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Histone deacetylase inhibitor MS-275 augments expression of a subset of IFN-γ-regulated genes in *Toxoplasma gondii*-infected macrophages but does not improve parasite control

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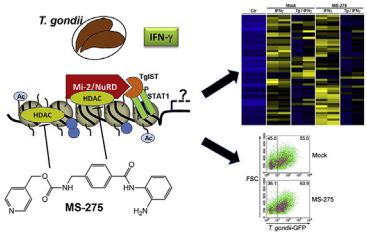
HIGHLIGHTS

- Inhibition of HDAC using the inhibitor MS-275 abolishes defective IFN-γ-regulated MHC class II in *T. gondii*-infected macrophages.
- MS-275 increases expression of a subset of IFN-γ-regulated genes in macrophages irrespective of infection.
- It does not restore the defective responsiveness of *T. gondii*-infected macrophages to IFN-γ at the transcriptional level.
- *T. gondii* inhibits the MS-275 induced transcriptome.
- Host defenses of macrophages against *T. gondii* are not augmented by MS-275.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxoplasma gondii is a ubiquitous apicomplexan parasite of mammals and birds and an important pathogen of humans. IFN-γ is the major mediator of host resistance against *T. gondii* but intriguingly, parasite-infected host cells including macrophages are severely impaired to respond to IFN-γ due to defective transcriptional activation of target genes. Here, we tested the possibility that the impaired responsiveness of *T. gondii*-infected macrophages to IFN-γ can be restored by inhibiting histone deacetylases (HDACs) using the class I-specific inhibitor MS-275. Treatment of RAW264.7 cells with MS-275 indeed increased MHC class II surface expression in infected and non-infected cells and largely abolished the inhibition of IFN-γ-regulated MHC class II expression exerted by *T. gondii*. Genome-wide transcriptome profiling revealed that MS-275 increased mean mRNA levels of IFN-γ-regulated genes particularly in non-infected macrophages. Transcript levels of 33% of IFN-γ secondary response genes but only those of a few primary response genes were also increased by MS-275 in *T. gondii*-infected cells. Importantly, the unresponsiveness of parasite-infected cells to IFN-γ was however not abolished by MS-275. Furthermore, MS-275 also up-regulated several anti-inflammatory cytokines or signaling molecules

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in *T. gondii*-infected macrophages. It additionally regulated expression of more than 2500 genes in non-infected macrophages expression of which was surprisingly counteracted by prior infection with *T. gondii*. FACS analysis and immunofluorescence microscopy revealed that MS-275 did not considerably diminish the number of parasite-positive cells or the intracellular replication in macrophages stimulated or not with IFN-γ. Thus, a supportive therapy using MS-275 appears inappropriate for treatment of toxoplasmosis.

1. Introduction

The apicomplexan *Toxoplasma gondii* infects warm-blooded animals including ~30% of humans worldwide. Although infection in humans is mostly asymptomatic or benign, it can lead to severe and even life-threatening illnesses including congenital toxoplasmosis after transplacental transmission or reactivated *Toxoplasma* encephalitis in immunocompromised patients (Montoya and Liesenfeld, 2004). *T. gondii* can also cause significant diseases, e.g. retinochorioiditis, in otherwise healthy individuals. Due to its extraordinary high prevalence, it is indeed now considered among the most important food-borne pathogens in the US (Hoffmann et al., 2012) and possibly other parts of the world.

After oral uptake, the parasite penetrates the intestinal epithelium and transforms into fast-dividing tachyzoites. This parasite stage is able to infect and replicate within any nucleated cell including monocytes and disseminates throughout its host. It elicits strong innate and adaptive immune responses that are able to control the tachyzoite stage. IFN-γ is the cytokine that is absolutely essential for efficient parasite control during acute toxoplasmosis (Lykens et al., 2010; Suzuki et al., 1988). However, concomitant differentiation of tachyzoites to slow-replicating and dormant bradyzoites enables the parasite to persist for the host's life predominantly in neural and muscular tissue. Extensive manipulation of its host cells including sentinels of the immune system is crucial for *T. gondii* in order to survive the acute stage of infection and to give rise to chronic toxoplasmosis (Lang et al., 2007). This includes the inhibition of IFN-γ-regulated gene expression on a genome-wide level in infected macrophages (Kim et al., 2007; Lang et al., 2006, Lang et al., 2012; Lüder et al., 1998, Lüder et al., 2001; Rosowski and Saeij, 2012).

Manipulation of host cell gene expression via epigenetic mechanisms is an emerging paradigm for various parasite-host interactions (Gomez-Diaz et al., 2012; Robert McMaster et al., 2016). We have previously shown that infection of macrophages with *T. gondii* strongly reduces the acetylation of histones H3 and H4 at promoters of distinct interferon (IFN)-γ-regulated genes (Lang et al., 2012). Binding of Brahma-related gene (Brg)-1, i.e. a core subunit of the chromatin remodeling complex Switch/Sucrose non-fermenting (SWI/SNF), to the IFN-γ-responsive promoter IV of the class II transactivator (CIITA) is also inhibited by infection (Lang et al., 2012). Since histone acetylation is generally associated with active gene expression, we proposed a parasite-triggered epigenetic mechanism being responsible for the unresponsiveness of *T. gondii*-infected macrophages to IFN-γ (Lang et al., 2012). In support of an epigenetic regulation, treatment of macrophages with histone deacetylase (HDAC) inhibitors prior to infection largely abolished the inhibition of IFN-γ-induced CIITA and MHC class II expression by *T. gondii* (Lang et al., 2012). HDACs reduce histone acetylation and their inhibition by HDAC inhibitors is generally assumed to activate gene expression. Whereas a general inhibition of the IFN-γ-responsiveness in infected cells via an epigenetic mechanism was challenged by others (Rosowski et al., 2014), *T. gondii* has been recently shown to recruit the Mi-2 nucleosome remodeling and deacetylase (Mi-2/NuRD) complex including HDAC1 and 2 to IFN-γ-responsive promoters thereby possibly repressing gene expression (Olias et al., 2016).

Macrophages respond to IFN-γ via the Janus kinase/Signal transducer and activator of transcription (JAK/STAT)-1 pathway. The transcriptional program initiated by IFN-γ is extraordinarily complex with several hundreds of genes being up- or down-regulated. Primary response genes including transcription factors of the interferon-regulatory factor (IRF) family are directly activated by phosphorylated STAT1 (pSTAT1) whereas genes activated in a second or even third wave of IFN-γ-mediated expression depend on additional transcription regulators, e.g. IRF-1 or CIITA. Gene expression requires an 'open' chromatin structure that allows binding of transcription factors, chromatin modifiers and the transcriptional machinery. Promoters however differ in their requirements for chromatin modifiers, i.e. histone-modifying enzymes and ATP-dependent multi-subunit chromatin remodeling complexes. Following LPS-mediated signaling, for example, primary response genes containing CpG islands in their promoters do not require SWI/SNF or Brg-1 whereas CpG-poor secondary response genes rely on SWI/SNF/Brg-1 to be expressed (Ramirez-Carrozzi et al., 2009; Ramirez-Carrozzi et al., 2006). Likewise, expression of the secondary response gene *ciita* in response to IFN-γ stimulation but not that of the primary response gene *irf1* depends on Brg-1 (Pattenden et al., 2002).

The different needs of inducible promoters including IFN-γ-responsive promoters suggest that HDAC inhibitors might modulate IFN-γ-mediated gene expression in *T. gondii*-infected macrophages in a gene-dependent manner. In this study, we therefore sought to investigate the effect of the HDAC inhibitor MS-275 on the defective IFN-γ-regulated gene expression in *T. gondii*-infected macrophages on a genome-wide level. We also determined whether enhanced responsiveness of infected macrophages to IFN-γ due to MS-275 treatment supports cell-autonomous immune responses in macrophages to more efficiently control intracellular parasite growth.

2. Materials and methods

2.1. Macrophages, parasites and infection

The murine leukaemic monocyte/macrophage cell line RAW264.7 (TIB 71; ATCC, Rockville, MD) was propagated in RPMI 1640 containing 4.5 g/L glucose, 10% heat-inactivated fetal calf serum (FCS), 1 mM sodium pyruvate, 10 mM HEPES, 100 U/mL penicillin and 100 μg/mL streptomycin (all cell culture reagents from Biochrom, Berlin, Germany). Tachyzoites of the *T. gondii* type II strain NTE (Gross et al., 1991) were co-cultivated in L929 murine fibroblasts as host cells in RPMI 1640, 1% FCS and antibiotics as indicated above. Flow cytometric analysis of parasite development was performed using a GFP-expressing mutant of the type II strain PTG/ME49 (kindly provided by Antonio Barragan, Stockholm,