InvivoGen Insight
INNOVATION WITHIN REACH
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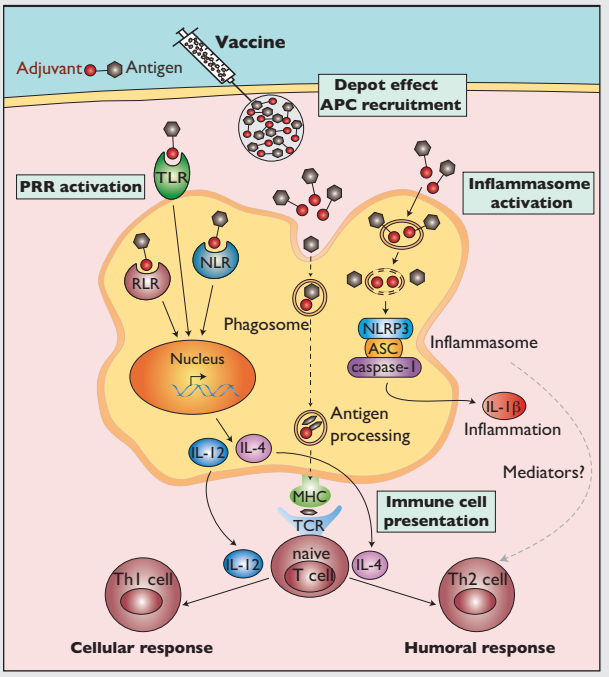
Trends in Vaccine Adjuvants



Adjuvants have been used for almost 80 years to enhance the immune response to vaccine antigens. Their development was initially purely empirical as the principles of adjuvant activity were largely unknown. Moreover, some adjuvants, such as complete Freund's adjuvant, were highly potent but associated with extreme reactogenicity precluding their use for human vaccines. Recent advances in the understanding of the interplay between the innate and the adaptative immune systems have shed light on the mechanisms of action of adjuvants and enabled the development of new adjuvants in a more rational manner.

Aluminium salts (referred to as alum) have been the only adjuvants licensed for human vaccines for decades and only a few others have been approved in the past 20 years (MF59®, AS04). These adjuvants were once thought to act primarily through the formation of a depot at the injection site, enabling enhanced antigen availability, activation of antigen presenting cells (APCs) and uptake by immune cells[1]. It is now becoming clear that they can also activate innate immunity pathways triggered by pattern recognition receptors (PRRs). The NLR pathway has been recently described as an important mechanism in alum adjuvancy[2]. Alum has been shown to activate the NLRP3 inflammasome although its role in alum-induced antibody response is controversial.

Members of nearly all of the PRR families are potential targets for adjuvants. These include Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). They signal through pathways that involve distinct adaptor molecules and intermediates leading to the activation of different transcription factors. These transcription factors, such as NF-κB and IRF3, induce the production of pro-inflammatory cytokines and chemokines and type I IFNs that increase the host's ability to eliminate the pathogen. These innate immune responses strongly influence the adaptative immune response, and thus can be harnessed to direct and enhance the response to vaccine antigens.

Alum is a very efficient adjuvant for diseases against which a neutralizing antibody response is needed, such as tetanus and diphteria. It leads to a very high antibody response with a Th2 profile[3, 4]. However, alum is a poor inducer of protective Th1 response, an important feature for the development of vaccines against intracellular pathogens[5]. A promising approach for the development of new adjuvants is the use of PRR agonists that can induce Th1-skewed responses by promoting a pro-inflammatory environment during the induction of the adaptative response. A number of PRR agonists are being tested for use as adjuvants. They include monophosphoryl lipid A (MPL), a derivative of *Salmonella minnesota* LPS, which is able to bind and activate TLR4, oligodeoxynucleotides containing specific CpG motifs (CpG ODNs) that are recognized by TLR9, and the TLR7/8 agonists imiquimod and resiquimod (R848)[3].

The idea of inducing potent and protective immune responses through the engagement of PRRs has led to the concept of combining PRR agonists and particulate adjuvants. Some formulations are already approved for human use, such as AS04, a formulation of alum and MPL, used in vaccines against human papilloma virus and hepatitis B virus. AS04 was shown to promote more proliferation of antigen-specific CD4+ T cells than alum alone[4]. MPL is believed to be responsible for AS04-induced innate responses, while alum is able to prolong the cytokine response to MPL. In experimental models, administration of other combinations, such as CpG ODNs with alum or MPL, has proven very effective[6, 7].

A better understanding of the mechanisms of immunopotentiation should allow improvments in the design of new vaccine adjuvants with enhanced efficacy and safety.

1. Morefield GL. *et al.*, 2005. Role of aluminum-containing adjuvants in antigen internalization by dendritic cells in vitro. Vaccine. 23(13):1588-95. **2. Li H. *et al.*, 2008.** Cutting edge: Inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. J Immunol. 181(1):17-21. **3. Coffman R. *et al.*, 2010.** Vaccine adjuvants: Putting innate immunity to work. Immunity 33(4):492-503. **4. Didierlaurent A. *et al.*, 2009.** AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol 183(10): 6186-97. **5. Mbow ML. *et al.*, 2010.** New adjuvants for human vaccines. Curr Opin Immunol. 22(3):411-6. **6. Siegrist C. *et al.*, 2004.** Co-administration of CpG oligonucleotides enhances the late affinity maturation process of human anti-hepatitis B vaccine response. Vaccine 23(5): 615-22. **7. Kim S. *et al.*, 2000.** Effect of immunological adjuvant combinations on the antibody and T-cell response to vaccination with MUC1-KLH and GD3-KLH conjugates. Vaccine 19: 530-7.

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Vaccine Adjuvants

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- **VacciGrade™ Adjuvants -** Suitable for preclinical studies
- **Quality Guaranteed -** Sterility and endotoxin level thoroughly tested

Alum and Emulsions

PRODUCT	DESCRIPTION	Th RESPONSE	RATIO	QUANTITY	CAT. CODE
AddaVax™	Squalene- Oil-in-water	Th2	1:1 (addaVax : antigen)	2 ml 5 x 2 ml	vac-adx-2 vac-adx-10
Alhydrogel 2%	Aluminium hydroxide gel	Th2	1:9 - 1:1 (alhydrogel : antigen)	50 ml 250 ml	vac-alu-50 vac-alu-250
IFA	Incomplete Freund's adjuvant Water-in-oil	Th2	1:1 (IFA : antigen)	10 ml 6 x 10 ml	vac-ifa-10 vac-ifa-60

AddaVax™ is a squalene-based oil-in-water nano-emulsion based on the formulation of MF59® that has been licensed in Europe for adjuvanted flu vaccines. Squalene is an oil more readily metabolized than the paraffin oil used in Freund's adjuvants. AddaVax™ promotes a significant increase in antibody titers with reportedly more balanced Th1/Th2 responses than those obtained with alum. MF59® is a registered trademark owned by Novartis Ag.

Alhydrogel 2% is an aluminium hydroxide (referred to as alum) wet gel suspension. Alum improves attraction and uptake of antigen by APCs. It has been suggested that the antigens absorbed on the aluminum salts are presented in a particulate form, making them more efficiently internalized by APCs. Alum increases Th2 antibodies but does not promote significant Th1 cellular response.

IFA (Incomplete Freund's adjuvant), a water-in-oil emulsion, is one of the most commonly used adjuvants in research. It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate). IFA does not contain killed *Mycobacterium tuberculosis* found in Complete Freund's Adjuvant and is thus less inflammatory. IFA induces a predominantly Th2 biased response through the formation of a depot at the injection site and the stimulation of antibody producing plasma cells.

PRR Ligands

PRODUCT	SPECIFICTY	DESCRIPTION	Th RESPONSE	WORKING CONCENTRATION	QTY	CAT. CODE
Flagellin FliC	TLR5 agonist	*S. typhimurium* recombinant flagellin	Th1 / Th2	1 - 10 µg/mouse	50 µg	vac-fla
Gardiquimod	TLR7 agonist	Imidazoquinoline compound	Th1	10 - 100 µg/mouse	5 mg	vac-gdq
Imiquimod	TLR7 agonist	Imidazoquinoline compound	Th1	10 - 100 µg/mouse	5 mg	vac-imq
MPLA	TLR4 agonist	Monophosphoryl Lipid A	Th1	2 - 20 µg/mouse	1 mg	vac-mpl
N-glycolyl-MDP	NOD2 agonist	N-glycolylated muramyldipeptide	Th1	5 - 30 µg/mouse	5 mg	vac-gmdp
ODN 1826	TLR9 agonist	CpG ODN, type B (murine)	Th1	20 - 50 µg/mouse	1 mg	vac-1826-1
ODN 2006	TLR9 agonist	CpG ODN, type B (human)	Th1	20 - 50 µg/mouse	1 mg	vac-2006-1
Poly(I:C)	TLR3 agonist	Polyinosine-polycytidylic acid	Th1	10 - 100 µg/mouse	10 mg	vac-pic
R848 (resiquimod)	TLR7/8 agonist	Imidazoquinoline compound -	Th1	10 - 100 µg/mouse	5 mg	vac-r848

Flagellin FliC is a recombinant flagellin protein encoded by the *fliC* gene from *Salmonella typhimurium*. Unlike other TLR agonists, flagellin tends to produce mixed Th1 and Th2 responses rather than strongly Th1 responses. It has been demonstrated that flagellin can act as a potent adjuvant in flu vaccines.

Gardiquimod, Imiquimod and R848 are imidazoquinoline compounds and agonists for TLR7 and TLR8. They are effective adjuvants by activating dendritic cells (DCs) and B cells to induce cytokines optimal for Th1 cell immunity, and antibody production.

MPLA (monophosphoryl lipid A), a TLR4 agonist, is a derivative of lipid A from *Salmonella minnesota* R595 lipopolysaccharide (LPS or endotoxin). MPLA is considerably less toxic than LPS whilst maintaining the immunostimulatory activity. When tested in animals models as a vaccine adjuvant, MPLA induces a strong Th1 response.

N-glycolyl-MDP is an N-glycolylated MDP (muramyl dipeptide) found in mycobacteria and a NOD2 agonist. N-glycolyl-MDP has been reported to display a stronger NOD2-mediated activity than N-acetyl-MDP found in most bacteria and thus to be a more potent vaccine adjuvant than N-acetyl-MDP.

Poly(I:C), is a synthetic double-stranded RNA that activates the immune response through TLR3 or RIG-I/MDA-5. It triggers the production of IL-12 and type I IFNs production, and improves MHC class II expression and cross-presentation of antigen, therefore promoting Th1 biased immunity.

ODN 1826 and ODN 2006 are synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG ODNs). CpG ODNs are recognized by TLR9, which is expressed exclusively on human B cells and plasmacytoid dendritic cells (pDCs), thereby inducing Th1-dominated immune responses. Pre-clinical and clinical trials have demonstrated that CpG ODNs can significantly improve vaccine-specific antibody responses.

Antibody Generation - pFUSE-CLIg & pFUSE-CHIg

pFUSE-CLIg and pFUSE-CHIg plasmids are designed to change a monoclonal antibody from one isotype to another human or murine IgG isotype therefore enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire IgG antibodies from fragment antigen-binding (Fab) or single-chain variable fragment (scFv) fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

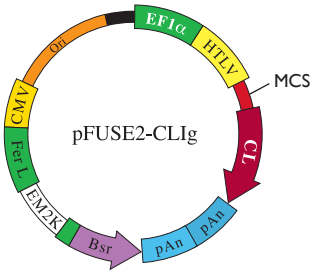
Description

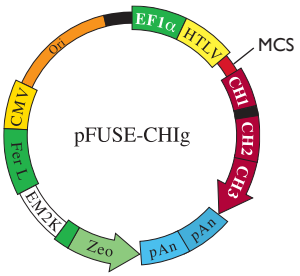
pFUSE-CHIg and pFUSE-CLIg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSE-CHIg and pFUSE-CLIg pair allows to generate an IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

- **pFUSE-CLIg plasmids** feature the constant region of the kappa or lambda light chain of human, mouse or rabbit origin (see table). They are selectable with blasticidin.
- **pFUSE-CHIg plasmids** feature the heavy chain constant region of the human, mouse or rabbit IgG isotypes (see table). pFUSE-CHIg plasmids are selectable with Zeocin™.

All pFUSE-CLIg and pFUSE-CHIg plasmids are now available **with or without the IL2 signal sequence**. Addition of this signal sequence allows the secretion of recombinant IgGs generated using Fab or scFv fragments selected from phage display libraries that lack a signal sequence.

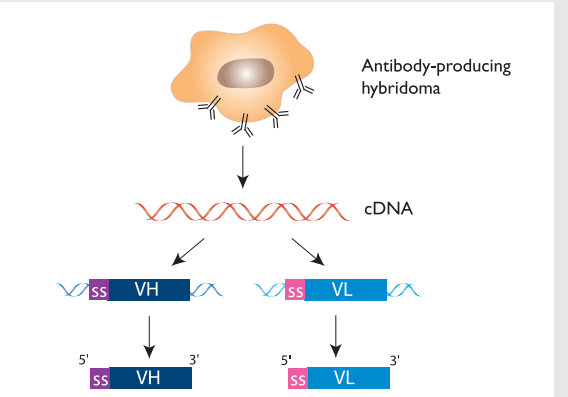
NEW Mutations in the CH2/CH3 domains of human IgG1 have been shown to alter the properties of this isotype. pFUSE-CHIg-hG1 featuring these mutations are now available. For more information, check www.invivogen.com/antibody-generation.



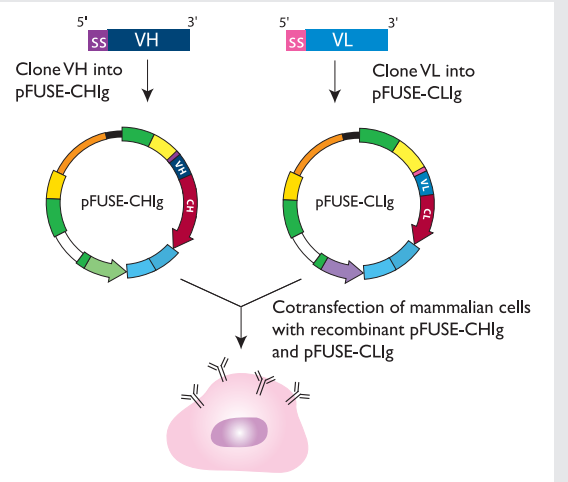


PRODUCT	ISOTYPE	CAT. CODE (No IL2ss)	CAT. CODE (With IL2ss)
pFUSE-CLIg			
pFUSE2-CLIg-hk	Human kappa	pfuse2-hclk	pfuse2ss-hclk
pFUSE2-CLIg-hl2	Human lambda 2	pfuse2-hcll2	pfuse2ss-hcll2
pFUSE2-CLIg-mk	Mouse kappa	pfuse2-mclk	pfuse2ss-mclk
pFUSE2-CLIg-ml1	Mouse lambda 1	pfuse2-mcll1	pfuse2ss-mcll1
pFUSE2-CLIg-ml2	Mouse lambda 2	pfuse2-mcll2	pfuse2ss-mcll2
pFUSE2-CLIg-rk1	Rabbit kappa 1	pfuse2-rclk1	pfuse2ss-rclk1
pFUSE2-CLIg-rk2	Rabbit kappa 2	pfuse2-rclk2	pfuse2ss-rclk2
pFUSE-CHIg			
pFUSE-CHIg-hG1	Human IgG1	pfuse-hchg1	pfusess-hchg1
pFUSE-CHIg-hG2	Human IgG2	pfuse-hchg2	pfusess-hchg2
pFUSE-CHIg-hG3	Human IgG3	pfuse-hchg3	pfusess-hchg3
pFUSE-CHIg-hG4	Human IgG4	pfuse-hchg4	pfusess-hchg4
pFUSE-CHIg-mG1	Mouse IgG1	pfuse-mchg1	pfusess-mchg1
pFUSE-CHIg-mG2a	Mouse IgG2a	pfuse-mchg2a	pfusess-mchg2a
pFUSE-CHIg-mG2b	Mouse IgG2b	pfuse-mchg2b	pfusess-mchg2b
pFUSE-CHIg-mG3	Mouse IgG3	pfuse-mchg3	pfusess-mchg3
pFUSE-CHIg-rG	Rabbit IgG	pfuse-rchg	pfusess-rchg

pFUSE-CHIg/pFUSE-CLIg-based antibody generation



1- Obtaining VH and VL sequences
To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons are sequenced.



2- Cloning into pFUSE-CHIg and pFUSE-CLIg
Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. When generating the insert for VH, a Nhe I site must be introduced at the 3' end to maintain the integrity of the constant region. Similarly, when generating the insert for VL, a Bsi WI (human VL) or Bst API (mouse VL) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

Contents

pFUSE-CLIg and pFUSE-CHIg plasmids are provided as 20 µg of lyophilized DNA. Each plasmid is supplied with 4 pouches of *E. coli* Fast-Media® Blas or Zeo (2 TB and 2 Agar).

Antibody Isotype Collections

- **Compare effector functions of different isotypes**
- **Study species-specificity of an Ig isotype**

Immunoglobulins (Ig) are divided in isotypes: nine in humans (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, IgE) and eight in mice (IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, IgD, IgE). Each isotype displays distinct structural and effector properties. These properties are key features in choosing the backbone for a therapeutic antibody. To help you decide which Ig isotype is the most suitable for your application, InvivoGen provides two well-known monoclonal antibodies, anti-hCD20 (rituximab) and anti-hTNF-α (adalimumab), available in the most common human and murine isotypes.

The anti-hCD20 and anti-hTNF-α isotype collections consist of monoclonal antibodies comprising the same variable region, that targets the human CD20 antigen or human TNF-α cytokine respectively, and the constant region of different isotypes. The constant region consists of the human or murine kappa light chain and the heavy chain of different isotypes. Eight human and three murine isotypes are available (see table).

Anti-hCD20 isotype collection

The Anti-hCD20 isotype collection features the variable region of rituximab. Rituximab is a mouse/human chimeric monoclonal antibody that targets the CD20 antigen found on the surface of malignant and normal B lymphocytes. Binding of rituximab to CD20 results in cell destruction through different mechanisms including direct signaling of apoptosis, complement activation and cell-mediated cytotoxicity. Rituximab has been approved by the FDA for the treatment of various lymphoid malignancies, including B-cell non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukaemia.

Anti-hTNF-α isotype collection

The Anti-hTNF-α isotype collection features the variable region of adalimumab. Adalimumab is a fully human monoclonal antibody against the pro-inflammatory cytokine TNF-α. Adalimumab binds to TNF-α and blocks its interaction with TNF receptors thereby downregulating the inflammatory reactions associated with autoimmune diseases. Adalimumab has been approved by the FDA for the treatment of various inflammatory diseases, such as rheumatoid arthritis and Crohn's disease.

Name	Types	Description
IgG	4	Major Ig in serum, placental transfer CDC (hIgG3>hIgG1>hIgG2>hIgG4; mIgG2a>mIgG1) ADCC (hIgG1≥hIgG3>hIgG2≥IgG4; mIgG2a>mIgG1)
IgM	1	Third most common serum Ig, first Ig to be made Good CDC, some ADCC
IgA	2	Major class in secretions, second most common serum Ig monomer in serum, dimer in secretions No CDC, some ADCC
IgE	1	Least common serum Ig, involved in allergic reaction Strong binding to Fc receptors on basophils, no CDC

The anti-hCD20 and anti-hTNF-α isotype collections have been generated by recombinant DNA technology. They have been produced in CHO cells and purified by different types of affinity chromatography: protein G for IgG isotypes, protein L for IgE and IgM, and peptide M for IgA isotypes. The activity of the anti-hCD20 and anti-hTNF-α antibodies has been tested by FACS analysis or neutralizing assays, respectively.

Each anti-hCD20 and anti-hTNF-α antibody is provided lyophilized from a 0.2 μm filtered buffered solution with stabilizers.

PRODUCT	ISOTYPE	QTY	CAT. CODE
Anti-hCD20 isotype collection			
Anti-hCD20-hIgG1	Human IgG1	100 μg	hcd20-mab1
Anti-hCD20-hIgG2	Human IgG2	100 μg	hcd20-mab2
Anti-hCD20-hIgG3	Human IgG3	100 μg	hcd20-mab3
Anti-hCD20-hIgG4	Human IgG4	100 μg	hcd20-mab4
Anti-hCD20-hIgM	Human IgM	100 μg	hcd20-mab5
Anti-hCD20-hIgA1	Human IgA1	100 μg	hcd20-mab6
Anti-hCD20-hIgA2	Human IgA2	100 μg	hcd20-mab7
Anti-hCD20-hIgE	Human IgE	100 μg	hcd20-mab8
Anti-hCD20-mIgG1	Mouse IgG1	100 μg	hcd20-mab9
Anti-hCD20-mIgG2a	Mouse IgG2a	100 μg	hcd20-mab10
Anti-hCD20-mIgA	Mouse IgA	100 μg	hcd20-mab11
Anti-hTNF-α isotype collection			
Anti-hTNF-α-hIgG1	Human IgG1	100 μg	htnfa-mab1
Anti-hTNF-α-hIgG2	Human IgG2	100 μg	htnfa-mab2
Anti-hTNF-α-hIgG3	Human IgG3	100 μg	htnfa-mab3
Anti-hTNF-α-hIgG4	Human IgG4	100 μg	htnfa-mab4
Anti-hTNF-α-hIgM	Human IgM	100 μg	htnfa-mab5
Anti-hTNF-α-hIgA1	Human IgA1	100 μg	htnfa-mab6
Anti-hTNF-α-hIgA2	Human IgA2	100 μg	htnfa-mab7
Anti-hTNF-α-hIgE	Human IgE	100 μg	htnfa-mab8
Anti-hTNF-α-mIgG1	Mouse IgG1	100 μg	htnfa-mab9
Anti-hTNF-α-mIgG2a	Mouse IgG2a	100 μg	htnfa-mab10
Anti-hTNF-α-mIgA	Mouse IgA	100 μg	htnfa-mab11

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