘導する．C 型 CpG は両方の性質を持っているが活性はやや弱い（いずれの型の CpG も TLR9 によって認識される）．CpG ODN がそれぞれの免疫細胞に存在する TLR9 によって認識されると，I 型 IFN や炎症性サイトカインの産生が誘導され，B 細胞の増殖，樹状細胞の成熟化のほかにもナチュラルキラー（NK）細胞の活性化も加わり，強力な Th1 型の獲得免疫反応（IgG2a の産生，Th1 細胞による IFN-γ 産生，細胞傷害性 T 細胞の細胞傷害活性）が惹起される．つまり，何らかの抗原とともに CpG ODN をアジュバントとして使用することで，以上のようなメカニズムから抗原単独で使用するよりもはるかに強力な Th1 型の獲得免疫が誘導できる．CpG ODN は非常に強く安全なアジュバントであるだけでなく，単独（抗原なし）での使用も可能である．実際にワクチンアジュバント，抗アレルギー薬，抗腫瘍薬としてさまざまな臨床治験が行われ，通常の注射による投与だけでなく，経鼻投与をはじめとする経粘膜投与[9]において，その有効性が一部で証明されている[10,11]．しかしながら，生物種間の TLR9 の発現パターンの違いなどから，依然としてヒトに対しては汎用されていない．

● monophosphoryl lipid A（MPLA）

MPLA はもともとサルモネラ菌のリポ多糖（lipopolysaccharide：LPS）から抽出された物質で，TLR4 がそのアジュバント効果に必須であることが知られている（ヒトの細胞では TLR2 も認識に関与することが報告されている）．MPLA のアジュバント効果はリポ多糖に匹敵するにもかかわらず，有害な副作用が生じにくいことが特徴でもある．その理由としてリポ多糖と比較して MLPA が，MyD88 依存性の経路をほとんど刺激せず TRIF 依存性の経路を主に刺激すること[12]，免疫抑制的に作用する IL-10 を多く産生する一方で炎症性サイトカインの IL-1β は誘導しにくいことなどが報告されている．MPLA は強力に CD4 T 細胞のプライミング・活性化を誘導するのが特徴で，主に Th1 型の反応を惹起する．グラクソ・スミスクラインは，MPLA を用いたコンビネーションアジュバントの開発に着手し，AS01（MPLA と QS21 をリポソームで包んだもの），AS02（MPLA と QS21 をエマルジョンで包んだもの），AS04（MPLA＋alum）を作製している（QS21，alum については後述する）．ヨーロッパでは AS04 を用いた hepatitis B virus（HBV）ワクチンとして FENDrix® やオーストラリアでは同じく AS04 を用いた human papilloma virus ワクチン，サーバリックス® が認可されている[6]．FENDrix® もサーバリックス® も筋肉注射にて投与されているが，MPLA は注射型だけではなく経粘膜免疫においてもアジュバント効果を発揮する．例えば，動物実験レベルでは経口もしくは経鼻ワクチンのアジュバントとしても効果が確認されている[13]．

表2 ワクチンアジュバント（合成）

合成アジュバント	自然免疫受容体
monophosphoryl lipid A（MPLA）	TLR2，TLR4
CpG ODN	TLR9
Pam3Cys-SK4	TLR1/2
MALP2	TLR2/6
poly I：C	TLR3，MDA5
imiquimod，resquimod	TLR7，TLR8
aluminium based salts	NALP3
chitosan	NALP3
細菌性類毒素（e.g. CT，LT）	？
サポニン（e.g. QS-21）	NALP3？
エマルジョン（e.g. MF59）	？

MALP2：macrophage-activating lipopeptide 2
poly I：C：polyinosinic-polycytidylic acid

自然免疫受容体によって認識される粘膜アジュバント

先述のとおり，自然免疫受容体のリガンドは，理論的にはすべてアジュバントとして使用できる可能性を有する．現在ヒトや動物で使用されているアジュバントで自然免疫受容体との関係が明らかにされているものは，まだわずかではあるが（表2），前述した「アジュバント効果にかかわる自然免疫受容体」で示したように，近年急速な勢いで自然免疫受容体とそのシグナル伝達経路が明らかになり，さら