Lipoarabinomannan and Lipomannan

**TLR0-dependent Immune Modulators**

Lipoarabinomannans (LAM) and Lipomannans (LM) are lipoglycans restricted to the Mycobacterium genus that act as potent modulators of the host immune response. They are found in the envelope of all mycobacteria species, such as the pathogenic strains M. tuberculosis and M. leprae, the vaccine strain, M. bovis BCG, the opportunistic strains M. avium and M. fortuitum, and the non-pathogenic strain M. smegmatis LM and LM, which induce different immune responses depending on the species they originate from, signal through TLR2. InvivoGen provides LAM and LM from M. smegmatis.

**Lipoarabinomannan - LAM-MS**

Lipoarabinomannans (LAM) display different immunomodulatory effects depending on their structure. PILAM, which are phosphatidyl-capped LAM and found in non-pathogenic species (M. smegmatis), are proinflammatory molecules whereas ManLAM, which are mannose-capped LAM and found in pathogenic species (M. tuberculosis), are anti-inflammatory molecules. LAM-MS is a PILAM and is known to activate macrophages in a TLR2-dependent manner. This activation is weak compared to that of LAMMS, its bioactive precursor (Fig. 1).

**Lipomannan - LM-MS**

Lipomannans (LMs) are composed of a mannan core and a glycosyl-phosphoinositol anchor and have a average MW of 5-6 kDa. LMs display strong inflammatory activity regardless of the species of Mycobacterium from which they are isolated. They induce the release of cytokines, such as TNF-α and IL-8, from differentiated cells in a TLR2 and MYD88-dependent manner. LM-MS is one of the most potent TLR2 ligand.

To test whether LAM-MS and LM-MS signal through TLR2 alone or require additional TLRs, such as TLR1 or TLR5, we performed TLR neutralizing experiments on HEK293 cells, that are known to express endogenous levels of TLR1 and TLR5, transfected with human TLR2 and human TLR1 and TLR5. Polyclonal antibodies against human TLR1 and TLR2, and a human TLR1 and TLR2, and a human TLR5 were used to block the responses induced by both LAM-MS and LM-MS whereas anti-TLR5 had no effect. These data suggest that LAM-MS and LM-MS require TLR1 and TLR2 to induce an immune response.

**Inside this issue:**

**Products**

- Fc Fusions - pFUSE-Fc
  - Wild-type Fc
  - Engineered Fc

- TLR Antibodies
  - Monomeric Antibodies
  - Polyclononal Antibodies

- TLR Ligands
  - Lipoarabinomannan
  - Lipomannan

**Review**

- IgG-Fc Engineering for Therapeutic Use

**IgG-Fc Engineering for Therapeutic Use**

Recombinant fusion proteins consisting of the extracellular domain of immunoregulatory proteins and the constant (Fc) domain of immunoglobulin G (IgG) represent a growing class of human therapeutics. The IgG class is divided in four isotopes: IgG1, IgG2, IgG3, and IgG4 in humans, and IgG1, IgG2s, IgG3s, and IgG4s in mice. They share more than 95% homology in the amino acid sequences of the Fc regions but show major differences in the amino acid composition and structure of the hinge region. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (FcRs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the targeted cells by triggering the complement cascade at the cell surface. IgGs exist in different levels of effector functions increasing in the order of IgG4s<IgG2s<IgG1s.<br>IgG3s. Human IgG3s display high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells. Under certain circumstances, for example when depletion of the target cell is undesirable, abrogating effector functions is required. On the contrary, in the case of antibodies intended for oncology use, increasing effector functions may improve their therapeutic activity. Modifying effector functions can be achieved by engineering the Fc regions to either improve or reduce their binding to FcRs. Numerous mutations have been made in the CH2 domain of IgG1 and their effect on ADCC and CDC tested in vitro. In particular, a mutation to alanine at C233 was reported to increase both ADCC and CDC. Increasing the tenness persistence of a therapeutic antibody is another way to improve its efficacy, allowing higher circulating levels, less frequent administration and reduced doses. This can be achieved by enhancing the binding of the Fc region to nonmual FcRs. FcRs which are expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation. Several mutations located at the interface between the CH2 and CH3 domains have been shown to increase the half-life of IgG1s. InvivoGen provides many of the engineered Fc regions mentioned in this short review. They are available in various forms, specificity specifically designed for the production of high levels of Fc fusion proteins in mammalian cells.

**Antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)**

IgGs are antibodies that consist of two heavy chains (H) and two light chains (L) that are made in the CH2 domain of IgG and their effect on ADCC and CDC tested in vitro. In particular, a mutation to alanine at C233 was reported to increase both ADCC and CDC. Increasing the tenness persistence of a therapeutic antibody is another way to improve its efficacy, allowing higher circulating levels, less frequent administration and reduced doses. This can be achieved by enhancing the binding of the Fc region to nonmual FcRs. FcRs which are expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation. Several mutations located at the interface between the CH2 and CH3 domains have been shown to increase the half-life of IgG1s. InvivoGen provides many of the engineered Fc regions mentioned in this short review. They are available in various forms, specificity specifically designed for the production of high levels of Fc fusion proteins in mammalian cells.
**TLR Antibodies**

### Mouse Monoclonal Antibodies - MAb-TLRs

InvivoGen provides a selection of monoclonal anti-TLR antibodies (MAb-TLR). These antibodies can be used for various applications: detection by flow cytometry, immunoprecipitation, or Western blot; immune assays, and neutralization by blocking the activation induced by the appropriate TLR ligand. MAb-TLRs are purified and lyophilized.

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<td>Neutralization</td>
<td>7</td>
<td>100 µg</td>
<td>mab-md9</td>
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### Rat Polyclonal Antibodies - Pab-TLRs

InvivoGen has developed new polyclonal anti-TLR antibodies (Pab-TLRs). Pab-TLRs have been generated by DNA vaccination. Wistar rats have received four hydrodynamic injections of a pFUSE-TLR-Flc plasmid, expressing the extracellular region of human TLRs fused to the Fc portion of human IgG2. The sera were harvested and the IgG fraction purified by Protein G affinity chromatography. Pab-TLRs are sterile, endotoxin-free (containing PfuSS-1), endotoxin-tested (≤ 0.01 EU/mg) and freeze-dried.

**All TLR antibodies are tested in house in neutralization assays.**

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**pFUSE-Fc**

Fc Fusion Proteins Made Easy

Fc-fusion proteins are chimeric proteins featuring the Fe region of an immunoglobulin fused to their C terminus. These soluble chimeras retain the activity of the native protein and present the advantages of a long half-life in the circulatory system; efficient mammalian expression and ease of purification. Fc-fusion proteins are useful research tools for many applications and hold promise as therapeutics. Furthermore, they can be used as antigens for vaccination applications. InvivoGen provides pFUSE-Fc, a family of plasmids featuring several Fc regions from various species: human IgG1, IgG2 and IgG4, mouse IgG1, IgG2a and IgG1c, rabbit IgG, rat IgG2b, and engineered Fc from human IgG1, IgG2, and mouse IgG2a.

Choose a pFUSE-Fc plasmid accordingly to your application:

- **Protein purification** - All pFUSE-Fc can be used for Protein A or Protein G affinity chromatography.
- **Long term expression in vivo** - Choose a pFUSE-Fc with an Fc engineered to display increased ADDC and CDC.
- **Therapeutic use with cell depletion activity** - Choose a pFUSE-Fc with an Fc engineered to display reduced ADDC and CDC.
- **Therapeutic use without cell depletion activity** - Choose a pFUSE-Fc with an Fc engineered to display reduced ADDC and CDC.

InvivoGen’s pFUSE-Fc plasmids feature a very innovative backbone with two unique promoters: EF1- prom/HTLV-1LTR and CMV enh/FH prom. They are expression independent of the cell cycle.

- **Ubiquitous** - Transferable in a variety of mammalian cells, including cells commonly used in protein purification systems (CHO, COS, HEK293).
- **pFUSE-Fc proteins** - the secretion of Fc-Fusion proteins. Two versions are available for each pFUSE-Fc:
  - **pFUSE-Fc1** is recommended when the protein of interest contains a native signal sequence.
  - **pFUSE-Fc2** contains a II2 signal sequence (II2ss) for the generation of Fusions derived from proteins that are not naturally secreted.

All pFUSE-Fc plasmids are provided as 20 µg of lyophilized DNA.

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Fc-fusion proteins are chimeric proteins featuring the Fc region of an immunoglobulin fused to their C terminus. These soluble chimeras retain the activity of the native protein and present the advantages of a long half-life in the circulatory system; efficient mammalian expression and ease of purification. Fc-fusion proteins are useful research tools for many applications and hold promise as therapeutics. Furthermore, they can be used as antigens for vaccination applications. InvivoGen provides pFUSE-Fc, a family of plasmids featuring several Fc regions from various species: human IgG1, IgG2 and IgG4, mouse IgG1, IgG2a and IgG1c, rabbit IgG, rat IgG2b, and engineered Fc from human IgG1, IgG2, and mouse IgG2a.

Choose a pFUSE-Fc plasmid accordingly to your application:

- **Protein purification** - All pFUSE-Fc can be used for Protein A or Protein G affinity chromatography.
- **Long term expression in vivo** - Choose a pFUSE-Fc with an Fc engineered to display an increased half-life.
- **Therapeutic use with cell depletion activity** - Choose a pFUSE-Fc with an Fc engineered to display increased ADDC and CDC.
- **Therapeutic use without cell depletion activity** - Choose a pFUSE-Fc with an Fc engineered to display reduced ADDC and CDC.

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pFUSE-Fc
Fc Fusions Made Easy

Fc-fusion proteins are chimeric proteins featuring the Fc region of an immunoglobulin fused to their C terminus. These soluble chimeras retain the activity of the native protein and present the advantages of a long half-life in the circulatory system, efficient mannose recognition and ease of purification. Fc-fusion proteins are useful research tools for many applications and hold promise as therapeutics. Furthermore, they can be used as antigens for vaccination applications. Invivogen provides pFUSE-Fc, a family of plasmids featuring several Fc regions from various species: human IgG1, IgG2 and IgG4, mouse IgG1, IgG2a and IgG2c, rabbit IgG, rat IgG2b, and engineered Fc from human IgG1, IgG2c and mouse IgG2a.

Choose a pFUSE-Fc plasmid according to your application:
- **Protein purification** - All pFUSE-Fc can be used for Protein A or Protein G affinity chromatography.
- **Long term expression in vivo** - Choose a pFUSE-Fc with an Fc engineered to display increased ADCC and CDC.
- **Therapeutic use with cell depletion activity** - Choose a pFUSE-Fc with an Fc engineered to display reduced ADCC and CDC.
- **Protein G affinity chromatography** - pAb-TLRs are sterile, azide-free, and lyophilized.
- **Strong** - High levels of expression. Production of Fc-Fusions is usually in the µg range.
- **Constitutive** - Expression independent of the cell cycle.
- **Ubiquitous** - Transferable in a variety of mammalian cells, including cells commonly used in protein purification systems (CHO, COS, HEK293).

All pFUSE-Fc plasmids are provided as 20 µg/mL in PBS. FAQs:
- **Therapeutic use without cell depletion activity** - pFUSE-FcFerL
- **Therapeutic use with cell depletion activity** - pFUSE-Fc
- **Protein purification** - pFUSE-FcFerL
- **Ubiquitous** - pFUSE-Fc
- **Strong** - pFUSE-Fc
- **Constitutive** - pFUSE-Fc
- **Ubiquitous** - pFUSE-Fc

TLR Antibodies
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<table>
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<tr>
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<td>FC, HK, WB</td>
<td>7</td>
<td>100 µg</td>
<td>mab-mtlr9</td>
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</tbody>
</table>

**FC** - flow cytometry, **IHC** - immunohistochemistry, **WB** - Western blot

* All TLR antibodies have been tested in-house for neutralization.

Rat Polyclonal Antibodies - PAb-TLRs
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<table>
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<tr>
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<td>Neutralization</td>
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<td>human TLR6</td>
<td>Neutralization</td>
<td>200 µg</td>
<td>pab-htlr6</td>
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</table>

Neutralization with PAb-TLRs: HEK293 cells expressing a given TLR and an NP-fused soluble TLR were incubated with 100 ng/mL MAb-TLR or PAb-TLR for 30 min prior to the addition of 100 ng/mL of cognate ligand. Cells were incubated for 4 h at 37°C. For Western blot, the supernatant was collected and Western blotted for TLR1, TLR5, TLR9, or ERK. For flow cytometry, cells were incubated for 30 min at 4°C with respective TLR antibodies followed by incubation for 30 min at 4°C with PE or FITC-conjugated secondary antibodies. Controls were incubated with an isotype-matched antibody that is negative for the respective mouse IgG or rat IgG isotype.

For up-to-date information on Invivogen’s products, visit www.invivogen.com

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Lipoarabinomannan and Lipomannan

TLR2-Dependent Immune Modulators

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Lipomannan - LM-MS

Lipomannans (LMs) are composed of a mannose core and a glycolyl-phosphoethanolamided anchor and have a mean MW of 6 kDa. LMs display strong inflammatory activity regardless of the species of Mycobacteria from which they are isolated. They induce the release of cytokines, such as TNF-α and IL-8, from differentiated cells in a TLR2 and MYD88 dependent manner. LM-MS is one of the most potent TLR2 ligand.

To test whether LAM-MS and LM-MS signal through TLR2 alone or require additional TLRs, such as TLR1 or TLR2, we performed TLR neutralizing experiments on HEK293 cells, that are known to express endogenous levels of TLR1 and TLR2, transfected with human TLR1 and TLR2 as well as an NF-κB inducible SEAP plasmid. Polyclonal antibodies against human TLR1 and TLR2 (PAb-hTLR6 and PAb-hTLR2) blocked the responses induced by both LAM-MS and LM-MS whereas PAb-hTLR1 had no effect (Fig. 2). These data suggest that LAM-MS and LM-MS require TLR1 and TLR2 to induce an immune response.

For further augment in its comprehensive TLR product line, InvivoGen is now providing TLR antibodies. These monoclonal or polyclonal antibodies can be used for detection or neutralization to determine whether a TLR is involved in the signaling of a given ligand such as lipoarabinomannan and lipomannosaminoglycan, two newly introduced TLR2 ligands. Furthermore, InvivoGen is expanding the pFUSE-Fc protein family by offering Fc regions from more IgG isotypes and also engineered Fc with altered properties to better suit your needs.

Inside this issue:

- Products
- IgG-Fc Engineering For Therapeutic Use

IgG-Fc Engineering For Therapeutic Use

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In the case of antibody-dependent cellular cytotoxicity (ADCC), the FcγR-2 or the first component of complement (C1q) depends on residues at positions 327, 330 and 331 greatly reduced activity. Under certain circumstances, for example when depletion of the target cell is undesirable, abrogating effector functions is required. On the contrary, in the case of antibodies intended for oncology use, increasing effector functions may improve their therapeutic activity. Modifying effector functions can be achieved by engineering the Fc regions to either improve or reduce their binding to FcγR or the complement factors.

Antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).