**Listeria monocytogenes as a probe of immune function**

Oleg Garifulin and Victor Boyartchuk

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### Abstract

For almost half a century, the mouse model of *Listeria monocytogenes* infection has been used to analyse both innate and adaptive components of immunity and to discover key immune genes. Vast accumulated knowledge about the disease in mice provides a unique framework for identifying and characterising immune molecules using a variety of experimental approaches. To illustrate the range of questions that can be addressed using modern genetics and genomics tools, the authors provide an overview of the analysis of components of immune signalling networks using the mouse model of *L. monocytogenes* infection.

### INTRODUCTION

*Listeria monocytogenes* is a Gram-positive bacterium which causes food-borne infections. In the majority of cases, immunocompetent individuals exposed to *L. monocytogenes* suffer only mild flu-like symptoms. In immunocompromised individuals, such as AIDS patients and patients undergoing chemotherapy, however, infection with this pathogen can be fatal. In addition, exposure to *L. monocytogenes* poses a serious risk during pregnancy and can lead to death of the foetus.¹

The mouse model of listeriosis has been widely used to understand the general mechanism of *L. monocytogenes* infection. The most important contributions of these studies concern the unique combination of features that this pathogen uses to evade and exploit the host immune system. To combat *L. monocytogenes* infection, the host has to utilise virtually every component of its immune system. Hence, since the early times of molecular immunology, the mouse model of *L. monocytogenes* infection has been used extensively to analyse both innate and adaptive components of immunity and to discover genes which play a key role in the control of these components.² Studies of *L. monocytogenes* infection were especially useful in analysing the immune cytokine and chemokine signalling networks which have to function effectively to combat virtually any infection. In this review, some of the general experimental approaches used to analyse *L. monocytogenes* infection in the mouse model are described along with some of the key signalling molecules studied using these approaches.

### L. MONOCYTOMGENES INFECTION

Several features of *L. monocytogenes* make it a unique model pathogen. This pathogen can infect a broad spectrum of target cell types, such as macrophages, hepatocytes, epithelial cells and endothelial cells. Entry of *L. monocytogenes* into host cells can be either passive (ie by macrophage internalisation) or active — mediated by internalins A and B. In addition, *L. monocytogenes* is well adapted to the intracellular vacuole/cytoplasm life cycle and uses polymerisation of host actin molecules for intracellular motility and as a way to facilitate its direct cell-to-cell spreading.¹

Contaminated food is the major source of *L. monocytogenes* infection in humans. Ingested *L. monocytogenes* is capable of crossing the mucosa to reach underlying tissue.³ There, it can be taken up by enterocytes or M cells near Peyer’s patches.⁴ By contrast with humans, mice
are quite resistant to intragastric infection with \textit{L. monocytogenes}. This generated some criticism of the murine model for the study of human listeriosis. Recently, however, the discovery of a mutation in murine E-cadherin explained the differences in induction of the disease between humans and mice.\textsuperscript{5} A Pro to Glu substitution at amino acid 16 prevents interaction of E-cadherin with bacterial internalin and hence does not allow intragastric uptake of bacteria in the mouse intestine. By changing this amino acid, it is now possible to engineer mouse lines which faithfully replicate all aspects of the human disease.\textsuperscript{6}

Since the mouse model of intragastric infection was only developed recently, experimental listeriosis in mice was traditionally induced by intravenous injection of a defined dose of bacteria. Within ten minutes of injection, the majority of the \textit{L. monocytogenes} accumulates in the liver and spleen. Up to 60 per cent of the bacteria are removed from the circulation by macrophages (Kupffer cells) resident in the liver.\textsuperscript{7} Although Kupffer cells are critical for clearance of \textit{L. monocytogenes} from the bloodstream, they seem to contribute little to the microbicidal activity in the liver in the early stages of infection.\textsuperscript{8} Instead, it seems that their primary role early in infection is to provide a surface to which \textit{L. monocytogenes} can bind. Bactericidal neutrophils, which are subsequently recruited to the surface of infected Kupffer cells by secreted cytokines/chemokines, then seem to be responsible for the majority of the initial killing of bound \textit{L. monocytogenes}.\textsuperscript{9} Whereas most of the \textit{L. monocytogenes} are killed by neutrophils, some bacteria avoid destruction by entering hepatocytes, which appear to be the primary site of multiplication of bacteria in the liver.\textsuperscript{10} In response to infection, hepatocytes produce numerous chemoattractants, resulting in the recruitment of additional neutrophils and the formation of microabscesses.\textsuperscript{11} The spreading of \textit{L. monocytogenes} from these microabscesses is controlled by blood-derived monocytes, which, by 48 hours after infection, surround the infectious sites and form granulomas.\textsuperscript{12} The final step in resolution of infection involves clearance of bacteria. This process is primarily mediated by cytotoxic CD8\textsuperscript{+} T cell lysis of \textit{L. monocytogenes}-infected cells.\textsuperscript{13}

Murine listeriosis is probably one of the most well-characterised bacterial infections. The components of immunity and the precise sequence of their action required for the containment and resolution of the infection are well known. This knowledge provides a unique framework to identify and characterise immune molecules using a variety of experimental approaches. From the early stages of the analysis of murine listeriosis, classical genetics approaches exploited phenotypic differences between inbred mouse strains to map the location of genes that determine differences in immune response to infection. Gene replacement approaches also greatly benefit from the well-characterised course of murine listeriosis, allowing relatively straightforward identification of the place and role in immune function of the gene under study. The advent of modern whole-genome analysis approaches, such as expression profiling and proteomics, further expands the utility of one’s knowledge of the murine model of listeriosis by allowing comprehensive characterisation of the disease on a molecular level.

**CLASSICAL GENETICS ANALYSIS**

The classical genetics approach to the analysis of infection models is unbiased and therefore is uniquely capable of identifying novel molecules. Classical genetics uses phenotypic differences in a series of mapping experiments to identify polymorphic molecules. The wide spectrum of sensitivities displayed to \textit{L. monocytogenes} infection by inbred mouse strains suggests the important role of genetic variability in the control of this infection (Table 1). These differences in
susceptibility of inbred mouse strains to *L. monocytogenes* infection were noticed over 20 years ago, prompting a series of genetic studies. In the absence of fine-mapping tools, however, only in a single case was one of the contributing factors identified. Analysis of recombinant inbred (RI) panels formed by the sensitive A/J and resistant C57BL/6J mouse strains revealed that the sensitivity of the A/J strain to *L. monocytogenes* was linked to the *Hc* locus on mouse chromosome 2. This locus encodes the C5 complement protein. Following cleavage with C5-convertase enzymes, the C5 protein produces C5a anaphylatoxin, which can attract macrophages and polymorphonuclear cells to the site of infection. A two base-pair deletion in the *C5* gene prevents production of this member of complement in the A/J strain. A C5 deficiency in A/J mice seemed to be a reasonable explanation for both the known defect in recruitment of macrophages to the site of inflammation and extreme sensitivity of this strain to *L. monocytogenes* infection. Moreover, follow-up studies of congenic A/J animals carrying an intact copy of C5 confirmed the important role of C5 in the inflammatory response. C5-sufficient A/J mice demonstrated only slightly increased inflammatory responses, however, and were unable to resist *L. monocytogenes* infections to the same extent as the C5 donor C57BL/6 strain. This suggests that even though C5 contributes to the control of *L. monocytogenes* infection, the overall sensitivity is determined by additional factors that are yet to be identified. The contention that C5-mediated signalling has simply an auxiliary role in the control of *L. monocytogenes* infection is further supported by the virtually identical resistance to *L. monocytogenes* infection of the two congenic Hc lines, B10D2/OSn (C5 deficient) and B10D2/NSn (C5 sufficient). Therefore, these studies indicate that the relative contribution of individual immune components to defence against *L. monocytogenes* is influenced by a number of additional genes which themselves differ between the mouse-strain backgrounds.

The creation of comprehensive genetic and physical maps of the mouse genome, and further development of molecular tools for rapid analysis of the allelic make-up of individual animals, have provided new opportunities for genetic analysis of *L. monocytogenes* infection. Recently, the differential susceptibility of the sensitive BALB/cByJ and resistant C57BL/6ByJ strains was analysed in a series of intercrosses. By using a novel approach to the genetic mapping of infectious disease traits developed by K. Broman, the present authors identified several quantitative trait loci showing strong

### Table 1: Range of sensitivities of inbred mouse strains to intravenous *Listeria monocytogenes* infection

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hc (allele)</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (colony-forming units)</th>
<th>Survival (%)</th>
<th>Time to death (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>+</td>
<td>9.0 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>NZB/WEHI</td>
<td>-</td>
<td>N/A</td>
<td>100</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>SJL/WEHI</td>
<td>+</td>
<td>2.5 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>-</td>
<td>14–16</td>
</tr>
<tr>
<td>[129]J</td>
<td>N/A</td>
<td>N/A</td>
<td>33</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>+</td>
<td>4.0 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
<td>14,16</td>
</tr>
<tr>
<td>A/J</td>
<td>-</td>
<td>1.0 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
<td>14,16</td>
</tr>
<tr>
<td>DBA/1 J</td>
<td>+</td>
<td>1.0 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>14,16</td>
</tr>
<tr>
<td>BALB/cj</td>
<td>+</td>
<td>3.9 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>CBA/H</td>
<td>N/A</td>
<td>5.0 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

*Hc allele (+) indicates that strain produces the C5 member of complement.

*Percentage of animals alive after intravenous infection with 1 x 10<sup>5</sup> colony-forming units.

Mutation in C5 member of the complement contributes to the sensitivity of A/J mice to *L. monocytogenes* infection

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*Hc allele (+) indicates that strain produces the C5 member of complement.

*Percentage of animals alive after intravenous infection with 1 x 10<sup>5</sup> colony-forming units.
linkage to sensitivity to infection. For two loci, the logarithm of the odds (LOD) scores, which characterise the strength of linkage, exceeded the experimentally determined thresholds of significance. On chromosome 5, the Listr1 locus appears to control the probability of survival trait. The Listr2 locus on chromosome 13 appears to contribute to both the probability of survival trait and the time to death trait. Interestingly, only animals carrying the BALB/cByJ allele at Listr2 and the C57BL/6ByJ allele at Listr1 displayed uniform mortality. This would indicate that the Listr1 allele from sensitive BALB/cByJ mice acts to increase resistance to L. monocytogenes infection. This observation is in line with previous reports that overall sensitive and resistant mouse strains carry a mixture of alleles, both deleterious and beneficial, for the control of infectious disease. The overall phenotype is the result of a complex interaction of these alleles. Thus, reassortment of parental alleles in the intercross progeny produces both super-sensitive and super-resistant animals.

Genetic polymorphisms affecting the innate immune response often have an effect on resistance to multiple pathogens. For example, studies of differences in susceptibility of inbred mouse strains to virulent Mycobacterium tuberculosis led to identification of the sst1 locus. Since the sst1 locus was found to affect innate immune function in the sensitive C3H inbred mouse strains, L. monocytogenes was selected as an additional pathogen for detailed analysis of the function of this locus. Incorporation of L. monocytogenes-based experiments greatly accelerated the pace of analysis of the sst1 locus and helped the eventual discovery of the lpr1 gene, which mediates innate immunity to tuberculosis. This discovery underscores the importance of the mouse model of L. monocytogenes infection for analysis of genetic factors determining the course and outcome of a wide range of infectious diseases. Hence, in addition to being an important human pathogen, L. monocytogenes can also be viewed as a probe which can be used to challenge mammalian immune function.

ANALYSING
L. MONOCYTogenES
INFECTION BY
MODIFYING THE HOST
GENOME

One of the most powerful experimental approaches currently available for addressing the role and function of individual molecules is direct modification of the host genome. In this approach, the gene of interest can be inserted into the genome, removed from it or replaced with a variant. Depending on the specific approach chosen, altered genes can be present in all cells of the host or only in selected cell populations. The critical step in all such genome modification approaches is being able to detect the phenotypic effect of the introduced modification. Ideally, a wide range of immune functions should be tested in the modified animal. Because host defence against L. monocytogenes requires the cooperative interaction of virtually all components of immunity, this pathogen has proven to be a nearly universal tool in phenotypic analysis of immune genes. This cooperation of host immune components in defence against infection is coordinated by the cytokine and chemokine signalling networks. Examples of the analysis of some critical signalling molecules are present below to illustrate typical approaches to studying immune function by mouse genome modification.

Transgenesis

The easiest way to study the effect of a gene on immune function in vivo is to induce its exogenous expression. This is usually achieved by injecting a DNA fragment into a fertilised egg or by incubating embryos with recombinant lentiviruses containing the gene of interest. Both approaches result in random integration of one or several copies of the

Resistance of inbred mouse strains to L. monocytogenes infection is determined by interaction of multiple genes

L. monocytogenes is an excellent tool for analysis of immune gene function
gene under study into the genome of transgenic animals, causing significant variation in gene expression levels in individual animals.

One common way to study gene function using transgenesis is to analyse the effects of overexpressing the gene. This is often achieved by placing the cDNA of the gene of interest under the control of a constitutive promoter. This approach has been used to establish the important role of monocyte chemoattractant protein-1 (MCP-1) signalling in the defence against L. monocytogenes infection. MCP-1 attracts monocytes, memory T lymphocytes and natural killer cells in vitro. To model sustained MCP-1 synthesis during the inflammatory response, several transgenic lines were created, in which MCP-1 was placed under control of the mouse mammary tumour virus long terminal repeat. Some of the transgenic lines generated produced high levels of MCP-1, but in general had no histological or haematological abnormalities. Of the three lines that produced a range of MCP-1 levels, the two with the highest levels were substantially more sensitive to L. monocytogenes infection than were wild-type mice or mice with low constitutive MCP-1 levels. The concentration-dependent induction of sensitivity suggests that overproduction of MCP-1 leads to desensitisation of target cells or neutralisation of the chemotactic gradient. Since, in all lines, macrophages did not appear to be inactivated — as measured by foreign body response — the resulting increase in sensitivity to L. monocytogenes indicates a requirement for specific MCP-1 signalling to generate a proper immune response.

The wide range of transgene expression levels in individual transgenic animals can be used to study the effect of concentration-dependent inhibition of a signalling pathway. This approach was used to analyse tumour necrosis factor-alpha (TNFα) signalling. TNFα is a pleiotropic cytokine critical to a wide range of signalling tasks, from cell trafficking to inflammation, and the inflammatory immune response to maintenance of lymphoid organ structure. Mice lacking TNFα or its major receptor, TNFR1, are extremely susceptible to L. monocytogenes infection. Soluble TNFR1–FcIgG3 fusion protein interferes with TNFα signalling. This was used to analyse the effect of partial inhibition of TNFα signalling by creating a series of transgenic lines producing different levels of the fusion protein. Mouse lines that express high levels of the fusion protein are significantly more susceptible to L. monocytogenes infection and die following administration of a dose that is sublethal to wild-type animals. By contrast, animals producing low amounts of the fusion protein are only partially sensitive. This observation further confirmed that unimpeded TNFα signalling is required for effective resistance to L. monocytogenes infection.

For more precise analysis of gene function, expression of a transgene can be restricted to a particular cell population by using a cell-type-specific promoter. This strategy was used to study the effects of ectopic production of interleukin (IL)-10 in vivo. Early cell culture-based studies identified a number of key cytokines which play a critical role in the control of L. monocytogenes infection. One of these cytokines, IL-10, is secreted by a wide variety of cell types and has pleiotropic stimulatory and suppressive activities in vitro. Macrophages stimulated with IL-10 have impaired listericidal activity and chemokine release is reduced. To test the effect of high levels of IL-10 secretion in vivo, a transgenic mouse was created in which human IL-10 was expressed under control of the mouse major histocompatibility complex class II EA promoter. This promoter restricts ectopic production of human IL-10 to the major histocompatibility complex class II EA-expressing cells, such as macrophages and B cells. Human IL-10 was selected because it is fully active in the mouse but, if necessary, can be selectively neutralised.
with anti-human IL-10 antibodies. Interestingly, the resulting transgenic mice have no gross abnormalities in their peripheral lymphocyte populations. Nevertheless, production of IL-10 by macrophages and B cells is sufficient to render transgenic mice highly susceptible to infection with \( L.\ monocytogenes \). Transgenic mice display uniform mortality when infected with a dose of bacteria that is sublethal to wild-type mice. This is consistent with the in vitro data and an earlier observation that deletion of IL-10 results in up to 50-fold reduction of bacterial burdens in both livers and spleens of infected animals. Therefore, analysis of transgenic lines overproducing IL-10 provides an independent line of evidence for the role of this cytokine in the defence against \( L.\ monocytogenes \).

**Gene targeting**

Although gene-targeting approaches are significantly more time and resource consuming than straightforward transgenesis, they allow a qualitatively higher level of precision in modifying the host genome. Gene targeting relies on homologous recombination to replace an endogenous copy of the gene with a custom-designed DNA construct. Depending on the nature of the construct, the endogenous copy of the gene can be deleted, resulting in a gene knockout, or replaced with a different variant of the gene, creating a knock-in line.

**Gene knockouts**

Gene knockouts are extensively used in studies of the role of immune signalling molecules in the control of \( L.\ monocytogenes \) infection (Table 2). As more gene knockouts are generated and analysed, more details are learned about the mechanisms that \( L.\ monocytogenes \) uses to overcome the immune defences of the host. An interesting recent development, brought on by the analysis of gene knockouts, is the establishment of the critical role of type I interferon (IFN) signalling in the control of \( L.\ monocytogenes \) infection. Recent studies demonstrate that inactivation of components of the IFN\( \beta \) signalling pathway leads to an up to 100-fold decrease in both liver and spleen bacterial loads. Analysis of mice deficient in the key IFN\( \beta \) transcription factor IRF3 or the type I IFN receptor IFNAR indicate that following \( L.\ monocytogenes \) infection, their splenocytes — unlike those of wild-type mice — do not succumb to type I IFN-mediated apoptosis. In addition, the concentration of the key cytokine, IL-12, is markedly higher in the serum of IFNAR\(-/-\) mice at day 1 following infection. This suggests that type I IFNs could mediate downregulation of the critical IFN\( \gamma \) regulator IL-12. IFN\( \beta \) appears to be a paracrine factor responsible for the splenic apoptosis observed in \( L.\ monocytogenes \)-infected mice. Indeed, \( L.\ monocytogenes \) upregulates such proapoptotic genes as TRAIL, PKR and Daxx in spleen and bone marrow macrophages of wild-type, but not IFNAR-deficient, mice. It appears that the bacterial pore-forming toxin listeriolysin is the key molecule inducing lymphocytes to undergo apoptosis and that type I IFN signalling acts to enhance this induction.

Type I IFNs do not seem to have an effect on induction of apoptosis or intracellular growth of \( L.\ monocytogenes \) in infected macrophages, although IFN\( \beta \) was shown to play an important role in sensitising macrophages for efficient, non-apoptotic \( L.\ monocytogenes \)-mediated cell death. In addition, \( L.\ monocytogenes \) is thought to induce IFN\( \beta \) expression in order to suppress accumulation of TNF\( \alpha \)-producing phagocytic cells (mainly CD11b\(^+\)) at sites of bacterial growth. Induction of IFN\( \beta \) expression by \( L.\ monocytogenes \) is dependent on the expression of listeriolysin and the presence of bacteria in the cytoplasm of infected cells, however, the actual host molecules that recognise the intracellular pathogen and then signal to induce IFN\( \beta \) expression remain to be identified.
Conditional genome modification
As illustrated above, many signalling molecules have pleiotropic effects and participate in the regulation of multiple processes. Therefore, to understand the exact role of such molecules, it is important to be able to reduce the complexity of the system. Current recombinase-based technology facilitates this task by allowing a gene of interest to be deleted in a subset of cell types. This conditional gene modification relies on site-specific recombinases such as Cre from bacteriophage P1 or FLP from the yeast *Saccharomyces cerevisiae*. Generally, coding sequences of the gene of interest are flanked by specific sequences recognised by the recombinases and are introduced into the genome by standard gene-targeting approaches. The resulting mouse lines that carry such a modified locus are then crossed to the lines that express a recombinase from a tissue-specific promoter. The tissue-specific expression of the recombinase defines the cell types in which the gene of interest is deleted. This approach was recently used to define the *in vivo* cell-type requirement for TNFα production during the course of *L. monocytogenes* infection. This was done using a series of mouse lines in which TNFα inactivation was cell lineage restricted. Inactivation of the TNFα gene only in macrophages and neutrophils was sufficient for almost complete loss of resistance to *L. monocytogenes* infection, to the extent similar to that observed in TNFα<sup>−/−</sup> mice. Analysis of mice in which the TNFα gene was inactivated in T cells demonstrated the requirement of T cell-derived TNFα for protection against high bacterial loads. Therefore, this *in vivo* analysis of cell-type restricted knockouts demonstrated the non-redundant role of neutrophils, macrophages and T cells in TNFα production and defence against *L. monocytogenes* infection.

### Table 2: Effect of genomic alterations involving some of the immune signalling molecules on sensitivity to *Listeria monocytogenes* infections

<table>
<thead>
<tr>
<th>Gene Modification</th>
<th>Effect on <em>L. monocytogenes</em> infection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Signalling molecules</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN&lt;sup&gt;γ&lt;/sup&gt; Knockout</td>
<td>Extremely susceptible</td>
<td>36,37</td>
</tr>
<tr>
<td>TNFα Knockout</td>
<td>Very susceptible</td>
<td>29,30,38</td>
</tr>
<tr>
<td>IL-6 Knockout</td>
<td>Highly susceptible</td>
<td>39</td>
</tr>
<tr>
<td>IL-10 Knockout</td>
<td>Increased resistance</td>
<td>35</td>
</tr>
<tr>
<td>IL-12 Knockout</td>
<td>Highly susceptible</td>
<td>36</td>
</tr>
<tr>
<td>IL-18 Knockout</td>
<td>Moderately susceptible</td>
<td>36</td>
</tr>
<tr>
<td>IL-12/IL-18 Knockout</td>
<td>Extremely susceptible</td>
<td>36</td>
</tr>
<tr>
<td>IL-15 Transgene</td>
<td>Increased resistance</td>
<td>40</td>
</tr>
<tr>
<td>CCL-2/MCP-1 Knockout</td>
<td>Extremely susceptible</td>
<td>41</td>
</tr>
<tr>
<td>IL-1ra Knockout</td>
<td>Transgene</td>
<td>Increased resistance</td>
</tr>
<tr>
<td>C5 anaphylatoxin</td>
<td>2 base pair deletion/gene replacement</td>
<td>Dispensable or slightly increase sensitivity</td>
</tr>
<tr>
<td>CCL3/MIP-1α Knockout</td>
<td>Dispensable for innate response; decreased adaptive immunity</td>
<td>44</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN&lt;sup&gt;γ&lt;/sup&gt;R Knockout</td>
<td>Highly increased resistance</td>
<td>47,48</td>
</tr>
<tr>
<td>IFN&lt;sup&gt;A&lt;/sup&gt; -1/2 Knockout</td>
<td>Knockout</td>
<td>Highly increased resistance</td>
</tr>
<tr>
<td>TNF-alphaR Knockout</td>
<td>Very susceptible</td>
<td>31</td>
</tr>
<tr>
<td>IL-1RI Knockout</td>
<td>Increased susceptibility</td>
<td>42,50</td>
</tr>
<tr>
<td>CXCR2/mIL-8Rh Knockout</td>
<td>Knockout</td>
<td>Enhanced resistance at early stage, but develop chronic infection</td>
</tr>
<tr>
<td>CX3CL1/Fractalkine Knockout</td>
<td>Knockout</td>
<td>Dispensable</td>
</tr>
<tr>
<td>CCR2 Knockout</td>
<td>Knockout</td>
<td>Extremely susceptible</td>
</tr>
<tr>
<td>CCR5 Knockout</td>
<td>Knockout</td>
<td>Strain specific, dispensable or slight increase in susceptibility</td>
</tr>
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**Expression of TNFα in macrophages and neutrophils is required for resistance to *L. monocytogenes* infection**

The expression of TNFα in macrophages and neutrophils is required for resistance to *L. monocytogenes* infection.
Gene knock-ins
Deletion of a gene is an effective, but not always optimal, way to study its function. Complications range from lethality caused by removal of essential components, to misleading phenotypes due to activation of compensatory mechanisms and to lack of a detectable phenotype in case of redundant genes. In addition, it is likely that most of the genetic variation observed in humans is caused by allelic variation and not by null mutations. Therefore, it is important to analyse the effect of allelic changes on the studied phenotype. An example of such a study is the analysis of the effect of an allelic substitution in the gp130 cytokine receptor subunit. The gp130 subunit is shared by receptors of the IL-6 family of cytokines, and a homozygous gp130 knockout is embryonically lethal.58 The ability of gp130 to transduce signals was studied by substituting a tyrosine at position 759 within its SHP2 binding domain with a phenylalanine. This mutant form of the gp130 gene was then used to replace the endogenous copy of the gene. Resulting knock-in mice were significantly more sensitive to L. monocytogenes infection and produced low levels of IFN-γ.59 This observation indicated an important role for gp130 in the early phases of the immune response to L. monocytogenes infection.

All of the in vivo genome modifications that we have described above are performed within the context of the allelic make-up of the particular mouse strain selected for the experiment. Therefore, the observed effect of the same modification in different strain backgrounds could vary. Analysis of the role of the CCR5 in control of L. monocytogenes infection illustrates this point. CCR5 is one of the chemokine receptors that binds the RANTES cytokine and MIP-1α. and is the only receptor for MIP-1α. Analysis of the CCR5 knockout on a C57BL/6 background indicates that signalling through CCR5 is dispensable for innate and adaptive immune responses to L. monocytogenes;53 however, the study of CCR5-deficient ICR mice demonstrated tenfold higher L. monocytogenes titres in the livers of knockout mice than in wild-type controls. Moreover, there was a marked increase in the production of IFNγ, granulocyte-macrophage colony-stimulating factor and IL-4 by CCR5-deficient activated T cells.54 It is quite possible that the differences in the observed effect of the CCR5 deletion on the course of L. monocytogenes infection are due to strain-specific allelic differences in the molecules interacting with CCR5. This observation provides further support for the idea that to gain a complete understanding of immune signalling, one will need to analyse not just individual molecules but the whole interacting complex.

WHOLE-GENOME ANALYSIS
The emergence of novel tools for whole-genome analysis opens new ways to study the course and outcome of infectious diseases. Array-based expression analysis allows detection of the expression levels of all known genes at any given stage of infection. This alone provides an enormous amount of information. For example, array-based analysis of gene expression changes induced following infection of bone marrow macrophages with L. monocytogenes identified two major categories of infection-induced genes. The cluster termed ‘early/persistent’ consists of nuclear factor-κB-dependent transcriptional responses, and the ‘late’ cluster is composed of IFN-responsive genes.60 One of the most exciting recent developments, however, combines expression profiling with traditional genetic mapping. This approach allows identification of genetic polymorphisms that directly control gene expression. Such genetic polymorphisms and the resulting differences in gene expression can be used as anchors that will tie the expression-interaction maps to genomic sequence. In one of the first
attempts to perform comprehensive characterization of the genetic control of differential gene expression, progeny of C57BL/6J and DBA/2J strains were analysed using Affymetrix GeneChip technology.61 This approach was further refined by incorporating genetically defined RI mouse and rat strains in the expression-mapping analysis.62–64 RI strains are formed by sequential brother–sister mating of progeny of phenotypically different mouse strains. As a result of this brother–sister inbreeding, initial recombination events are fixed and all alleles become homozygous. This process results in a set of animals with randomly interspersed homozygous regions of parental alleles. Since each recombination event is fixed, the set can be used repeatedly for genetic mapping under a variety of conditions, reducing experimental error. Furthermore, a high-resolution map has already been built for several RI strains.65

In the authors’ laboratory, RNA expression profiles of the resistant C57BL/6ByJ and the sensitive BALB/cByJ animals are used to identify genetic lesions that control sensitivity to L. monocytogenes infection. This pair of differentially susceptible strains was used to create a panel of 13 CXB RI mouse strains.66 To map the locations of polymorphisms controlling differential gene expression, one matches the distribution of the differential gene expression values, obtained by real-time polymerase chain reaction in all of the 13 CXB strains, to their allelic pattern. When the pattern of expression values of a gene matches the pattern of parental alleles at its locus, it indicates that the controlling polymorphism is located in cis. This expression–mapping approach has already permitted the identification of a candidate gene in the Listr1 locus.

While modern gene expression profiling approaches provide a wealth of information, they still characterise only a subset of genetically determined changes induced by infectious disease, such as L. monocytogenes infection. The authors believe that the next step in genetic studies of mouse models of infectious diseases is incorporation of modern proteomic analysis. For example, serum proteomics is becoming a powerful tool for the study and diagnosis of various diseases.67 In the course of L. monocytogenes infection, most of the signals are carried through the bloodstream. Therefore, analysing the serum proteome throughout the course of infection in differentially susceptible mouse strains, and combining this analysis with mapping approaches, will expand researchers’ ability to understand the genetic control of susceptibility to infection.

The ubiquitous Gram-positive pathogen L. monocytogenes has been extensively used in experimental biology for over 40 years. Moreover, over the course of those years, the mouse model of L. monocytogenes infection continues to provide new insights into the biology of the mammalian immune system. The immune system relies heavily on cytokine/chemokine signalling networks that are complex and redundant. By combining modern whole-genome approaches with classical genetics, the authors have created new tools which can help to untangle the complex patterns of interactions required for an effective immune function. Because defence against L. monocytogenes infection relies heavily on signalling between components of the host immunity, the authors believe that this model of infectious disease is well suited to providing a framework for such a comprehensive analysis of interconnected signalling events.

References
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