Interferon-γ Listericidal Action Is Mediated by Novel Rab5a Functions at the Phagosomal Environment*

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Control and clearance of Listeria monocytogenes infection is an interferon-γ-dependent process. The listericidal mechanism of action involves activation of NADPH oxidase and inducible nitric-oxide synthase to produce reactive oxygen and nitrogen intermediate radicals, respectively. Recently, we have described in a nonpathogenic model of L. monocytogenes (hemolysin negative mutant strain) that the interferon-γ-inducible GTPase Rab5a contributed to Listeria destruction induced in resting macrophages. Here, we report in a pathogenic model of L. monocytogenes (hemolysin-positive strain) that Rab5a plays a central role in Listeria destruction induced by interferon-γ and within the phagosomal environment. These findings reveal the importance of Rab5a as the responsible factor mediating the listericidal action of interferon-γ. Active Rab5a causes remodeling of the phagosomal environment, facilitates the translocation of Rac2 to LM phagosomes, and regulates the activity of this GTPase. Rac2 activation and translocation governs the phagocyte NADPH oxidase activity and the consequent reactive oxygen intermediate production that leads to killing of the pathogen.

L. monocytogenes is an intracellular facultative bacterium able to invade phagocytic cells and is responsible for severe pathologies in immunocompromised people, newborns and pregnant women (1). L. monocytogenes entry into the host cell is an active process involving several protein components. After a short phagosomal period (~30 min), L. monocytogenes escapes to the cytosol, avoids intracellular killing, and replicates (reviewed in Ref. 2). The L. monocytogenes survival mechanism involves two steps: (i) live bacteria avoid phagosome maturation by inactivation of the endosomal trafficking regulator Rab5a, which blocks the recruitment of lysosomal proteins to the phagosomes (Lamp-1 and cathepsin-D) (3) and (ii) secretion by L. monocytogenes of listeriolysin and PI-PLC lyses the phagosomal membrane, translocates L. monocytogenes to the cytoplasm, and consequently, allows for L. monocytogenes intracellular survival (4).

Control of L. monocytogenes infection and clearance is an interferon-γ (IFN-γ)³-dependent process. IFN-γ priming of macrophages (MØs) recruited at the inflammatory site triggers their listericidal abilities (5). IFN-γ signaling modulates the expression and activation of more than 200 proteins (6). However, to date, only a few of these molecules have been shown to exert a direct role in pathogen elimination (7). Among these are (i) IGTP, a GTP-binding protein relevant for Toxoplasma clearance (8) and (ii) Nramp1, a MØ-restricted lysosomal protein involved in Leishmania, Salmonella, and Mycobacterium spp. clearance (9). In addition, IFN-γ induces the production of reactive oxygen (ROI) and nitrogen (RNI) intermediates with microbicidal activity (10). From this set of molecules, only ROI and RNI have been shown to restrict L. monocytogenes growth (10, 11), while the other two molecules (i.e. IGTP or Nramp1) play no role at all in L. monocytogenes clearance (8, 9).

Recently, we have shown that in resting MØs the inhibition of Rab5a synthesis allows for intracellular survival of a listerialysin-defective L. monocytogenes mutant, that under normal Rab5a levels is unable to grow and fails to escape from the phagosome (12). Furthermore, we have also described that IFN-γ signaling up-regulates Rab5a function (13). However, at this stage, no correlation between the induction of ROI and RNI by IFN-γ and the Rab5a function has been established. Here, we show that Rab5a is a key molecule for the IFN-γ promoted clearance of a pathogenic L. monocytogenes strain at the phagosomal stage. We show that Rab5a, in the GTP form, controls the recruitment of active Rac2 to the transformed L. monocytogenes phagolysosome and the assembly of the phagocyte NADPH oxidase with the production of toxic radicals. These Rab5a-mediated actions compromise Listeria viability within the phagolysosomes and further L. monocytogenes intracellular survival.

EXPERIMENTAL PROCEDURES

Cells and Reagents—J774 cells and protease peptide-elicited peritoneal MØs from Balb/c mice were cultured in Dulbecco’s modified Eagle’s medium, 5% fetal calf serum, 2 mM l-glutamine, and 50 μg/ml gentamicin. Phosphothioate Rab5a antisense (5′-TGC GCC TCG ACT AGC) and sense (5′-CAT GT) and phosphothioate Rab5a control (5′-ACA TCG CTA GTC GAG GCG CA) (20-mer) were from Isogen Bioscience BV (Maarseen, The Netherlands). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: IFN-γ, interferon-γ; CFU, colony-forming units; GST, glutathione S-transferase; HBE, homogenization buffer; HBSS-g, Hank’s balanced salt solution containing 10 mM glucose; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; MØ, macrophage; HRP, horseradish peroxidase; PBD, p21-activated kinase-derived binding domain; GTP-γS, guanosine 5′-3-O-(thio) triphosphate or guanosine 5′-O-(3-thiophosphosphate); PI-PLC, phosphatidylinositol phospholipase C.

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Holland). Mouse recombinant IFN-γ was purchased from Roche Molecular Biochemicals; 35S translabel (10 mCi/ml) was from Amersham Pharmacia Biotech; horseradish peroxidase (HRP), superoxide dismutase, and ferricytochrome c were from Sigma; and brain heart infusion was from Difco Laboratories.

Transfection and Overexpression of Rab5 Constructs in J774 Cells—Rab5a: Q79L, Rab5a:S34N, and Rab5c:Q80L. C. dianisimidate subclones were electroporated into pcDNA3 using EcoRI/BamHI sites. Cells (5 × 105) were transfected by electroporation (150 V, 800 microfarads, 129 ohms) for 24 h. Overexpression was checked on cell lysates with specific antibodies at 8 and 24 h, respectively.

Assays and Listericidal Activity—The following antibodies were used. Mouse monoclonal anti-Rab5a (4F11) was described in Ref. 3. Polyclonal rabbit anti-Rab5c was a gift from M. Zerial (EMBL, Heidelberg, Germany). Rabbit anti-Rab7 was a generous gift from A. Wandinger-Ness (University of New Mexico, Albuquerque, NM). Rabbit anti-Rac2 was developed in rabbits (R7859) (14), and rabbit anti-cathepsin-D (3) was a gift from P. D. Stahl (Washington University, St. Louis, MO). Rabbit anti-Rac2-H was a gift from I. V. Sandoval (Centro de Biologia Molecular “Severo Ochoa,” Madrid, Spain). Rat anti-mouse Lamp-1 monoclonal antibody (1G11) was a gift from D. G. Russell, (Washington University, St. Louis, MO). Biotinylated rat anti-mouse TfR (CD71) was purchased from Caltag, and secondary peroxidase-conjugated antibodies (goat anti-mouse, anti-rabbit, or anti-rat) were from Amersham Pharmacia Biotech. Streptoavidin-peroxidase-conjugated antibody was purchased from Caltag Laboratories (San Leandro, CA). Biotinylated rat anti-mouse TfR (CD71) was purchased from Becton Dickinson. Biotinylated rat anti-mouse Lamp-1 monoclonal antibody (1G11) was a gift from D. G. Russell, (Washington University, St. Louis, MO). Biotinylated rat anti-mouse TfR (CD71) was purchased from Caltag, and secondary peroxidase-conjugated antibodies (goat anti-mouse, anti-rabbit, or anti-rat) were from Amersham Pharmacia Biotech. Streptoavidin-peroxidase-conjugated antibody was purchased from Caltag Laboratories (San Leandro, CA).

GST-PBD, the p21-activated kinase-derived binding domain for activated Rac2 proteins, was expressed in E. coli BL-21 strain. Recombinant proteins were incubated with 5 μM isopropyl-β-D-thiogalactoside for 3 h at 37 °C and purified with glutathione-agarose according to the manufacturer’s instructions (CLONTECH, Palo Alto, CA).

Antisense and IFN-γ-Treatment of Cells—Introduction of antisense and sense phosphotidyl oligonucleotides onto J774 cells or peritoneal M0s (5 × 105/ml) was performed as described (12) with a Baxter BTX-603 electroporator and the following settings: 220 V, 800 microfarads, 75 ohms. Cells were set on culture plates for 6 h at 37 °C and washed and incubated (+) or not (-) with 100 units/ml IFN-γ for 16 h.

Immunoprecipitation—This assay was performed essentially as previously described (16). In brief, proteose peptone-peritoneal peritoneal M0s (or J774 cells) treated with Rab5a antisense/sense oligonucleotides were plated onto 96-well plates at 3 × 104 cells/well in Dulbecco’s modified Eagle’s medium without phenol red plus 50 μg/ml gentamicin. A solution of 80 μM ferricytochrome c in HBSS-g containing the corresponding stimulus was added (10 and 100 units/ml IFN-γ). Cells were incubated at 37 °C in 5% CO2 for 4 h in change in absorbance (A550 nm) obtained in well samples was subtracted from those wells incubated with the same stimulus in the presence of superoxide dismutase (10 μg/ml). Results were expressed as nmol of O2 produced/106 cells in quadruplicate wells. Results were representative of at least seven independent experiments.

RESULTS AND DISCUSSION

L. monocytogenes is a facultative intracellular parasite able to infect and replicate inside nonactivated M0s. Once in the phagosome, the bacterium secretes listeriolysin and PI-PLC to lyse the phagosome and escape to the cytoplasm, where it replicates (4). The activation of M0s by IFN-γ provokes an increase in the phagocytic rate, partially prevents the escape of L. monocytogenes to the cytosol (17), and triggers the production of toxic radicals (i.e. ROI and RNI), which lead to the elimination of the microorganism (10).

Analysis of phagosomes from infected M0s pulsed with IFN-γ has established that this lymphokine promotes phagosome maturation (16) and up-regulates the levels of endosomal/lysosomal proteins, such as cathepsin-D (21) and Rab5a (13), and down-regulates endosomal markers such as the mannose and the transferrin receptors, TIR (22, 23), while other Rab remains unaffected (i.e. Rab5b, Rab5c, Rab7, or Rab11) (13). However, until now, the link between the IFN-γ-induced microbicidal effects and the up-regulation of Rab5a has remained elusive.
IFN-γ Reduces Intraphagosomal Listeria Viability and Promotes Phagosomal Maturation—Since IFN-γ triggers the listericidal activity of MØs, we first investigated its action in infected MØs at the phagosomal stage. As shown in Fig. 1, among the typical endosomal markers analyzed in *L. monocytogenes* isolated phagosomes, Rab5a was clearly increased after IFN-γ treatment (+); whereas Rab5c or Rab7 was unmodified and Tfr was down-regulated. Similar data have been reported in studies of whole cell extracts (13, 23). Isolated phagosomes from MØs not treated with IFN-γ (−) lacked lysosomal markers such as cathepsin-D, Lamp-1, and Limp-II. In contrast, all of these markers were increased in phagosomes from MØs previously pulsed with IFN-γ (+). These results suggested that IFN-γ up-regulated the interactions of phagosomes with late endosomes and lysosomes rather than with early endosomes. IFN-γ promotion of phagosomal maturation was accompanied by a significant reduction of the pathogen viability inside the phagosomes (1-h phagosomes in Fig. 1, bar graphs). These results argue that a more rapid entry of this lysosomal environment into the phagosomal space contributes to the killing of the pathogen.

**IFN-γ Promotion of Phagosomal Maturation and Lysosomal Protein Transport Is Regulated by Rab5a**—Our earlier data showed that within the phagosome, *Listeria* avoided phagosome maturation by blocking the Rab5a activity (3, 12). Since IFN-γ signaling increases the synthesis and enhances the activity of this small GTPase (13), we next studied whether promotion of phagosomal maturation by IFN-γ was mediated by Rab5a and designed the following experimental strategy. Rab5a synthesis was first blocked using phosphothioate antisense oligonucleotides targeted to the Rab5a translation initiation codon, and then an IFN-γ pulse was given (12).

Analysis of phagosomes from MØs treated with Rab5a antisense oligonucleotides showed that this treatment effectively blocked Rab5a expression on phagosomes (A − versus S − cells in Fig. 2A). Moreover, Rab5a antisense treatment also blocked Rab5a expression even in phagosomes of MØs pulsed with IFN-γ (+) (compare A + with S + cells and S − cells with S + cells, respectively; Fig. 2A). The inhibition was thus effective at the level of protein synthesis; as shown in Fig. 2B, this reduction mainly affected membrane-bound Rab5a.

Parallel studies of Rab5c and Rab7 levels showed that their expression in phagosomes was not affected by the Rab5a anti-
sense treatment, either alone or in combination with a pulse of IFN-γ (Fig. 2A). On the other hand, the increase in the levels of Lamp-1, Limp-II, and cathepsin-D in phagosomes induced by IFN-γ in control cells (S+), were almost completely abolished in cells treated with Rab5α antisense oligonucleotides (A+). Experiments performed with lysosensor green as a pH indicator showed that IFN-γ treatment decreased MØ vesicle pH, but independently of Rab5α antisense or sense treatment (data not shown).

These observations highlighted two important conclusions: (i) Rab5α acquires a novel function upon IFN-γ stimulation that affects the transport of lysosomal proteins to phagosomes, and (ii) the normal *L. monocytogenes* strategy of blocking transport of newly synthesized lysosomal proteins to phagosomes (3) can be overcome by the increase in Rab5α function promoted by IFN-γ.

IFN-γ induction of Rab5α synthesis has been shown to promote the binding of the GTP form of Rab5α to membranes (13). This argues that the Rab5α form that controls the transport of lysosomal proteins to the phagosomes should be the GTP form. Our data are also in agreement with a recent report showing a role for rabenosyn-5, a Rab5-GTP-interacting effector protein, that affects the transport of lysosomal proteins to the phagosomes (10).

**Rab5α Mediates the IFN-γ-induced Listericidal Abilities at the Phagosomal Stage—**When intracellular growth of *L. monocytogenes* was studied, there was an inverse correlation between viability, expressed as the replication index, and Rab5α levels. The highest replication index corresponded to Rab5α antisense-treated cells (Fig. 2C, filled bars). Interestingly, these Rab5α antisense-treated cells pulsed with IFN-γ (A+) relative to the controls (S−); the replication index values were 22.5 in A− cells, 9 in A+ cells, and 6.2 in S− cells (Fig. 2C, white bars). This Rab5α antisense treatment blocks the listericidal effect of IFN-γ as shown by the replication index values of 9 in A+ cells compared with a replication index value of 0.18 in S+ cells (Fig. 2C, white bars). These results cannot be explained by different *L. monocytogenes* ingestion rates on Rab5α antisense- or Rab5α sense-treated cells (12). Similarly, intraphagosomal *L. monocytogenes* viability, expressed as CFU from isolated phagosomes after 1 h of infection, inversely correlated with Rab5α expression on *L. monocytogenes* phagosomes. The highest viability corresponded to A− phagosomes with the lowest Rab5α levels. The lowest viability corresponded to S+ phagosomes with the highest Rab5α levels, as shown by the viability values ranging from 29 × 10^4 CFU in A− cells to 16 × 10^4 CFU in A+ cells, 9 × 10^4 CFU in S− cells, and 2.7 × 10^4 CFU in S+ cells (Fig. 2C, filled bars).

We also estimated lysosomal protein synthesis after antisense or sense treatment in the presence or absence of IFN-γ. As shown in Fig. 3, synthesis of Lamp-1 or Limp-II was not affected by any of the treatments. IFN-γ increased the synthesis of cathepsin-D, as previously reported (21), independently of Rab5α antisense or sense treatments. These results confirmed that Rab5α regulated the transport of lysosomal proteins to phagosomes induced by IFN-γ but not the synthesis of lysosomal proteins.

Taken together, these results show that IFN-γ-promoted phagosomal maturation depends on Rab5α that more importantly mediated the listericidal effect of IFN-γ on phagosomes, leading to a significant decrease in *L. monocytogenes* viability. Rab5α Regulates the IFN-γ-promoted Interactions of *L. monocytogenes* Phagosomes with Lysosomes—Until now, transport from late endosomes/lysosomes to phagosomes has been suggested to be Rab5α-mediated in resting phagosomes (25). However, no report has implicated Rab5α in these transport events from activated MØs, nor has the effect of IFN-γ in stimulating this process been shown. To analyze the role of Rab5α and IFN-γ in this event, we studied the transfer of HRP from preloaded early endosomes (100 µg/ml, HRP uptake for 5 min) or lysosomes (100 µg/ml, HRP uptake for 5 min and 2 h chase) into *L. monocytogenes* phagosomes. *L. monocytogenes* phagosomes were isolated from cells treated with antisense/ sense oligonucleotides and pulsed with IFN-γ as above. The results shown in Fig. 4 indicated that the Rab5α antisense treatment significantly blocked the transfer of HRP from early endosomes to phagosomes. However, under these conditions, IFN-γ treatment did not promote the fusion between early endosomes and *L. monocytogenes* phagosomes (Fig. 4, white
In summary, the experiments shown in Figs. 2–4 indicated that IFN-γ promoted the delivery of lysosomal proteins to *L. monocytogenes* phagosomes via a process that was clearly dependent on Rab5a. These results link Rab5a action to the clearance of *L. monocytogenes* by phagocytes. Moreover, the simplest model to explain the IFN-γ effect on *L. monocytogenes* phagosome maturation is that it promotes the Rab5a-mediated fusion of phagosomes with late endosomes/lysosomes. These data were in accordance with those reported with the yeast homologue, Ypt51p, or the allelic form, Vps21p, on late transport events (26–28) as well as in fusion events of latex bead phagosomes with lysosomes from resting MØs (25).

Rab5a Acts Upstream of Rac2 and ROI Production in the IFN-γ Signaling Pathway—The results presented above clearly indicate that Rab5a regulated the IFN-γ-induced listericidal abilities of MØs at the phagosomal stage. To date, the listericial mechanisms induced by different signals including IFN-γ rely on the production of ROI and RNI toxic molecules (5, 11, 29, 30). Production of ROI radicals requires translocation of Rac2 to the membranes to assemble an active phagocyte NADPH oxidase complex (31), a process triggered by IFN-γ. Only active Rac2 (GTP form) is known to be required to activate the phagocyte NADPH oxidase to produce ROI (32). We next analyzed whether Rab5a was involved in the activation of Rac2 and production of ROI radicals regulated by IFN-γ. First, we observed that IFN-γ-induced the translocation of Rac2 to the phagosomes, and this was almost completely abolished by treatment of MØs with Rab5a antisense (Fig. 5A). These results strongly suggested that in the pathway of IFN-γ signaling, Rab5a action acted upstream of rac2. For ROI production, the NADPH oxidase enzyme needs to bind and activate onto the phagosomal membranes (31). NADPH oxidase activation correlates with the recruitment of the active form of Rac2 to the membranes (32). Recently, a protocol has been described to quantify activated Rac2 in whole cells using the binding domain of the p21-activated kinase 1 that exclusively binds Rac2-GTP (Fig. 5B, GST-PBD lanes) (15). Using this protocol, we observed that the amount of activated Rac2 in Rab5a antisense-treated cells was significantly lower than in sense-treated cells, both in the presence and absence of IFN-γ (Fig. 5B, GST-PBD-IP lanes), while the total Rac2 levels remained constant (Fig. 5B, +GTP-γS lanes and Rac2 lanes). The same results were observed when the study was repeated in isolated *L. monocytogenes* phagosomes after Rab5a antisense/sense and IFN-γ treatment (data not shown). This observation suggests that measurement of Rac2 activation on whole cell extracts was a valid indicator of the translocation of GTP-active Rac2 to the phagosomes. More interestingly, these findings indicate that Rab5a mediates the IFN-γ-induced Rac2 activation and translocation to the phagosomes. With respect to this, it is not inconceivable that Rab5a-Rac2 may act in conjunction. In fact, Rab5a and the Rac family have been reported to act together in coordinating the process of (re)assembly of stress fibers and focal adhesions (33) as well as in coordinating EGF receptor signaling and trafficking (34).

Activation of the phagocyte NADPH oxidase leads to a functional enzyme able to produce ROI toxic molecules (31). Next, we studied the role of Rab5a on the IFN-γ-induced production of ROI radicals by a functional phagocyte NADPH oxidase. For this purpose, we used the same Rab5a antisense/sense strategy in the presence and absence of an IFN-γ pulse as above. For ROI production, we used peritoneally elicited MØs due to their higher ROI production levels. Nonetheless, experiments performed in the J774 MØ cell line showed similar results (an average of five different assays were performed (data not shown). As shown in Fig. 5C, which shows one representative.

**Fig. 5.** Rab5a acts upstream of Rac2 and ROI production in the IFN-γ signaling pathway. A, J774 cells were treated with Rab5a antisense/sense and IFN-γ as above. Isolated phagosomes were solubilized, and Western blots were developed with rabbit R786/9 anti-Rac2 antibody. B, J774 cells treated as above and assayed for Rac2 activation (GST-PBD lanes) (lanes labeled as -GTPγS). Controls (lanes labeled as +GTPγS) corresponded to total Rac2 protein able to be activated. Whole cell Rac2 levels are shown in lanes labeled as rac2. C, protease peptone-elicited peritoneal MØs (or J774 cells) were treated with Rab5a antisense/sense oligonucleotides as above and assayed for O2− production as under “Experimental Procedures.” Stimuli were 10 or 100 units/ml of IFN-γ/well for 60 min at 37 °C. Absorbance (A550) of each value was subtracted from superoxide dismutase values, and results are expressed as nmol of O2−/106 cells in quadruplicate wells. Results corresponded to a representative experiment out of seven.
experiment (out of seven), production of ROI correlated perfectly with Rac2 activation and was inhibited by Rab5a antisense treatment. A 1.4-fold inhibition was observed in A− cells compared with S− cells. As expected, IFN-γ treatment induced ROI production in control cells, but interestingly, this ROI induction was 1.6-fold inhibited by the Rab5a antisense treatment. The effect of Rab5a antisense treatment on ROI production correlated well with the effect shown on Rac2 activation (Fig. 5B) and translocation to the phagosomes (Fig. 5A). The data show that Rab5a regulates the IFN-γ-mediated Rac2 activation, both by enhancing its translocation from the cytosol to the phagosomes and by locking Rac2 in its active GTP conformation.

To address whether Rab5a synthesis alone was sufficient to trigger the effects observed on phagosome-lysosome fusion (Fig. 4), we transiently overexpressed both the active and inactive forms of Rab5a into J774 cells; we also included the active form of another Rab isoform expressed onto L. monocytogenes phagosomes, Rab5c, as a control (5a:Q79L, 5a:S34N, and 5c:Q80L, respectively).

Overexpression protocols after 24 h of transfection gave 5–7-fold increased levels above controls (cells transfected with vector alone) (Fig. 6A). Phagosomes (after 30 min of infection) from these cells showed that transfer of HRP from lysosomes was particularly enhanced in Rab5a:Q79L-transfected cells (Fig. 6B). These results were in accordance with those previously reported for Rab5a:WT-transfected cells (13). Overexpression with Rab5a-inactive form (5a:S34N) inhibited the phagosome-lysosome fusion enhancement, even below control levels. However, overexpression with the active form of Rab5c, Q80L, showed a very low levels of phagosome-lysosome fusion, similar to control cells and to results reported previously (13). These data argue that Rab5a synthesis and its activation in the GTP form were sufficient to promote phagosome-lysosome fusion.

We also checked whether Rab5a synthesis was able to control the observed rac2-GTP recruitment to phagosomes (Fig. 5B). To do this, we isolated phagosomes from cells overexpressed with Rab5a or Rab5c cDNAs, as in Fig. 6A, and analyzed the induction of Rac2 activation and translocation to the phagosomes, using the same protocol as used in Fig. 5B. As shown in Fig. 6C, Rac2 recruitment was promoted in Rab5a:Q79L-overexpressed cells and was diminished below control levels in Rab5a:S34N-overexpressing cells. It should be noted that cells overexpressing the active Rab5c:Q80L form showed levels similar to the controls. These results clearly indicate that Rab5a-GTP, exclusively, controls the recruitment of Rac2-GTP and that Rab5a action is upstream of Rac2.

It is also interesting that another cytokine, the granulocyte colony-stimulating factor, could control the growth of the pathogen Brucella abortus also by regulating the interactions with the endosomal compartment (35). This interaction may transfer bacteria from a relative nonhostile environment to one that contains reducing agents, acid hydrolases, and oxygen radicals (35). It can be speculated that these ROI, elements of the respiratory burst, also present in the endocytic compartments, can then reach both the Listeria- and Brucella-containing phagosomes under each cytokine situation and compromise the bacterial growth inside the cells.

In summary, our results are the first to implicate a small GTPase, Rab5a, in pathogen clearance by phagocytes and to show that this function is induced by IFN-γ action. The novelty of this Rab5a-IFN-γ-mediated function resides in the regulation by this GTPase of two sequential processes in the phagosome. First, Rab5a-GTP promotes phagosomal maturation by regulating the transport of lysosomal proteins to the phagosome. Second, it regulates Rac2 activation and the assembly of the phagocyte NADPH oxidase to produce toxic free radicals. The combined effects of these Rab5a actions are a more effective destruction of pathogens. Finally, these Rab5a novel functions acquired by the IFN-γ treatment acted together with another IFN-γ-mediated feature on L. monocytogenes phagosome (i.e. the blockage of the action of the two membrane lytic L. monocytogenes proteins, listeriolysin and PI-PLC).

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