INFECTION AND IMMUNITY, Jan. 2002, p. 416–418 Vol. 70, No. 1
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.1.416–418.2002

STK Receptor Tyrosine Kinase Regulates Susceptibility to Infection with *Listeria monocytogenes*

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Received 15 June 2001/Returned for modification 23 August 2001/Accepted 4 October 2001

We have previously identified the STK receptor tyrosine kinase as a key regulator of macrophage activation and cell-mediated immune responses. Here we demonstrate that, although MSP activation of STK inhibits NO production by macrophages in response to heat-killed *Listeria monocytogenes,* STK-deficient mice exhibit increased susceptibility to infection with *Listeria*.

The STK receptor tyrosine kinase (15, 23), a member of the MET family of RTKs, is expressed on a variety of epithelial cell populations, hematopoietic progenitor cells, and tissue-resident macrophages (15, 16, 22). STK is the murine homologue of the human RON receptor and the ligand for STK, as well as RON, is macrophage-stimulating protein (MSP) (31). MSP is expressed primarily in the liver (6), where it is synthesized and secreted as an inactive, single-chain precursor. This pro-MSP can be activated by proteolytic conversion to a heterodimer by enzymes of the coagulation cascade (32). Stimulation of peritoneal macrophages by MSP results in shape change, chemotaxis, and phagocytosis (27, 28). Moreover, MSP has been shown to suppress nitric oxide (NO) production by activated peritoneal macrophages in vitro (30). The ability of MSP to inhibit NO production by macrophages in response to cytokines and lipopolysaccharide (LPS) has been shown to require phosphatidylinositol 3-kinase (1) and is associated with decreased transcriptional activity of IRF-1, resulting in decreased expression of inducible nitric oxide synthase (iNOS) (17).

Knockout studies have suggested a role for STK in placental and ovarian development (19, 29). Furthermore, mice with a targeted deletion in exon 1 of the gene encoding STK are resistant to Friend virus-induced erythroleukemia, confirming the identity of STK as the Friend virus susceptibility gene, *Fv2* (20). Previously, we and others have shown that activated macrophages from STK-deficient mice (3, 19, 29) produce elevated levels of NO, both in vitro and in vivo, rendering them more susceptible to endotoxic shock. Based on these observations, we proposed a model in which MSP becomes activated after LPS administration, thereby activating STK signaling in macrophages and limiting the tissue damage associated with septic shock by attenuating NO synthesis.

While MSP has been shown to inhibit NO production by peritoneal macrophages in response to stimulation with gamma interferon (IFN-γ) and LPS, the ability of MSP to regulate NO production in response to gram-positive bacteria has not been previously demonstrated. In order to determine whether MSP inhibits NO production in response to *Listeria* spp., we harvested primary peritoneal macrophages from wild-type and STK-deficient mice and stimulated them with 100 U of IFN-γ/ml plus heat-killed *Listeria monocytogenes* (HKLM) (50 bacteria/macrophage), in the presence or absence of 100 ng of MSP/ml. After 36 h, the levels of NO in the supernatant were assayed by reacting 100 μl of supernatant with an equal volume of Greiss reagent as described previously (3). MSP alone has no effect on the production of NO (data not shown). However, the data from these studies demonstrate that, in addition to its role in regulating NO production in response to LPS, MSP inhibits NO production in response to IFN-γ plus HKLM (Fig. 1A) and that this regulation requires the STK receptor.

To determine whether the ability of STK to suppress NO production in response to HKLM would result in increased resistance to infection in mice with a targeted mutation in STK, we challenged the $STK^{-/-}$ mice and their control littermates with *L. monocytogenes*. One day prior to inoculation, serial 1:10 dilutions of *L. monocytogenes* EDG were grown in Trypticase soy broth at 37°C in shaking cultures (125 rpm) for 15 h. On the day of infection, the optical densities of the *Listeria* dilutions were determined, and an infectious dose of 4.82×10^5 in 0.2 ml of phosphate-buffered saline (PBS) was administered by tail vein injection. $STK^{-/-}$ mice exhibited increased susceptibility to infection with 2×10^5 to 4×10^5 *Listeria* organisms compared to their littermate controls (Fig. 1B). The mortality in these animals occurred primarily between days 3 and 4 after the initial infection, corresponding to the time that T-cell-mediated immunity is triggered.

To determine the bacterial load, the livers and spleens were homogenized on day 3 or 4 after infection, in 8 and 9 ml of PBS, respectively, for 30 s at speed 5. Serial dilutions of the homogenate were plated on Trypticase soy agar, and the number of bacteria/organ was determined by colony counts. As shown in Table 1, the increased susceptibility of the STK-deficient mice to 2×10^5 to 4×10^5 *L. monocytogenes* was accompanied by an increase in the bacterial load in livers, but not the spleens, of these animals on day 3. However, the

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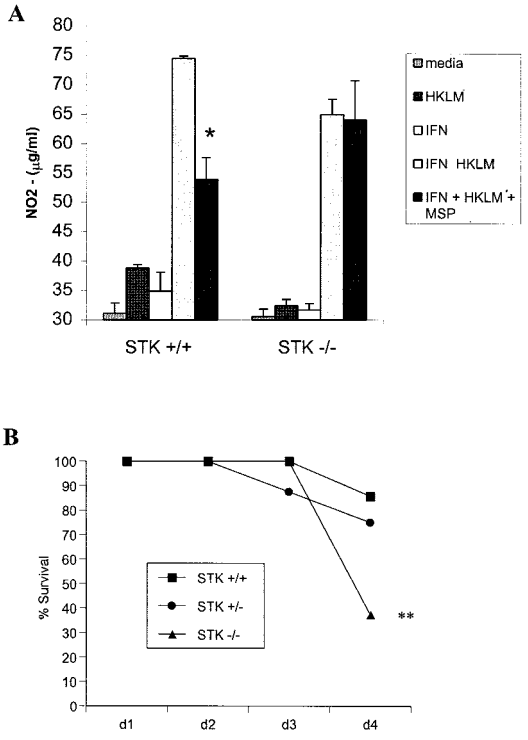

FIG. 1. Increased susceptibility of $STK^{-/-}$ mice to infection with *L. monocytogenes.* (A) Resident peritoneal macrophages from $STK^{+/+}$ and $STK^{-/-}$ mice were treated with HKLM in the presence or absence of 100 U of IFN-γ and 100 ng of MSP/ml. After 36 h of stimulation, the levels of NO in the supernatant were assayed by using Greiss reagent. (B) $STK^{+/+}$ ($n = 7$), $STK^{+/-}$ ($n = 8$), and $STK^{-/-}$ ($n = 8$) mice were injected intravenously with an infectious dose of 2×10^5 to 4×10^5 *L. monocytogenes*, and the survival of these animals was monitored for 4 days. ✱, $P < 0.01$; ✱✱, $P < 0.02$.

bacterial burden in the livers of surviving STK-deficient animals on day 4 was indistinguishable from that of wild-type controls. These data suggest that the difference in susceptibility may lie in the innate ability of macrophages to maintain the bacterial load at a level which is not lethal until the onset of T-cell-mediated events. At a lower *Listeria* dose (1.3×10^4), we failed to observe a statistically significant difference in the bacterial burden in either the livers or the spleens of STK-deficient mice.

Targeted mutations in a number of genes involved in the regulation of NO production also affect susceptibility to infection with *Listeria.* Mutations in genes that encode members of the IFN-γ (14, 18) and tumor necrosis factor alpha (TNF-α) pathways (21, 24, 25) render mice more susceptible to *Listeria*. Both of these signaling pathways appear to be essential for optimal activation of iNOS expression and subsequent NO production by macrophages. Furthermore, disruption of the iNOS gene itself renders mice more susceptible to infection with *Listeria* (33), highlighting the pivotal role for NO in the immune response to *Listeria*. The observation that the STK-deficient mice are more susceptible to infection with *Listeria*, despite the role of STK in limiting NO production, suggests that the enhanced sensitivity of these mice to *Listeria* could be secondary to increased inflammatory damage. Alternatively, the STK-deficient mice may harbor a defect in other pathways essential for efficient bacterial clearance.

Interleukin-6-deficient mice are more susceptible to *Listeria* due to inefficient neutrophilia (5), and mice depleted for neutrophils exhibit exacerbated listeriosis in the liver but not in the spleen or peritoneal cavity (2). These data suggest a crucial role for neutrophils in the hepatic cell-mediated immune response to *Listeria*. Recent studies have also highlighted the role of reactive oxygen intermediates in the course of listeriosis (7, 10). In addition, studies utilizing the TNF receptor p55 knockout mice have found that, despite normal leukocyte recruitment, inflammatory cytokine production, nitric oxide synthesis, and oxidative burst formation, these mice are highly susceptible to *Listeria* infection, suggesting a role for NO- and reactive oxygen intermediate-independent mechanisms of killing (9). Furthermore, IFN-γ treatment of peritoneal macrophages has been shown to restrict listerial growth due to limited escape of the *Listeria* from the phagocytic vacuole by a reactive nitrogen-independent mechanism (4). Therefore, antibacterial effector mechanisms other than NO could be targets of the MSP/STK signaling pathway.

In addition to a potential role in the cytotoxic response of macrophages to infection, STK could also regulate susceptibility to *Listeria* by influencing the uptake of *Listeria* by host cells. MSP has been shown to promote phagocytosis of C3bi-coated sheep erythrocytes through the CR3 receptor (27). We have shown that the STK receptor associates with CR3 and that MSP-induced CR3-mediated phagocytosis requires the STK receptor (M. A. Lutz and P. H. Correll, unpublished data). *Listeria* that are phagocytosed by macrophages via the CR3 receptor are readily killed in the absence of inflammatory mediators (8). Furthermore, we have recently demonstrated upregulation of scavenger receptor A (SR-A) by primary peritoneal macrophages in response to MSP (A. C. Morrison and P. H. Correll, unpublished data). SR-A knockout mice are more susceptible to endotoxic shock (13) and infection with *Listeria* (8), a phenotype strikingly similar to the phenotype of the STK-deficient mice. In addition to traditional routes of entry, the MET receptor has recently been shown to mediate uptake of *Listeria* by hepatocytes and other epithelial cells by an InB-dependent mechanism (26). *Listeria* lacking InB fail to effectively colonize the liver, and STK has recently been shown to interact with the MET receptor (11, 12). Therefore, the possibility exists that STK regulates the uptake of *Listeria* by hepatocytes through interactions with MET. Future studies

TABLE 1. Increased bacterial burden in the livers of $STK^{-/-}$ mice[a]

Dose	Day	Organ	Mean bacterial burden ± SD (n) in:		
			$STK^{+/+}$ mice	$STK^{+/-}$ mice	$STK^{-/-}$ mice
2×10^5–4×10^5	3	Liver	6.8 ± 1.4 (10)	7.2 ± 1.0 (11)	8.4 ± 1.1* (10)
		Spleen	7.1 ± 0.7 (10)	7.3 ± 0.6 (11)	7.5 ± 0.5 (10)
	4	Liver	5.8 ± 1.3 (8)	6.1 ± 1.7 (10)	6.2 ± 1.0 (6)
		Spleen	6.5 ± 0.9 (8)	6.6 ± 1.1 (10)	7.1 ± 0.7 (6)
1.3×10^4	3	Liver	4.71 ± 1.91 (8)	5.04 ± 1.90 (8)	5.22 ± 1.60 (7)
		Spleen	6.04 ± 1.15 (8)	5.59 ± 1.25 (8)	5.82 ± 1.31 (7)

[a] $STK^{+/+}$, $STK^{+/-}$, and $STK^{-/-}$ mice were injected intravenously with an infectious dose of *L. monocytogenes*. The bacterial burden ($\log_{10}$/organ) in the liver and spleen was determined on days 3 and 4 after infection. *, $P < 0.01$.

will be aimed at further delineating the mechanism by which STK regulates the host response to infection with *Listeria*.

This work was supported by Public Health Service grant AI43367 from the National Institute of Allergy and Infectious Disease (P.H.C.) and by a Terry Fox Program Project Grant of the National Cancer Institute of Canada (A.B.).

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Editor: V. J. DiRita