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# Human parvovirus B19 infection leads to downregulation of thyroid, estrogen, and retinoid hormone receptor expression

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ABSTRACT

Erythrovirus B19 (B19V) is a member of the family *Parvoviridae*. Infection with B19V has been linked to a variety of diseases including erythroid, thyroid, neurological and autoimmune diseases. Here we show that infection of primary CD36+ cells with B19V coincides with downregulation of thyroid, retinoid, and estrogen hormone receptors. In addition we show changes in expression of a variety of related downstream signaling genes participating in cancer and cardiac-related diseases in B19V-infected erythroid primary cells.



## Introduction

Erythrovirus B19 (B19V) belongs to the family *Parvoviridae*. Discovered in 1975, B19V is now known to persist in many cells and organs of the human body (Norja et al., 2008), however it has been shown to replicate only in erythroid progenitor cells (EPCs; Ozawa et al., 1986). The B19V genome contains a single promoter, p6, that regulates expression of the major non-structural protein NS1, two structural proteins VP1 and VP2, and two minor non-structural proteins 11 kDa and 7.5 kDa (Heegaard and Brown, 2002).

NS1 plays a major role in the viral life cycle (Raab et al., 2002; Astell et al., 1987). It trans-activates the p6 promoter and other viral and cellular promoters such as HIV-LTR, IL-6, and TNF-α (Fu et al., 2002; Sol et al., 1999) as well as induces apoptosis (Zhi et al., 2006; Ozawa et al., 1988).

B19V causes a variety of diseases such as transient aplastic crisis (Pattison et al., 1981), fifth disease (Anderson et al., 1983), polyarthritis (Kerr, 2000), thrombocytopenia (Osaki et al., 1999), non-immune hydrops fetalis (Levy et al., 1997), and myocarditis (Dettmeyer et al., 2003). It is associated with multiple brain disorders (Barah et al., 2003) and autoimmune diseases (Lehmann et al., 2003). Recently, B19V infection has been strongly associated with Hashimoto's thyroiditis and thyroid cancer by our laboratory and others (Adamson et al., 2011; Wang et al., 2008, 2010), but the molecular mechanism of interaction between B19V and thyroid signaling is unknown. Interestingly, it has been shown that NS1 of the related parvovirus, minute virus of mice (MVM), upregulates the expression of thyroid hormone receptor alpha (THRα; Vanacker et al., 1996). Thyroid hormone (T3) was also shown to increase the cytotoxicity of MVM (Vanacker et al., 1993).

Here we demonstrated that B19V infection of CD36+ EPCs leads to downregulation of THRα, and that NS1 is sufficient to induce this downregulation. We also showed that B19V infection modulates the expression of estrogen and retinoid receptors, as well as a variety of related downstream signaling genes involved in cancer and cardio-vascular pathways.

## Results

To understand the influence of B19V infection on THRα expression we utilized CD36+ EPCs. The cells were either mock infected

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or infected with wild-type B19V. Cells were collected immediately after infection, and 48 hours post infection (hpi). THRα mRNA level was assessed by a conventional RT-PCR utilizing THRα specific primers. As shown in Fig. 1(a) infection with B19V led to down-regulation of THRα mRNA.

In order to quantify the differences in mRNA expression, qRT-PCR was performed using pre-designed TaqMan® gene expression assays for THRα and GAPDH (Applied Biosystems, Foster City, CA). Fig. 1(b) shows that significant downregulation of THRα expression was observed in cells infected with B19V.

Next, we examined whether B19V NS1 expression is responsible for the downregulation of THRα expression. Since primary cells are difficult to transfect we utilized the erythroleukemia cell line K562. We constructed a plasmid expressing either NS1 or GFP (as a negative control) driven by the B19V p6 promoter. Cells were collected at 6, 24, and 48 hours post transfection (hpt), and total RNA and protein were isolated. Fig. 2(b) shows a moderate decrease of THRα mRNA level in cells expressing NS1 48 hpt. Fig. 2(a) demonstrates the similar result on protein level, starting with 24 hpt.

In order to examine whether such an effect occurs only in the erythroid system strictly or is a common property of NS1, we decided to utilize immortalized normal thyroid cells N-thy-ori. Fig. 2(c) shows the decrease of the THRα protein level in cells expressing NS1. Interestingly in this case antibody was able to detect two isoforms of THRα—THRα1 and THRα2.

In order to exclude the possibility of the observed effect being due to overexpression of NS1, and to mimic more natural conditions, we constructed a recombinant adeno-associated virus serotype 2 expressing either GFP or NS1 under B19V p6 (rAAV2-p6-GFP and rAAV2-p6-NS1). As can be seen in Fig. 2(d) the THRα mRNA level is significantly downregulated in cells infected with rAAV-p6-NS1 compared to mock infection 48 hpi.

THRs belong to the superfamily of nuclear receptors (NRs). The members of this superfamily control the expression of many genes

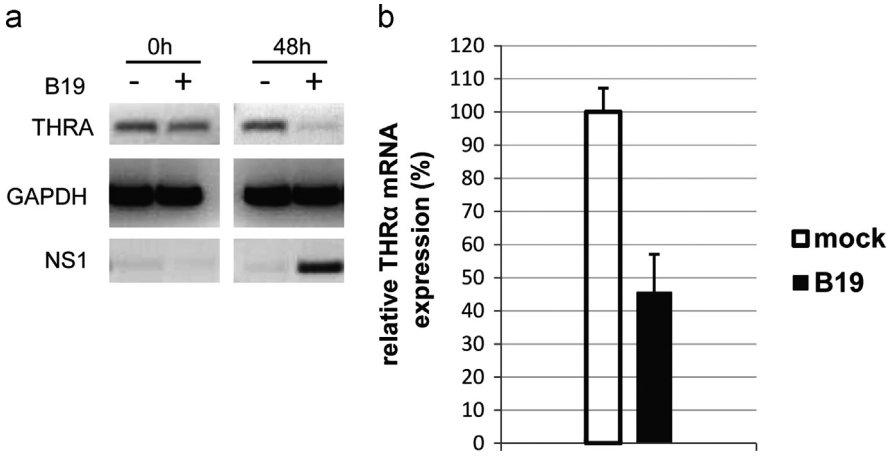


**Fig. 1.** Influence of B19V on THRα expression in CD36+cells. (a) THRα mRNA level assessed by RT-PCR using total RNA from CD36+cells mock infected (−) or infected with B19V (+). GAPDH mRNA was used as a loading control. NS1 mRNA expression was observed 48 hpi. (b) THRα mRNA levels assessed by qRT-PCR 48 hpi [$n$ (number of technical replicates)=4, mock SD (standard deviation)=0.071, B19V SD=0.011. $p$ ($p$ value)=0.001].

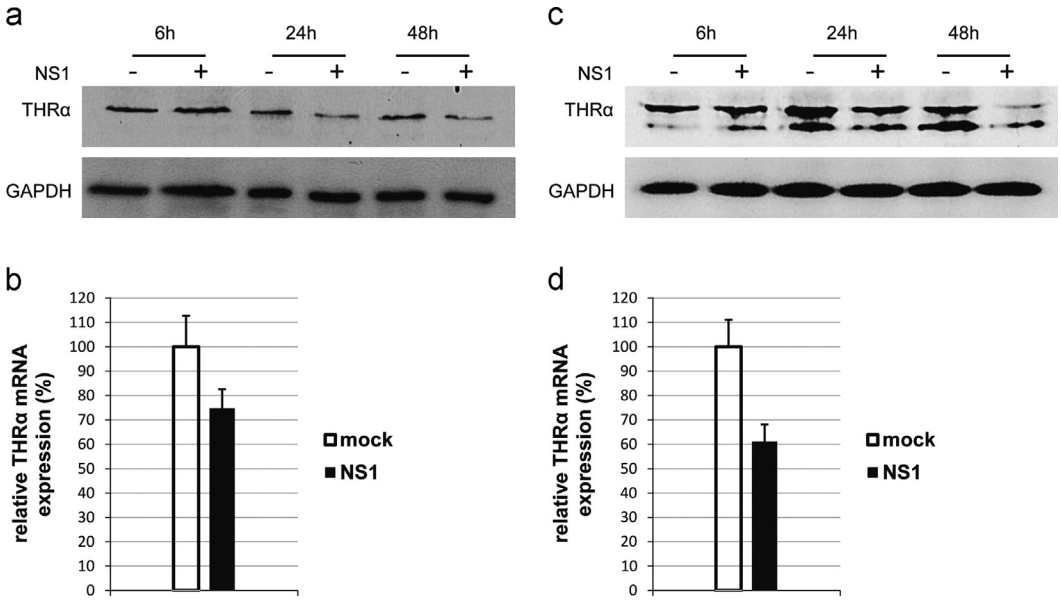


**Fig. 2.** Influence of B19V NS1 on THRα expression in K562 (a, b) and N-thy-ori (c, d) cells. (a) Protein level was assessed by Western blot, and GAPDH was utilized as a loading control. (b) mRNA Level was assessed by qRT-PCR using total RNA from cells mock-transfected or transfected with NS1 48 hpt. ($n$=3, mock SD=0.108, NS1 SD=0.101, $p$=0.006). (c) Protein level was assessed by western blot, and GAPDH was utilized as a loading control. (d) Infection of N-thy-ori cells with rAAV2-p6-GFP (mock) or rAAV2-p6-NS1 (NS1); THRα mRNA level was assessed by qRT-PCR 48 hpi ($n$=3, mock SD=0.092, NS1 SD=0.123, $p$=0.008).