CCR5 in multiple sclerosis : expression, regulation and modulation by statins

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Statins and CIITA

Statins and control of *MHC2TA* gene transcription

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Stabins and control of MHC2TA gene transcription

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To the editor:

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, are potent inhibitors of the mevalonate pathway, which governs biosynthesis of isoprenoids and cholesterol [1]. Because of their lipid-lowering effects, statins are widely used in medical practice for the treatment of coronary disease. Recently it has been suggested that statins also have immunomodulatory properties and may, in this way, have a role in the regulation of immune responses [2,3]. Statins reduce IFN-γ-induced and constitutive membrane expression of major histocompatibility complex (MHC) class II molecules in addition to modulation of proinflammatory cytokine expression and expression of other immune relevant molecules in different cell types [4-7].

Initiation of immune responses relies on the presentation of peptide in the context of MHC class II molecules by 'professional' antigen-presenting cells (APCs) to $CD4^+$ T cells. Therefore, MHC class II molecules are expressed constitutively on APCs whereas on 'non-professional' APCs their expression can be induced by IFN-γ or after activation, as observed in activated human T-lymphocytes. Essential for transcription of genes encoding MHC class II is the class II transactivator (CIITA, encoded by *MHC2TA*) [8]. Because of the required regulated control of MHC class II molecules, to ensure the initiation of proper immune responses against pathogens, *MHC2TA* expression itself is tightly regulated through the activation of tissue-specific promoter elements of which *MHC2TA* promoter I (*MHC2TA*-PI) is used specifically in dendritic cells, whereas

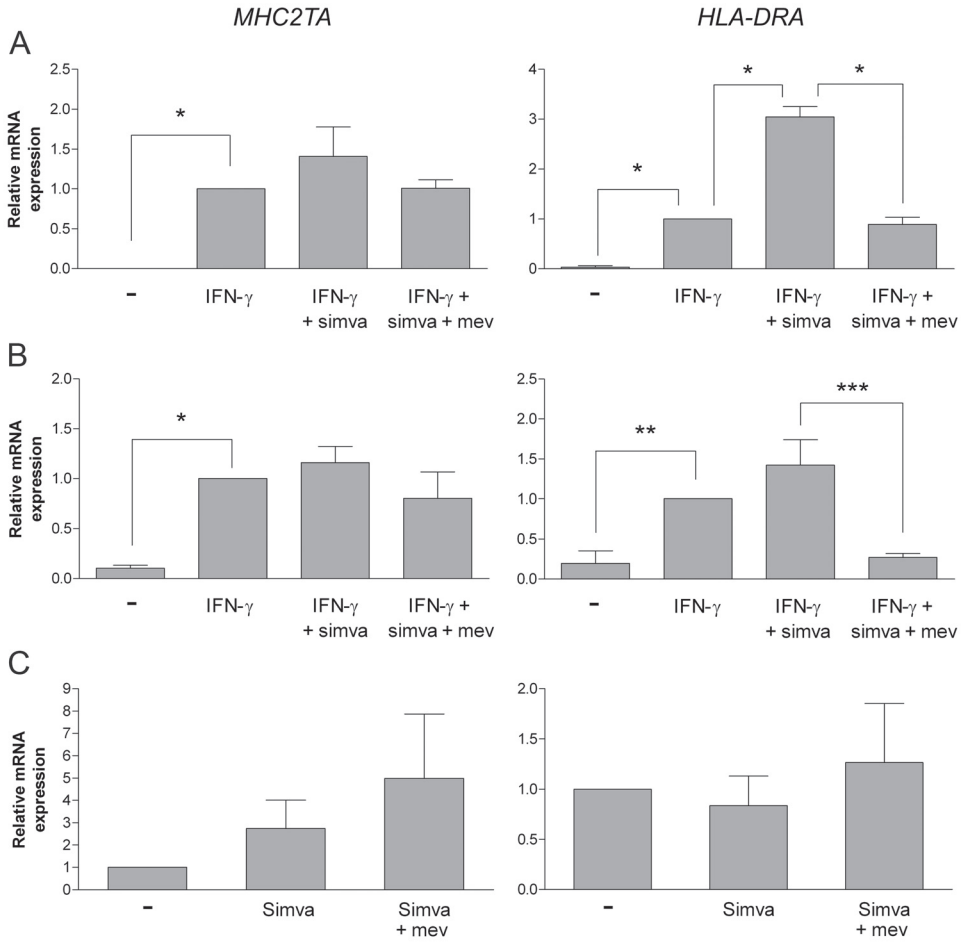

Figure 1. Simvastatin does not inhibit *MHC2TA* or *HLA-DRA* transcription. Real-time PCR determination of mRNA levels for *MHC2TA* and *HLA-DRA* in (A) HeLa (cervical carcinoma) cells and (B) U937 monocyte cells treated with IFN-γ alone or in combination with simvastatin (simva) or simvastatin and L-mevalonate (mev) and (C) human activated primary T cells treated with simvastatin or simvastatin and L-mevalonate. We calculated expression levels after these treatments relative to (A and B) IFN-γ induced expression and (C) unstimulated conditions using the comparative Ct method (comparative expression level = $2^{-(\text{stimulated } \Delta Ct - \text{control } \Delta Ct)}$, ΔCt corrected for *18S* expression). Data shown as mean + s.e.m. of three independent experiments and statistically significant differences according to two-tailed Student *t*-test: * $P < 0.01$, ** $P < 0.05$, *** $P < 0.1$.

MHC2TA-PIII is activated in several cell types of the hematopoietic lineage (*e.g.*, B cells) [8]. *MHC2TA*-PIV is the principal IFN-γ-responsive promoter and is activated after exposure of non-bone marrow-derived cells to IFN-γ [8].

It has been shown that statins specifically mediate their effect on IFN-γ-induced MHC class II molecule expression through downregulation of transcription of the IFN-γ-induced PIV-isoform of *MHC2TA* [4]. On the other hand, it has been shown that statins also affect the IFN-γ-mediated induction of the PI-isoform [5] and downregulate membrane expression of MHC class II molecules in B cells, which is mediated by the PIII promoter [6]. Based on these observations it would seem that statins affect the activity of all *MHC2TA* promoters, which could explain the downregulation of cell-surface MHC class II expression in a variety of different cell types [4-6].

We have investigated the effect of statins on cell-surface expression of MHC class II molecules with emphasis on *MHC2TA* mRNA transcript levels by real-time RT-PCR and on activation of *MHC2TA* promoters in reporter assays in a variety of different cell types. The results of our experiments do not show a downregulatory effect of simvastatin on *MHC2TA* transcription, under the same or similar experimental conditions as used in previous studies (Figure 1) [4,5]. This is corroborated by the observation that simvastatin does not interfere in IFN-γ-induced activity of *MHC2TA*-PIV, nor in activity of *MHC2TA*-PIII (Figure 2). In contrast, under the conditions used in the previously mentioned reports [4-6], we do observe a reduction of constitutive and IFN-γ-induced cell-surface expression of MHC class II molecules after simvastatin treatment of various cell types used in our studies. This suggests that an alternative mechanism may contribute to the observed inhibition of MHC class II cell-surface expression by simvastatin.

Further suggestions for an alternative mechanism by which statins display immunomodulatory properties comes from the reported observations that statins, in addition to their effect on cell-surface expression of MHC class II molecules, also inhibit expression of immunoregulatory molecules such as adhesion molecules, costimulatory molecules and chemokine receptors, diminish the production of proinflammatory cytokines and chemokines, and reduce adhesion and proliferation of various cell types [5-7]. We have made similar observations and have found that statins, in addition to MHC class II molecules, also inhibit membrane expression of a number of additional molecules that have important roles in immunity (Kuipers et al., unpublished observations). Subsequent investigations have provided evidence in support of the notion that the effect of statins on cell-surface MHC class II expression is caused by disruption of cholesterol-containing microdomains (H. Kuipers, P. Biesta, T. Groothuis, J. Neefjes, A. Mommaas, P. van den Elsen, unpublished data). These so-called 'lipid rafts' are important for the intracellular transport, recycling and concentration to the cell-surface of MHC class II molecules [9]. In general, glycosylphosphatidylinositol-linked proteins are characteristic components of biochemically defined lipid rafts and rely on the integrity of these cholesterol-containing vesicles for transport and function at the cell-surface. This explains that through disruption of lipid rafts statins have a

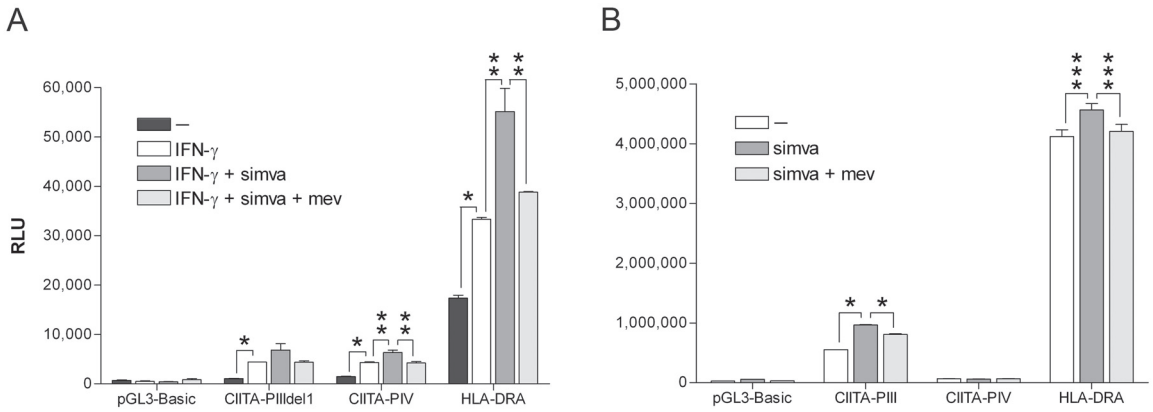

Figure 2. Simvastatin does not reduce *MHC2TA*-PIII, *MHC2TA*-PIV or *HLA-DRA* promoter activity. **A** U937 monocyte cells transfected with luciferase reporter constructs containing the *MHC2TA*-PIIIDEL1 (which includes the IFN-γ inducible upstream region of *MHC2TA*-PIII), *MHC2TA*-PIV or *HLA-DRA* promoters treated with IFN-γ alone or in combination with simvastatin (simva) or simvastatin and L-mevalonate (mev). **B** Raji B cells transfected with luciferase reporter constructs containing the *MHC2TA*-PIII, *MHC2TA*-PIV or *HLA-DRA* promoters, treated with simvastatin alone or in combination with L-mevalonate. Data shown as mean relative light units (RLU) + s.e.m. of one representative of three independent experiments. Asterisks indicate significance of differences according to two-tailed Student *t*-test: * $P < 0.01$, ** $P < 0.05$, *** $P < 0.1$. The pGL3-Basic luciferase reporter plasmid served as a control.

much broader effect, impairing membrane expression and function of a variety of different proteins.

In conclusion, we feel that the immunomodulatory properties of statins do not seem to be caused through specific inhibition of *MHC2TA* transcription mediated by the various isotypic promoters. More probably, we hypothesize that disruption of lipid rafts, essential components in a variety of immune processes essential for antigen presentation and subsequent T-cell activation, explains the downregulatory effects of statins on cell-surface expression of MHC class II and other immunoregulatory molecules. This notion is in line with observations made by others [10].

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