

Heg Inc RNA and Cdk1 mRNA in mononuclear cells and regulation of autoantibody levels

Abstract

We studied Heg Inc RNA and Cdk1 mRNA in mononuclear cells in untreated and treated patient with Graves disease and in normal subjects. Heg was inversely correlated with TRAb in untreated patients and inversely correlated with CD14 mRNA in treated patients and in normal subjects. These and other findings suggest that endogenous Heg may reduce expression of CD14 mRNA and thereby reducing inflammation and autoantibody levels. The concentration of Cdk1 mRNA decreased 50% during Thiamazole treatment. Concentrations of TRAb decreased correspondingly. Conclusions: These and other findings indicate that Heg Inc RNA and Cdk1 have an effect on autoantibody levels achieved without affecting the risk of developing autoimmune disease. Our results also support the hypothesis that inhibition of Cdk1 or CK2 by new drugs may inhibit the production of autoantibodies and autoimmunity in the organism.

Keywords: autoantibodies, Heg Inc RNA, Cdk1 mRNA, CD14 cells, thiamazole treatment, diabetes

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Introduction

We found some years ago an unknown non-coding RNA fragment in mononuclear cells designated Heg Inc RNA (European Nucleotide Archive: EU137727.1).^{1,2} Heg RNA was located to chromosome 1, antisense to and overlaps a larger part of exon 7 from Nucks mRNA. Nucks (nuclear ubiquitous casein kinase and cyclin-dependent substrate) plays a major role in the regulation of transcription and is a substrate for Cdk1. Heg and other RNA products were quantified by RT-PCR-HPLC.¹ The mean concentration of Heg Inc RNA in normal subjects were 0.15 ± 0.01 amol/ μ g of DNA.² Surprisingly we found that Heg Inc RNA was close and negatively correlated with concentrations of TSH receptor autoantibodies (TRAb) in patients with untreated Graves disease. We also study Cdk1 and CK2 mRNAs (homo sapiens casein kinase I and II), which both are members of the cyclin-dependent kinase cascade. Cdk1 is important for cell division and differentiation.³ Cdk1 was positively correlated with concentrations of TRAb, but only provided Heg was included in the analysis. We did not find other RNA fragments, which correlated with TRAb. The interaction between Heg Inc RNA and Cdk1 mRNA explained a large and high significant part of the variation in TRAb. It is easy to understand why Cdk1, which regulates cell cycle, correlates with TRAb. The mechanism of action for Heg, on the other hand, is not known. Heg may reflect effects of Nucks, but there was no correlation between Nucks and TRAb. Gene expression in mononuclear cells after incubation for 20 hours led to the expected increase in GCR and NF- κ B mRNAs, whereas Heg, Nucks mRNA and Cd14 mRNA were only slightly changed. A general inhibition of gene expression reduced the concentration of both Nucks mRNA and Heg significant.

Preliminary trials suggested that there was a correlation between Heg and CD14 mRNA. We found a significant and negative relationship between CD14 and Heg in normal subjects. A similar relationship was observed in patients treated for Graves' disease. There are several types of monocytes including CD14 cells. CD14 and CD16 are co-receptors for toll-like receptors (TLR) 4 and 7, which are necessary for regulation of the immune system.^{4,5} TLR7 becomes activated

by single stranded RNA. However, we did not analyze CD16 cells. Mononuclear cells were incubated with a fragment of Heg Inc RNA. While there was no change in the control experiment, the concentration of CD14 decreased to very small values after addition of Heg. There was also a decrease in CD14 mRNA after addition of LPS. Both exogenous and endogenous Heg were thus related to CD14. However, the effect of endogenous Heg cannot be compared with the effect of endogenous Heg. First, it was necessary to make the cell membrane permeable to nucleotides with lipofectamine. Lipofectamine had no effect on the basal Heg concentration, which indicates that this is of endogenous origin. Furthermore, the added fragment comes via the cytoplasm and is likely rapidly to come into contact with toll-like receptors. We also found that the single stranded exogenous Heg increased the concentration of TLR7 mRNA in mononuclear cells. Further study showed that CD14 cells contained a high concentration of TLR7 mRNA and far more than contents in dendritic cells and CD8 cells. The decrease in CD14 mRNA after addition of exogenous Heg is probably a consequence of activation of the CD14 protein and the subsequent shedding of the C14 protein and increase in soluble CD14. CD14 cells may also develop into macrophages and dendritic cells. Endogenous Heg may be located to the nucleus and Heg may due to its location and affinity bind to a protein in the CD14/toll-like receptor complex and activate the system, which will decrease CD14 mRNA. High levels of endogenous Heg is associated with low levels of CD14 mRNA, which may cause a low degree of inflammation and low levels of TRAb. Similarly, low concentrations of Heg may cause a high degree of inflammation and of TRAb. Our findings suggest that the observed gene expression is important for regulation of TRAb. This refers not only to the relationships observed, but also to the fact that the RNAs found reflect proteins, which are important for cell cycle and differentiation of cells. Heg Inc RNA is antisense to and overlaps a large part of exon 7 of Nucks mRNA. The Nucks protein is particularly important for regulation of access to the chromatin. However, it is important to understand that our findings have little to do due with the risk of developing autoimmunity, but rather with the regulation with the level of antibodies, which can be obtained. Are there similar changes in gene expression in Type 1 diabetes, which is

also an autoimmune disease Table 1.⁶ In patients with Type 1 diabetes the purpose of research is primarily to prevent the development of autoantibodies, which destroy islet cells. Patients who develop Graves disease are only treated, after the disease has developed. In many patients, it is possible to normalize the condition treatment with antithyroid drugs.⁷

Table 1

Serial no	Concepts
1	Heg Inc RNA in mononuclear cells was inversely correlated to TRAB in untreated patients with Grave's disease and inversely correlated to CD14 mRNA in treated patients and in normal subjects. These and other findings indicate that endogenous Heg can reduce expression of CD14 mRNA and thereby reducing inflammation and autoantibody levels.
2	Cdk1 mRNA showed a tendency to a positive relationship with TRAB in untreated patients. However, the concentration decreased 50% during thiamazole treatment. Concentrations of TRAB decreased correspondingly.
3	These and other findings indicate that Heg Inc RNA and Cdk1 RNA have an effect on levels of autoantibodies achieved without affecting the risk of developing autoimmune disease.

In our study, we found that the concentration of autoantibodies decreased about 50% during treatment with thiamazole, whereas Heg showed no change. It is well known that antithyroid drugs have a certain immunomodulating effect, often believed to be due to a decrease in thyroid hormones and a reduction in thyrocyte-immunocyte signaling.⁸ However, a controlled clinical trial supported the hypothesis of a direct immunosuppressive effect of methimazole in patients with Graves disease.⁹ It is difficult to believe that the pronounced decline of 50% in the concentration of Cdk1 mRNA in mononuclear cells during treatment may be due to changes in the thyroidea gland. The level of autoantibodies still remained increased during treatment with thiamazol. Furthermore, concentrations of Cdk1 mRNA were only marginally increased in untreated patients with Graves disease. The inhibition of Cdk1 mRNA may be due to a pharmacological effect of thiamazol in mononuclear cells. It is therefore of interest to investigate gene expression in mononuclear cells in Type 1 diabetes to see, if there are similar changes as in Graves disease. It is possible although speculative, that antithyroid drugs may induce some reduction in the autoantibody levels in patients with Type

1 diabetes. Our results support the hypothesis that inhibition of Cdk1 or CK2 by new drugs may inhibit the production of autoantibodies and autoimmunity in the organism.

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None.

Conflict of interest

Author declares there is no conflict of interest.

References

- Christensen NJ, Habekost G, Bratholm P. A RNA transcript (Heg) in mononuclear cells is negatively correlated with CD14 mRNA and TSH receptor auto antibodies. *Clinical & Experimental Immunology*. 2008;154(2):209–215.
- Christensen NJ, Habekost G, Bratholm P. Regulation of TSH Receptor Auto antibodies by a long Non-coding RNA (Heg) and Cdk1—A Review. *British Journal of Medicine & Medical Research*. 2013;3(3):508–516.
- Wells AD, Morawski PA. New roles for cyclin-dependent kinases in T cell biology: linking cell division and differentiation. *Nature Reviews Immunology*. 2014;14(4):261–269.
- Deane JA, Pisitkun P, Barrett RS, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation immunity. *Immunity*. 2007;27(5):801–810.
- Frank L, Veerdonk, Netea MG. Diversity: A Hallmark of Monocyte Society. *Immunity*. 2010;33(3):289–291.
- Jacobsen LM, Haller MJ, Schatz DA. Understanding Pre-Type 1 Diabetes; The Key to Prevention. *Frontiers in Endocrinology*. 2018;9:1–8.
- Christensen NJ, Habekost G, Bratholm P. Decrease in TSH receptor auto antibodies during ant thyroid treatment: Relationship with a long noncoding Heg RNA and Cdk1 mRNA in mononuclear cells. *ISRN Endocrinology*. 2011.
- Volpe R. The Immunomodulatory Effects of Anti-thyroid Drugs are Mediated via Actions on Thyroid Cells, Affecting Thyrocyte-immunocyte Signalling A Review. *Current Pharmaceutical Design*. 2001;7(6):451–460.
- Lechpammer M, Lukac J, Lechpammer S, et al. Antithyroid drug-induced immunomodulation in Graves' disease patients. *Acta Medica Croatica*. 2002;56(1):21–26.