The Role of Natural Killer Cells in the Defense against *Listeria monocytogenes* Lessons from a Rat Model

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Key Words

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Abstract

Ly49 receptors in rodents, like killer cell immunoglobulin-like receptors in humans, regulate natural killer (NK) cell activity. Although inhibitory Ly49 receptors clearly recognize classical major histocompatibility complex class I (MHC-I) molecules, the role for the activating Ly49 receptors has been less well understood. Here, we discuss recent data from a rat model for listeriosis. Rats depleted of NK cells, or more specifically the Ly49 receptor-bearing cells, showed increased bacterial loads in their spleen. Athymic nude rats with no functional T cells but increased numbers of Ly49-expressing NK cells were more resistant to infection, indicating a central role of NK cells in early immune defense against *Listeria* in this species. *Listeria* infection of macrophages or enteric epithelial cells led to upregulation of MHC-I, including nonclassical (Ib) molecules not regularly recognized by T cells. We have shown that activating Ly49 receptors are more efficiently stimulated when binding to upregulated class Ib antigens on infected cells. From this we postulate that activating

Ly49 receptors may have a sentinel function in the early immune response against *Listeria* in detecting diseased cells 'flagged' by increased MHC-Ib expression.

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The Bacterium

Listeria monocytogenes is a facultative intracellular bacterium that was independently discovered in 1926 by a research group in the United Kingdom and nearly concurrently by a group in South Africa [1]. *Listeria* is a rod-shaped Gram-positive bacterium that has the ability to transform from a saprophytic to an intracellular life, and therefore, can live in e.g. soil but also as an intracellular parasite in several animal species [2]. The genome size of *L. monocytogenes* is approximately 3 million base pairs, including 2,853 protein-coding genes – amongst them 133 surface proteins, 68 lipoproteins and 11 hydrophobic tail proteins [1, 3]. *Listeria* is able to proliferate in temperatures ranging from 4 to 37°C and it encodes thermoregulated virulence genes. These genes are controlled by the transcriptional activator PrfA working optimally at 37°C, which is a key factor for the transition from the saprophytic to the intracellular stage [2].

Listeria Infections in Humans

Listeria is widespread in nature, but seldom causes clinical infections in healthy individuals apart from occasional disease outbreaks. Although *Listeria* infection is rare (7 per 1,000,000 healthy people each year), the overall mortality rate of systemic infection is high (approximately 25%) compared to other pathogens such as *Salmonella* (<1%) [3, 4]. *Listeria* may cause sepsis and spread to the nervous system causing cranial nerve palsies, encephalitis, abscesses or meningitis. Since *Listeria* infections are relatively rare in humans, it represents little threat to the population as a whole. However, immunocompromized individuals such as transplanted patients or persons with AIDS are particularly vulnerable. Furthermore, pregnant women are at risk, since *Listeria* can infect fetuses, particularly in the third trimester, by penetrating the endothelial layer of the placenta. This may lead to premature labor, stillbirth, abortions and neonatal infections [5].

Since there are no vaccines against *Listeria*, knowledge about the mode of infection, virulence factors, spread of the bacterium and putative immune responses against it are important factors when considering preventive measurements for patients at risk. Humans are often exposed to *Listeria* through contaminated food products, such as unpasteurized dairy products, incompletely cooked meats or vegetables contaminated from soil. Also, animals may be carriers of *Listeria* without having symptoms of infection [1, 6]. Since clinical infection usually is sporadic rather than epidemic in humans, it is difficult to identify immunogenetic factors that predispose to resistance or susceptibility. The strategies *Listeria* use to infect host cells have largely been identified in animal models. Below, we describe some mechanisms that *Listeria* uses to evade host defense mechanisms, bearing in mind that there may be considerable species variations.

Mechanisms by Which *Listeria* Infects Cells and Evades the Immune System

Listeria is usually eliminated by the immune system in the gut. The cells and secreted substances that contribute to this defense are not well described, except that CD8⁺ T cells in the epithelium may play a role [1]. However, bacteria that escape these initial defense mechanisms may give rise to serious systemic infections. *Listeria* primarily infects macrophages (M ϕ), dendritic cells (DCs), hepatocytes and intestinal epithelial cells, but fibroblasts, endo-

thelial cells, neurons and even lymphocytes including natural killer (NK) cells may be susceptible [7, 8]. *Listeria* is taken up by M ϕ by binding to polysaccharide receptors or type I M ϕ scavenger receptors [4, 6]. Uptake in intestinal epithelial cells is dependent on interactions between *Listeria* surface protein internalin A and epithelial cadherin (E-cadherin) [1]. After internalization, *Listeria* must be able to escape from the vacuole/phagosome before fusion with lysosomes in order to survive. This key process is dependent on listeriolysin O, an enzyme that lyses the endosomal membrane and is a central virulence factor for *Listeria* [8]. The escape from phagosomes is enhanced by bacterial phospholipase C [9]. Listeriolysin O also induces apoptosis of activated lymphocytes. Once in the cytosol, *Listeria* uses the cellular machinery to move around. It induces directed polymerization of actin by the actin assembly-inducing protein, thus pushing itself through the cytoplasm. In the same manner, the bacterium moves from cell to cell covered by a layer of the host cell membrane protecting it from extracellular immune defense mechanisms such as acute phase proteins, complement factors and antibodies. The innate and adaptive immune mechanisms that protect infected individuals from disease are incompletely understood [10]. Especially, the role of *Listeria*-derived peptides has been poorly investigated in humans. Several animal experimental models have been developed to study *Listeria* infections. The mouse has been widely utilized, although there are several fundamental differences between rodents and man in how the bacterium is internalized in the intestine and the mode of spread to various organs [5]. Therefore, extrapolation from animal models to man should be done with caution.

Immune Responses to *Listeria* in Mice

The mouse has been used as a model organism for studying interactions between *Listeria* and the immune system [1, 11]. The first evidence that cellular immune responses were instrumental in resistance to *Listeria* came from adoptive transfer of lymphocytes from infected to naive mice [12, 13]. Interestingly, the ability of *Listeria* to induce potent innate and adaptive immune responses has been exploited in cancer immunotherapy [14, 15].

Innate immune responses are rapidly triggered upon *Listeria* infections and are essential for host survival [1, 16]. Within the first 24 h after infection, neutrophils gather at the infection site [17, 18]. They migrate from the bone

marrow into the liver and spleen, which are the primary sites of *Listeria* growth upon systemic infections. Here, the neutrophils contribute to bacterial killing and destruction of infected cells. Neutrophil depletion studies have shown that these cells are important in the earliest phase of the infection [1, 19]. In the spleen, infection of M ϕ in the marginal zones induces secretion of the monocyte chemotactic protein 1, which in turn induces a CCR2-dependent recruitment of specialized monocytes from the bone marrow. These monocytes differentiate into tumor necrosis factor (TNF)- α and inducible nitric oxide synthase-producing DCs, called 'TipDCs' [20, 21]. Mice deficient in CCR2 are more susceptible to *Listeria* growth in the spleen which may be the result of a lack of TipDCs in the spleen [20–22]. NK cells represent an important source of interferon (IFN)- γ production at infectious loci containing infected M ϕ and TipDCs [23]. The exact mechanism by which NK cells are recruited here is unclear, but may partly result from a CCR5-dependent response to M ϕ inflammatory proteins 1 α and 1 β secreted by activated M ϕ [23]. It is unclear whether the NK cells derive from local spleen cells, or are recruited from the bone marrow [24]. Although IFN- γ production by NK cells contributes to the innate immune resistance, memory cytotoxic T lymphocytes (CTLs) seem to be the dominant IFN- γ producers in mice [25]. Recent studies have shown the importance of antigen-presenting cells in controlling both the innate and adaptive immune responses to *Listeria* [23, 26]. Although innate immune responses may limit the infection as shown in severely combined immunodeficient mice, it is clear that long-term sterilizing immunity is dependent on an intact adaptive immune system [27].

M ϕ are activated at the site of infection, and this is necessary for their ability to kill *Listeria*. How activated M ϕ kill *Listeria* is not entirely clear, but the generation of reactive nitrogen and oxygen metabolites is thought to be central [11]. M ϕ produce TNF- α and interleukin (IL)-12 necessary for NK cell activation and IFN- γ secretion. In addition, activated DCs produce IL-12 and IL-18 that may also stimulate NK cells [28]. IFN- γ mediates multiple effects including activation of M ϕ . Its importance is evident in mice lacking the IFN- γ receptor, which are highly susceptible to *Listeria* infection [29]. Conversely, type I IFNs, important in the viral defense, have been reported to impair antibacterial responses [30–32], although recent experiments have shown that type I IFNs may have protective effects in the absence of MyD88-mediated signaling [33]. Direct antibacterial activity by NK cells has also been shown, although the mechanisms are unclear

[34, 35]. TNF- α is another cytokine essential for primary resistance to *Listeria* [23].

DCs and M ϕ present *Listeria* peptides to T cells [17]. DCs are required for priming of naive *Listeria*-specific CD8⁺ T cells [14], an ability which M ϕ do not have [36]. CTL responses against cells presenting *Listeria* peptides on either MHC-Ia or MHC-Ib molecules contribute to resistance to this pathogen [1]. Class Ia molecules can present a range of *Listeria*-derived peptides generated by proteasome degradation of *Listeria*-derived proteins in the cytosol [1]. CTL responses to listeriolysin O and p60 peptides have been well characterized. Of the class Ib molecules, particular emphasis has been put on H2-M3. This MHC-Ib molecule promiscuously presents a range of formylated peptides from bacteria to CTLs and may identify cells infected with a wide range of bacteria that are dependent on formylated methionin for protein synthesis [1]. These two modes of CTL recognition may be temporally separated. The class Ib response may precede the class Ia response and has more of the feature of a nonadaptive immune response with little immunological memory, while the class-Ia-restricted CTLs predominate in the secondary memory response to *Listeria* and bring along sterilizing immunity [1]. However, these mechanisms do not account for all resistance connected to MHC-I presentation of *Listeria* peptides, since MHC-Ia-deficient mice are able to mount protective immune responses to formyltransferase-deficient *Listeria* strains [37, 38]. However, the MHC-Ib molecules conferring this resistance have not been identified. In the spirit of the next section we would suggest that these class Ib molecules might be ligands for MHC-I receptors on NK cells. Furthermore, there is evidence that most CTLs recruited to the site of infection are not specific for *Listeria* peptides. However, they may produce large amounts of IFN- γ important for the clearance of the bacterium [39, 40].

NK cells produce IFN- γ and may be important for the early defense against *Listeria* [41]. However, the role of NK cells has been subject to controversies, as both beneficial and detrimental effects of NK cells have been observed. Mice deficient in DAP12, necessary for signaling through many activating receptors on NK and myeloid cells [42, 43], and mice deficient in type I IFN production important for activation of NK cells [32] were more resistant to *Listeria*. Furthermore, NK1.1⁺ cells, being mostly NK cells, have been shown to impede resistance to *Listeria* [44]. Finally, NK cells may produce the anti-inflammatory cytokine IL-10 during systemic *Listeria* infections [45, 46]. These controversies may depend on the ex-

Fig. 1. A schematic view of the rat MHC (RT1). Ia = Classical class I genes; Ib = nonclassical class I genes. Note the similarities with mouse H2 (not shown) in the overall organization of the MHC, except that H2D/L genes are lacking in the rat. The class Ia region may contain 1, 2 or 3 loci depending on the rat strain. The C/E region also varies greatly in gene content between rat strains.

perimental systems studied, and *Listeria* models in other species may help understand these discrepancies.

In rodent infection models, *Listeria* is usually given intravenously since rodents cannot easily be infected via the oral route. Even with high doses of bacteria, oral infection seldom leads to lethality [5]. It has been discovered that mouse E-cadherin is unable to interact with internalin A due to the amino acid [47] at position 16 in E-cadherin (glutamic acid in mice, proline in humans). This probably explains why rodents might be carriers of the bacteria without getting systemic infections.

Listeria Models in the Rat

Early studies in the rat showed that T cells from *Listeria*-infected rats conferred resistance when transferred to naive hosts, and this resistance showed MHC restriction [48]. However, until recently, the role of the innate immune system has been given little attention.

We caught an interest in the role of NK cells in resistance to *Listeria* since infection of Mφ and intestinal epithelial cells led to an increased MHC-I expression, especially of nonclassical class I, that could conceivably influence NK cell recognition. We have previously shown that some nonclassical MHC-I molecules are ligands for NK cell receptors. These molecules are encoded by a string of genes telomeric of the class II/III region (fig. 1) [49, 50]. Based on immunogenetic and functional studies, the ligands for several activating and inhibitory Ly49 receptors have been mapped to the proximal part of this nonclassical class I region, termed 'RT1-CE' (fig. 1) [51–55]. Rat as opposed to mouse NK cells protect against *Listeria* infection in vivo [56] and are also stimulated by *Listeria*-in-

fected cells in vitro [57, 58]. The protective effect of NK cells in rats may be related to the relatively large number of Ly49 receptors in rats as compared to mice [59]. The rat MHC-I region is also organized differently from the mouse as described in the next section.

The Rat MHC (RT1) Complex

The whole genome of the Brown Norway (BN) rat strain was sequenced in 2004 [60]. The RT1 complex of BN rats consists of 4 Mb and contains at least 220 genes [50]. The MHC-I molecules can be subdivided into classical (Ia) and nonclassical (Ib) molecules. They vary in expression patterns, polymorphisms and functions. The classical MHC molecules are encoded from the RT1-A region in the left flank of the MHC, while the nonclassical ones are encoded from the RT1-CE, RT1-N, RT1-M1, RT1-M4, RT1-M3A, RT1-M3B and RT1-M2 regions telomeric to the class II/III region (fig. 1) [50]. The centromeric class Ia region (RT1-A) and the first telomeric class Ib gene cluster (RT1-CE) located on either side of the central MHC class II/III region show the largest degree of homology with the mouse, but H2-D/L orthologues do not exist in the rat [61]. However, both these regions contain genes that encode ligands for NK cell receptors. The classical RT1-A region contains one, two or three MHC-I genes depending on the haplotype. For instance, the RT1^k and RT1^u haplotypes contain one, RT1^c two and RT1ⁿ three expressed MHC-Ia genes [62–65]. Rat NK cell receptors may show exquisite specificity for certain class I alleles. As an example, the inhibitory rat Ly49i2 NK cell receptor recognizes only one of the RT1-A molecules in the PVG strain (RT1-A1^c) [53, 66].

Fig. 2. Schematic presentation of the rat NKC. Note that considerable polymorphisms exist, especially in the *Ly49* region, between different rat strains.

The *RT1-CE* region contains 16 nonclassical Ib genes in the *RT1^u* haplotype [50, 67]. Thirteen of these are probably expressed [50]. Other haplotypes may express different numbers of *RT1-CE* genes and molecules, so it is difficult to define alleles within this region. The function of these nonclassical MHC-I molecules has not been well characterized. They are less polymorphic than classical class I molecules and often have a restricted tissue distribution and low surface expression. However, unlike the stress-induced ligands for the NKG2D receptor (MICA in humans and RAE in mice), the *RT1-CE* molecules are clearly expressed, also on normal unstressed cells like hematopoietic cells. Some *RT1-CE* molecules can present endogenous peptides recognized by CTLs [68]. *Ly49* receptors have been shown to recognize MHC-I-encoded molecules both from the *RT1-A* and *RT1-CE* regions [51–55]. The second telomeric class I gene cluster, *RT1-N*, also contains 16 class Ib genes in the BN rat. There are close similarities between the mouse and rat M region class I genes. The M3 molecule, being orthologous to the mouse H2-M3, probably presents N-formylated peptides to CD8⁺ T cells; however, its role in resistance to infections in rats remains to be determined. The framework gene *mog* marks the telomeric border of human leukocyte antigen, but the border of the *RT1* complex extends further distally. The class III (also called ‘central’) region encodes a diverse array of structurally unrelated genes both in mice and rats, including complement factors, *TNF*, *Hsp70* and *TAP*, many of which are involved in the innate and adaptive immune responses [50].

The NK Cell Gene Complex

The human, mouse and rat NK cell gene complexes (NKC) are located on chromosomes 12, 6 and 4, respectively. The murine NKC encodes lectin-like molecules that can be classified into families of highly homologous members. An overview of the rat NKC is depicted in figure 2. These receptors are encoded by clusters of closely linked genes that usually are separated from the other

gene families. One exception are the intertwined *Nkrp1* and *Clr* (C-type lectin related) genes [69]. Between families, the receptors share some general structural features but display limited homology [70]. The rat NKC spans from the *Nkrp1a* to the *Ly49i8* gene (fig. 2) [71]. The BN rat NKC contains about 67 killer cell lectin-like receptor genes. Of particular interest is the telomeric region encoding *Ly49* receptors. The number of *Ly49* genes differs widely between NKC haplotypes. As a general rule, with reservations about the limited number of rat and mouse strains so far investigated, the rat NKC seems to contain more *Ly49* genes than the mouse [59]. As opposed to the mouse, many of these *Ly49* receptors contain a charged amino acid in the transmembrane region and are predicted to have an activating function.

Rat *Ly49* Receptors Recognize Both Classical and Nonclassical MHC-I Molecules

In rats, *Ly49* receptors recognize both classical and nonclassical MHC-I molecules. Previous studies have suggested that both activating and inhibitory rat *Ly49* receptors recognize polymorphic ligands encoded within the *RT1-CE* region [72]. Inhibitory *Ly49* receptors additionally bind MHC-Ia molecules. Based on the genomic sequences of BN rats, the rat NKC contains 13 inhibitory and 8 activating *Ly49* receptors [59]. The *Ly49* stimulatory receptor 3 (*Ly49s3*) was the first activating receptor to be characterized [54]. It recognizes nonclassical MHC-I ligands and is present in the high NK alloresponder rat strains PVG and AO, but not in low alloresponder rat strains such as BN and DA. *Ly49s3* surface expression is markedly downmodulated in the presence of ligand(s) [73]. The *Ly49i5* inhibitory receptor recognizes ligands encoded within the nonclassical class I region of the *RT1^u* and *RT1^l* haplotypes, while the structurally related stimulatory *Ly49s5* receptor recognizes ligands from the *RT1^u* haplotype. *Ly49s5* is expressed in the high NK alloresponder PVG strain, but not in low alloresponder strains [55].

Listeria Infection in Rats – The Role of NK Cells

Rats depleted of NK cells with the pan-NK monoclonal antibody 3.2.3 towards NKR-P1A (the rat homologue of mouse NKR-P1C) showed increased bacterial loads in the spleen after system administration of the bacterium [56]. Similar observations were made when a subset of NK cells encompassing the majority of Ly49 receptor-bearing cells [56] (termed 'Ly49s3⁺ NK cells') [73, 74] was depleted. These experiments suggested that NK cells were involved in the resistance to *Listeria*. Experiments with athymic rats, lacking functional T cells but with increased number of NK cells [75], further confirmed this finding. These rats resisted *Listeria* more efficiently than normal rats, i.e. the bacteria had been cleared from the spleen already at day 3 after infection. This was somewhat surprising, since the innate immune system does not induce complete clearance of the bacteria in severely combined immunodeficient mice. However, there are significant differences between mice and rats in the repertoire of Ly49 receptors. Especially the large repertoire of activating Ly49 receptors in PVG rats [54] and the increased frequency of Ly49 receptor-bearing NK cells in athymic nude rats derived from PVG [76] [unpubl. observations] may explain this dichotomy.

The specific role played by Ly49s3⁺ NK cells in the resistance to *Listeria* has not yet been pinpointed, but an expansion of this subset in the spleen during *Listeria* infection has been observed [24, 56], which may result from either a local expansion or direct recruitment from other tissues. However, DA rats that lack most Ly49 receptors [77] cleared *Listeria* as efficiently as did PVG rats [56], suggesting that Ly49-independent resistance mechanisms exist. Other NK cell receptors, such as NKG2D, may contribute by recognizing stress-induced ligands on *Listeria*-infected cells as shown in mice [78]. That Ly49 receptors are not the sole innate receptors contributing to *Listeria* resistance was further confirmed in experiments where both Ly49s3⁺ and Ly49s3⁻ NK cells responded to *Listeria*-infected cells in vivo with IFN- γ production [57].

For further studies of the role of Ly49 receptors in *Listeria* resistance in vitro, two rat cell lines have been used: R2 M ϕ [58] of the RT1^d haplotype and CC531 colon carcinoma epithelial cells (RT1^u haplotype) [57]. In contrast to normal cells that are easily destroyed by *Listeria* infection, infection of these lines was easily achievable. In vitro infection of the cells increased the expression of both MHC-Ia and MHC-Ib molecules [57, 58]. This is in contrast to infection with other intracellular pathogens like

cytomegalovirus, which either downregulate MHC-I or instruct the infected cell to express 'decoy' MHC-I-like molecules that inhibit NK cells.

In vitro experiments with reporter cell lines, expressing Ly49s5, Ly49s4 or Ly49i4, strongly indicated that increased expression of RT1-CE molecules on infected cells may be detected by Ly49 receptors since blocking with antibodies against RT1-CE-encoded molecules also blocked the interaction with the cognate stimulatory or inhibitory Ly49 receptor [57, 58]. However, since each NK cell expresses several stimulatory and inhibitory NK cell receptors, the overall result of NK cell recognition of a target cell will depend on several NK cell receptor MHC-I interactions [79]. The Ly49s4 and Ly49i4 receptors recognize the same ligand(s). Often signals generated by inhibitory NK cell receptors dominate over their stimulatory counterparts. However, if the activating receptors for a given MHC ligand are expressed by different NK cell subsets than their inhibitory counterparts, the chance that activation is overridden by inhibitory receptors is minimized. This is the case for NK cells expressing Ly49s5 and Ly49i5 present on different subpopulations of NK cells [55]. Although it remains to be proven, presumably also Ly49s4 and Ly49i4 may be expressed by different subsets of NK cells. In vitro IFN- γ stimulation of CC531 and R2 cells led to increased MHC-I expression and enhanced Ly49 receptor responses similarly to *Listeria*-infected cells. Taken together, this suggests that the increased receptor response was caused by a general upregulation of MHC-Ib whether induced by *Listeria* or cytokines.

The upregulation of MHC-I molecules on infected cells both in vitro and in vivo indicates that the in vitro data are relevant for interpretation of the in vivo data. Incubation of bulk spleen mononuclear cells (including NK cells) with *Listeria*-infected cells (R2 and CC531) induced IFN- γ production in NK cells. However, this did not point to an independent role of NK cells, as no IFN- γ production was observed in parallel experiments with sorted NK cells. This suggests that other factors are needed for NK cell activation. Apparently, other cells, such as DCs, may be required for NK cell activation and production of IFN- γ , as has been shown in the mouse. Supernatants from *Listeria*-infected cells did not induce any IFN- γ response [57] showing that soluble factors alone were incapable of activating NK cells and that direct contact with accessory cells may be required for IFN- γ production by NK cells. It has been suggested that full activation of NK cells requires priming, cytokines and contact with antigen-presenting cells [28, 80].

A general upregulation of MHC-Ib molecules may act as a 'danger' signal for NK cell recognition. IFN- γ stimulation of R2 cells or CC531 cells upregulated their MHC-I expression similarly to *Listeria* and also led to increased NK cell receptor responses. Therefore, a general upregulation of nonclassical MHC-I, whether induced by microbial infection or IFN- γ , was sufficient to explain the results obtained with the reporter cells. However, R2 and CC531 cells do not themselves produce significant amounts of IFN- γ , suggesting that in vitro infection with *Listeria* upregulates MHC-I independently of IFN- γ . The situation may be different in vivo, where bystander cells may produce enough IFN- γ for upregulation to occur [55]. The precise role that Ly49 receptor interactions with MHC-I plays in bacterial resistance in vivo also remains to be determined. The depletion experiments with either the pan-NK antibody 3.2.3 or the monoclonal antibody DAR 13 reacting only with the NK cell subset expressing Ly49 were highly suggestive, but did not prove that Ly49 receptors were instrumental for protection. Experiments investigating the effect of NK cell depletion in rats deficient in Ly49 expressing NK cells still await to be done.

A Novel Role of MHC-Ib in Ly49 Recognition – Future Aspects

The *Listeria* model in rats has given the opportunity to gain new knowledge about putative novel functions of nonclassical MHC-I molecules. As already mentioned, expression of stress-induced NKG2D ligands may lead to T and NK cell activation [81, 82]. The upregulation of MHC-Ib molecules on *Listeria*-infected cells may show some mechanistic similarity to the NKG2D ligands up-

regulated during stress or infection and act as 'danger' signals. This is also supported by the fact that MHC-Ib molecules in the rat are usually not targets for CTL responses. Their upregulation during infection may lead to recognition and killing by NK cells expressing the cognate-activating receptor. The mechanism of upregulation is still enigmatic: whether it is only cytokine driven (IFN- γ) or might be dependent on more sophisticated mechanisms such as *Listeria*-derived peptides remains to be determined.

Altogether, data from the rat model suggest that NK cells play an important role in the early defense against *Listeria* infection. NK cells may directly recognize infected cells through the interaction between Ly49 receptors and their MHC-I ligands, or by other receptors for ligands which are upregulated on *Listeria*-infected cells. A complex organism like *Listeria* obviously requires activation of several effector mechanisms of the immune system to be effectively eliminated. NK cell recognition of MHC-Ib molecules on *Listeria*-infected cells may contribute to the defense at an early stage of the infection. However, it should be kept in mind that activation of resting NK cells may require synergy between multiple receptors present on a single cell [83], and therefore, it would be of interest to investigate if activation of other NK cell receptors like NKG2/CD94, NKG2D and NKp46 is needed for NK cell recognition of infected cells through Ly49 receptors.

Although clinical *Listeria* infections are relatively rare in the population and present no threat as pandemics, the lessons to be learned from immunity to *Listeria* may also apply to other intracellular bacteria and give new insight into the multiple mechanisms that the immune system employs to combat such infections.

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