ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 20 (2005) 961 – 968

Anti-inflammatory nuclear receptor superfamily in multiple sclerosis patients from Sardinia and Sweden

Xuan Liu,[a,1] Knut R. Steffensen,[b,1] Alessandra Sanna,[c] Giannina Arru,[c] Maria Laura Fois,[c] Giulio Rosati,[c] Stefano Sotgiu,[c] Hans Link,[a] Jan-Åke Gustafsson,[b] and Yu-Min Huang[a,*]

[a]*Neurotec Department, Division of Neuroimmunology, Karolinska Institute, 141 86 Stockholm, Sweden*
[b]*Department of Biosciences, Karolinska Institutet at NOVUM, Huddinge, Sweden*
[c]*Institute of Clinical Neurology, University of Sassari, Sassari, Italy*

Received 5 March 2005; revised 17 April 2005; accepted 6 June 2005
Available online 14 July 2005

Several nuclear hormone receptors have been associated with inflammatory reactions. Particularly, liver X receptors (LXRs) have recently been identified as key transcriptional regulators of genes involved in lipid homeostasis and inflammation. LXRs are negative regulators of macrophage inflammatory gene expression. Multiple sclerosis (MS), a demyelinating disease of the central nervous system of unknown cause, is characterized by recurrent inflammation involving macrophages and their inflammatory mediators. Sweden belongs to the countries with a high MS incidence. In Italy, the MS incidence is lower, except on the island of Sardinia where the incidence is even higher than in Sweden. Subjects from Sardinia are ethnically more homogeneous, and differ from Swedes also regarding genetic background and environment. We studied mRNA expression of several nuclear hormone receptors in blood mononuclear cells (MNC) from female patients with untreated relapsing-remitting MS from Sassari, Sardinia, and Stockholm, Sweden. Sex- and age-matched healthy controls (HC) were from both areas. mRNA expression was evaluated by quantitative real-time PCR. We found altered mRNA expression of LXRs, estrogen receptors (ERs), and androgen receptor (AR) in MS. mRNA expression of both LXRα and LXRβ is lower in MS from Stockholm but not from Sassari. In particular, LXRα mRNA expression was significantly lower in MS from Stockholm as compared with all groups in the study including MS from Sassari. Low levels of ERα mRNA are seen in MS from both Stockholm and Sassari. The splice variant ERβcx showed significantly higher mRNA expression in MS from Sassari and Stockholm as compared with corresponding HC. In particular, ERβcx mRNA in MS from Sassari was remarkably higher as compared with all other groups in the study. Higher levels of AR mRNA are present in HC from Sassari. The findings indicate that the expression levels of anti-inflammatory nuclear receptor superfamily genes in MS appear to reflect both ethnic and environmental influences.

Keywords: Multiple sclerosis; Liver X receptors; Estrogen receptors; Androgen receptor; Sardinia

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system of unknown aetiology. The clinical course is usually characterized by relapses and remissions which, sooner or later, are followed by disease progression and, finally, severe disability. There is evidence for an altered immune balance in MS patients both within the central nervous system and cerebrospinal fluid (CSF) compartments and systemically. The extent of such changes is still under study and their role remains enigmatic, as the pathogenesis of MS is also elusive. Difficulties to identify the cause and to clarify the pathogenesis can in part be attributed to the complex disease traits that involve both genetic and environmental factors. MS has profound heterogeneity in epidemiological distribution, genetic susceptibility, clinical manifestations, pathological lesion patterns, immunological alterations, and clinical responses to the various therapies that are offered and that are at best only partially effective. Taken together, such a complexity seems to indicate a pathogenic heterogeneity in MS.

Prevalence studies point to a north–south gradient for MS in Europe, with a clearly higher prevalence in e.g. Scandinavia compared to countries in the south (Kurtzke, 1997). There is an unexplained clustering of MS on the Italian island of Sardinia when compared to the rest of Southern Europe, reaching prevalence numbers that may even exceed those from e.g. Sweden. The populations on Sardinia and in Sweden are environmentally as well as ethnically distinct (Underhill et al., 2000; Sotgiu et al., 2004). Sardinians are genetically more homogeneous and geographically more isolated compared to Swedes. Studies comparing such diverse populations could provide pieces of information to identify risk factors common to MS and, thereby, increase our understanding of the disease pathogenesis.

In a recent study, we presented evidence for the existence in MS in general of a pro-inflammatory state that is reflected by enhanced expression of the cytokine IL-12p40 in both Sassari on Sardinia and Stockholm, Sweden (Huang et al., 2005). A remarkable heterogeneity was, however, observed between Sassari and Stock-

* Corresponding author.
E-mail address: yu-min.huang@neurotec.ki.se (Y.-M. Huang).
[1] The first two authors contributed equally.
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2005.06.001

holm for the expression of tumour necrosis factor-alpha (TNF-α), IL-6, and indoleamine 2,3 dioxygenase (IDO). These findings indicate heterogeneity traits of MS that may also have clinical implications for e.g. future individualized MS therapy.

The present study aims at investigating mRNA expression of nuclear hormone receptors (NHRs) which are associated with inflammatory signalling. NHRs represent a large superfamily of transcription factors with a plethora of biological functions including development, metabolism, and homeostasis (Nagpal, 2003; Robinson-Rechavi et al., 2003). In particular, liver X receptors (LXRs) and their relevant target genes have been shown to play an important role not only in cholesterol and lipid metabolism, but also in inflammatory processes and innate immunity (Chawla et al., 2001; Joseph et al., 2004; Steffensen and Gustafsson, 2004). LXRα null mice develop enlarged, cholesterol-loaden livers and elevated serum cholesterol levels upon exposure to high-cholesterol diets (Repa and Mangelsdorf, 2002). LXRα,β null mice accumulate cholesterol in liver, lung, spleen, vessel walls, and CNS even on a normal diet with age (Schuster et al., 2002; Wang et al., 2002). LXR ligand inhibits atherosclerosis development in murine models of atherosclerosis (Joseph et al., 2002). Recently, a role for LXRs has been described in macrophage biology (Landis et al., 2002; Joseph et al., 2003), including the protective functions of innate immunity and the reduction of inflammation (Joseph et al., 2004).

The biological actions of LXRs relate in part to their ability to regulate ATP-binding cassette transporter A1 (ABCA1) (Schmitz and Drobnik, 2002) and the stearoyl CoA desaturase (SCD-1) (Wang et al., 2004). Other NHR involved in inflammatory reactions are three peroxisome proliferator-activated receptor (PPAR) paralogues (PPARα, β/δ, and γ) (Chinetti et al., 2004), the retinoic acid receptor paralogues (RARα, β, and γ), and the retinoid X receptor paralogues (RXRα, β, and γ) (Costet et al., 2003; Peng et al., 2004).

Among NHRs, estrogen receptors (ERs) (Offner, 2004) and the steroid/NHR superfamily such as androgen receptor (AR) (Palaszynski et al., 2004) have recently been reported to have anti-inflammatory functions, and considered to have immunoprotective effects in MS experimental animal models and, possibly, in MS (Offner, 2004). ERα is also involved in LXR network and play a roll in lipid metabolism.

In this study, inflammation-related NHRs, LXRs, and their target genes, and the sex hormone receptors ERs, and the steroid/NHR superfamily are examined at gene levels in highly matched MS patients and healthy controls (HC) from two different parts of the world with distinct populations and environments.

Materials and methods

Patients

Fifteen MS patients from Sassari and 22 from Stockholm were enrolled in the study. According to the study design, all MS patients were females and fulfilled the Poser criteria for clinically definite MS. The average age of the MS patients from Sassari and Stockholm is 31 ± 6 (20–40) and 32 ± 7 (19–45) years, respectively. All patients were diagnosed as having relapsing-remitting MS, and all were in the phase of clinical remission for at least 6 months at the time of sampling. All patients had MS-like lesions on MRI of the brain and oligoclonal IgG bands in the CSF while undetectable in corresponding serum. The average levels of the expanded disability status scale (EDSS) were 2 ± 1 (0–4) and 2 ± 1 (0–3.5), respectively. None of the patients had ever been treated with the disease-modulating drugs interferon-β or Copaxone, and none had received steroids for the last 6 months prior to inclusion in the study. None of the patients included had ever received any other immunosuppressive drugs. Fifteen age-matched female HC from Sassari and 22 from Stockholm were enrolled during the same time periods as the MS patients. The average age of HC from Sassari and Stockholm was 30 ± 5 (24–44) and 32 ± 7 (21–44) years, respectively. The blood sampling period in Sassari was between May 2002 and June 2003, and in Stockholm between Feb. 2002 and June 2003.

Preparation and storage of mononuclear cells

All the materials and reagents used in the study were from the same source and the same batch. 30 ml of blood from MS patients and HC was obtained in heparinized tubes. Mononuclear cells (MNC) were separated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). Cell viability as measured by Trypan blue exclusion always exceeded 95%. MNC were divided into aliquots of $5–10 \times 10^6$ cells and pelleted by centrifugation. The cells were then lysed in 1 ml of TRIZOL reagent (Gibco, Paisley, UK), quickly frozen in liquid nitrogen, and stored at −70°C until further use.

Isolation of total RNA, DNAse I treatment, and cDNA synthesis

Total RNA was isolated with RNeasy® Mini Kit (Qiagen, Valencia, CA) according to the standard protocol. The concentration and quality of the purified total RNA were determined spectrophotometrically at OD_{260} nm, and the $OD_{260/280}$ ratio was calculated. To eliminate any residual genomic DNA, the RNA samples were incubated with DNAse I (1 U/1 μg of RNA; Invitrogen, Carlsbad, CA) for 15 min at room temperature, and inactivated by addition of 1 μl of 25 mM EDTA to the reaction and heated at 65°C for 10 min. Synthesis of single-strand cDNA was carried out using SuperScript™ II RNase H^- Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions. The reaction was incubated at 25°C for 10 min, at 43°C for 60 min and at 70°C for 15 min.

Real-time polymerase chain reaction and PCR analysis

Totally, 19 genes were selected for the study. PCR primers for these selected genes and for β-actin used as internal control were designed using Primer Express® Software version 2.0, a software program specially provided with the 7700 SDS (Applied Biosystems, Foster City, CA). Primer sequences are shown in Table 1.

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay on the basis of SYBR Green I (Ramos-Payan et al., 2003) was performed using ABI PRISMER 7700 Sequence Detection System (Perkin-Elmer–Applied Biosystems, Foster City, CA). For each gene specific primer pairs, a dissociation curve analysis was conducted to verify the specificity of the primer pairs. Briefly, Master Mix (90 μl) containing 200 nM primers, 45 μl 2× qPCR™ Mastermix Plus for Sybr™ Green I (Eurogentec, Ghent, Belgium), and 2 μl cDNA (20 ng) were mixed before aliquoting in triplicate to a 96-well microtiter plate. The cDNA was amplified under the following universal conditions: one cycle at 50°C for 2

Table 1
The sequence of primers for real-time RT-PCR (all primers 5′–3′)

Primer	Forward primer	Reverse primer
LXRs and their relevant genes		
LXRα	TGCCCCATGGACACCTACAT	CCAGCTGACGGCATTTG
LXRβ	CCTACCACGAGTTCCCTGGAT	TCCTTTACAGTGGGTGAAGAAGAAG
PPARα	CCTCCTCGGTGACTTATCCTGT	TTCGATGTTCAATGCTCCACTG
PPARδ(β)	CATGGAGCAGCCACAGGAG	AAGTGCATGCTGTGGTCCC
PPARγ	GAAGAGCCTTCCAACTCCCTC	GAACTCCATAGTGAAATCCAGAAGC
RARα	TCTTCATCACCAGCAAAACGC	GTCACCAACCGAGCAGG
RARβ	CCACTGGACCATGTAACTCTAGTGTC	CATCAAGAAGGGCTGGAAAAAA
RARγ	GAAGACCCTGCCATTCCACA	CCTTCTCCTTTGTCCCCTTTTT
RXRα	TTTCCGTTGCTGTTTATCGATG	AGTATTCAGAGCAGATGAAGATGTCAC
ABCA1	TGTCCAGTCCAGTAATGGTTCTGT	CGAGATATGGTCCGGATTGC-3′
SCD-1	GAGTACCGCTGGCACATCAA	ATGGCGGCCTTGGAGACT
Estrogen receptors		
ERα	ATCCTGATGATTGGTCTCGTCT	GGATATGGTCCTTCTCTTCCAGA
ERβ	TCCATGCGCCTGGCTAAC	CAGATGTTCCATGCCCTTGTTA
ERβ-Cx	TCCATGCGCCTGGCTAAC	CCATCGTTGCTTCAGGCAA
Other hormone receptors		
AR	TCACCAAGCTCCTGGACTCC	CGCTCACCATGTGTGACTTGA
PR	CCAGCATGTCGCCTTAGAAAGT	CATCCAGTGCTCTCACAACTCTGA
GR	ACTTGGAGGATCATGACTACGCTC	TGCAGTAGGGTCATTTGGTCATC
β-Actin	ACGAGGCCCAGAGCAAGAG	TCCCCGTTGGCGATGAT-3′

min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min.

PCR data analysis

To correct for variations in input RNA amounts and efficiency of reverse transcription, an endogenous 'housekeeping' gene, β-actin, was also quantified and used to normalize the results. For each sample, the normalized cycle threshold (Ct) value was obtained by subtracting the Ct value of gene of interest from the Ct value of beta-actin (normalized Ct = Ct of beta-actin-Ct of gene of interest). The results are presented as relative fold changes with β-actin as internal control.

Post-PCR data mining

All PCR assays showed intra- and inter-assay variations of less than 5%. Statistical analysis was performed using the GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). To compare the significance between all MS patients and all HC, unpaired *t* test or Mann–Whitney test (nonparametric distribution) were used according to the distribution of data variances. A P value < 0.05 was considered to indicate a significant difference between groups. For multiple comparisons between subgroups, parametric ANOVA test was used for normally distributed data, followed by Bonferroni's test if $P < 0.05$. Otherwise, Kruskal–Wallis test was performed, followed by Dunn's multiple comparison test. All values are given as mean ± SEM.

Results

mRNA expression of LXRs and their target genes

LXR alpha (LXRα) and LXR beta (LXRβ) are highly homologous transcription factors belonging to the same sub-family of NHR. LXRs function as master regulators of cholesterol metabolism and control cholesterol efflux (Joseph et al., 2002) and it was recently discovered that LXRs have protective function in innate immunity (Joseph et al., 2004). In this study, we used quantitative real-time polymerase chain reaction (PCR) to determine mRNA expression levels of both LXRα and LXRβ in blood MNC in female MS patients compared to age-matched female HC from Sassari and Stockholm.

LXRα mRNA was significantly lower in all MS patients (n = 36) as compared with all the HC included (n = 36) (Fig. 1A; $P < 0.05$). Using multiple comparisons, lower levels of LXRα mRNA were confined only to the MS patient group from Stockholm. MS patients from Stockholm showed remarkably lower LXRα mRNA (2.6 ± 0.2, Fig. 1A) as compared with HC from Stockholm (3.4 ± 0.2, $P < 0.01$), MS patients (3.8 ± 0.2, $P < 0.001$), and HC (4.0 ± 0.2, $P < 0.001$) from Sassari. No difference was detected between the MS patients and HC from Sassari, and the HC from Stockholm.

mRNA expression of LXRβ did not differ between the groups comprising all MS patients (n = 37) and all HC (n = 37) (Fig. 1B). However, the MS patients from Stockholm showed lower levels of LXRβ mRNA (2.9 ± 0.3) as compared with HC from Stockholm (4.1 ± 0.4; $P = 0.02$) as was seen for LXRα. The MS patients and HC from Sassari showed equal levels of LXRβ mRNA (4.2 ± 0.6 vs. 4.1 ± 0.5, $P > 0.05$).

Several genes have been shown to be under direct or indirect transcriptional control of LXRs either via their cognate response elements in their respective promoters or via other factors (Steffensen and Gustafsson, 2004). Of particular interest are the ABCA1 and SCD. But in this study, we did not find significant differences in expression patterns of ABCA1 or SCD-1 between the whole group of MS patients and all the HC. Nor did we observe any differences between MS patients and HC from Stockholm or from Sassari (data not shown). Similar negative observations were made for other NHRs including PPARα, β/δ, and γ; RARα, β, and γ; and RXRα, β, and γ (data not shown).

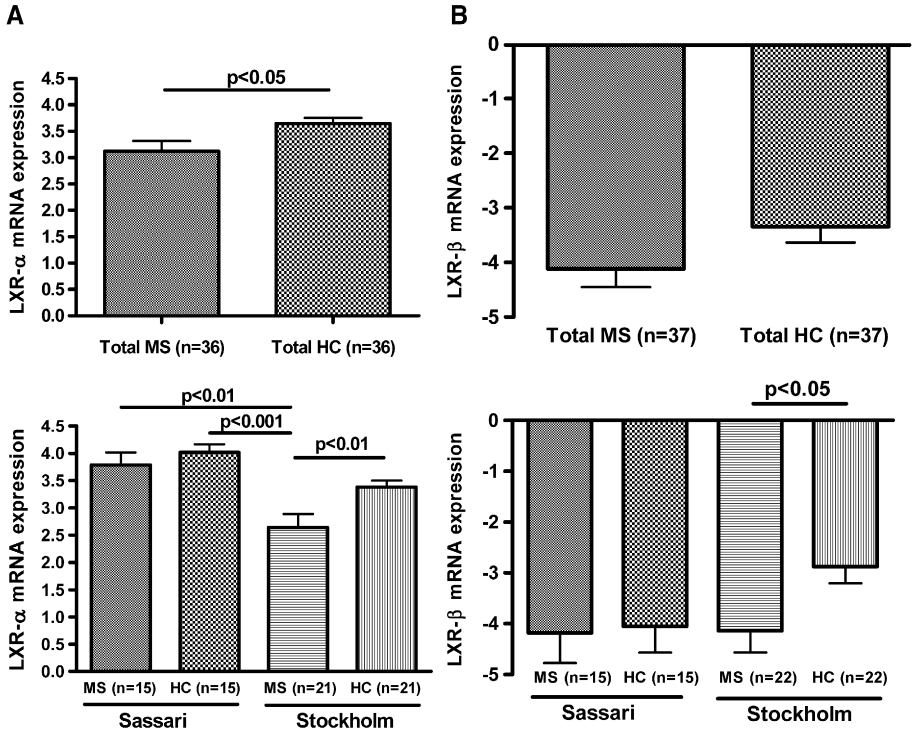

Fig. 1. Liver X receptor (LXR) mRNA expression in blood mononuclear cells (MNC) from patients with multiple sclerosis (MS) and healthy controls (HC). mRNA levels were quantified by real-time RT-PCR. mRNA values are normalized to β-actin. Values represent mean ± SEM. Each sample is examined in triplicate. (A) Lower levels of LXRα mRNA are detected in the group of all MS patients compared to all HC, and confined to MS patients from Stockholm as compared to other groups in the study. (B) LXRβ mRNA is lower in MS than in HC from Stockholm.

mRNA expression of estrogen receptors

The estrogen receptors ERα and ERβ also belong to the NHR superfamily and are estrogen inducible transcription factors. Estrogen can inhibit the clinical signs and histopathological lesions of experimental autoimmune encephalomyelitis (EAE) (Polanczyk et al., 2003), and is used in clinical trials to treat MS (Offner, 2004). Using mutant mice in which ERα has been inactivated, it was found that ERα was responsible for the estrogen-mediated inhibition of EAE (Garidou et al., 2004). Most recently, ERs have been shown to regulate the severity of EAE in female as well as male mice (Polanczyk et al., 2004). Estrogen also inhibits lipid uptake and reduces the development of atherosclerosis (Kramer and Wray, 2002). ERβcx does not bind any ligand, nor does it activate transcription. ERβcx preferentially heterodimerizes with ERα, inhibiting ERα DNA binding and subsequent ERα signalling. All 3 estrogen receptor variants were examined for mRNA expression by quantitative real-time PCR in MS patients and HC from both Stockholm and Sassari.

The whole group of MS patients showed a tendency of low ERα mRNA expression (−0.6 ± 0.3, $n = 37$), but without a statistically significant difference as compared with the whole group of HC (−0.06 ± 0.3; $n = 36$; $P = 0.5$; Fig. 2A). Upon subgrouping, the MS patients and HC based on their origin, HC from Sassari showed higher ERα mRNA expression (0.6 ± 0.5) as compared with MS from the same area (−0.6 ± 0.3; $P < 0.05$), and with MS (−0.6 ± 0.5; $P < 0.05$) and HC (−0.5 ± 0.3; $P < 0.05$) from Stockholm. ERα mRNA expression was similar among MS patients from Sassari and MS patients and HC from Stockholm (Fig. 2A). No difference was observed in mRNA expression of ERβ between the whole group of MS patients and all HC, nor between the subgroups of MS patients and corresponding HC from Sassari or from Stockholm (data not shown).

The whole group of MS patients showed significantly higher ERβcx mRNA expression as compared to the whole group of HC (3.9 ± 1.0 vs. 1.8 ± 0.3; $P < 0.05$) (Fig. 2B). Upon subgrouping, MS patients from Sassari showed higher levels of ERβcx mRNA expression as compared with corresponding HC (6.3 ± 2.2 vs. 3.1 ± 0.4, $P < 0.05$), and with MS patients (2.3 ± 0.4) and HC (0.8 ± 0.4) from Stockholm ($P < 0.05$ for both comparisons). MS patients from Stockholm showed significantly higher ERβcx mRNA expression compared with corresponding HC ($P < 0.05$).

mRNA expression of other steroid/NHR superfamily members

The androgen receptor (AR), the glucocorticoid receptor (GR), and the progesterone receptor (PR) are also members of the steroid/NHR superfamily. They are involved in the lipid metabolism and the reduction of inflammation with the linkage to LXRs. Therefore, these 3 receptors were also included in this study.

In accordance with the results obtained for ERα mRNA expression, the whole group of MS patients showed a tendency of lower AR mRNA expression (1.3 ± 0.3, $n = 37$) as compared with all HC (1.9 ± 0.3, $n = 36$), but without reaching statistical significance ($P = 0.2$; Fig. 3). Multiple comparison analysis showed that HC from Sassari had higher AR mRNA expression (2.9 ± 0.2) as compared with MS patients from the same area (1.4 ± 0.3, $P < 0.05$), and MS patients (1.3 ± 0.4, $P < 0.05$) and HC (1.1 ± 0.3, $P < 0.01$) from Stockholm.

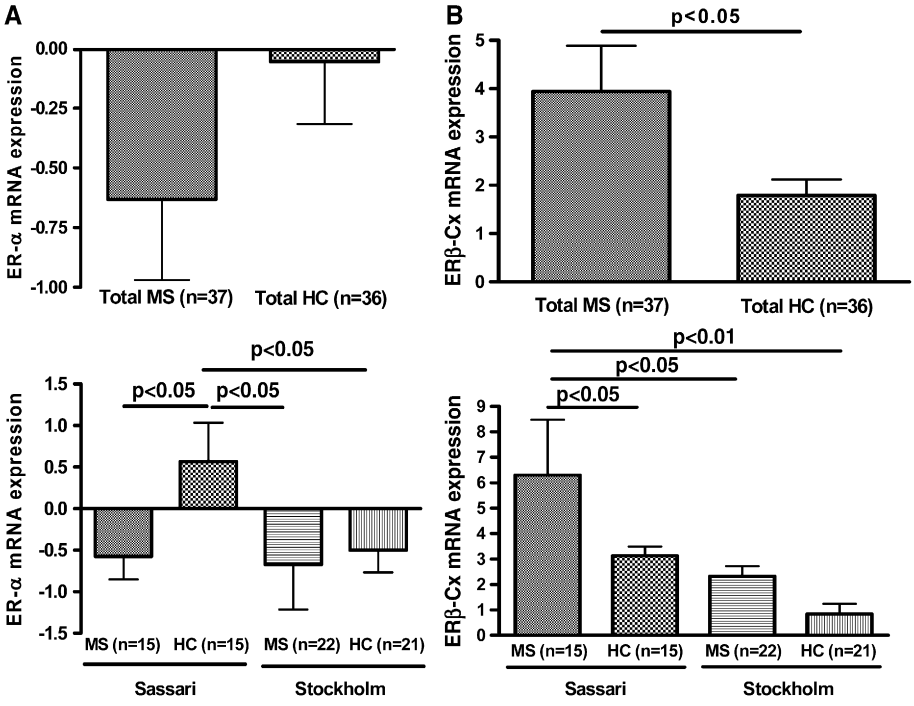

Fig. 2. Estrogen receptor (ER) mRNA expression by MNC from MS and HC. (A) Levels of ERα mRNA are lower in all the MS patients examined as compared with all HC without statistical significance. HC from Sassari have higher levels of ERα mRNA when compared with the MS from Sassari and subjects from Stockholm. Such a difference was not observed between MS and HC from Stockholm. (B) The splice variant ERβcx mRNA is significantly higher in all MS as compared with all HC. MS from Sassari has remarkably higher ERβcx mRNA than HC from the same area, and from both MS and HC from Stockholm. MS from Stockholm have higher ERβcx mRNA than HC from the same area ($P < 0.05$).

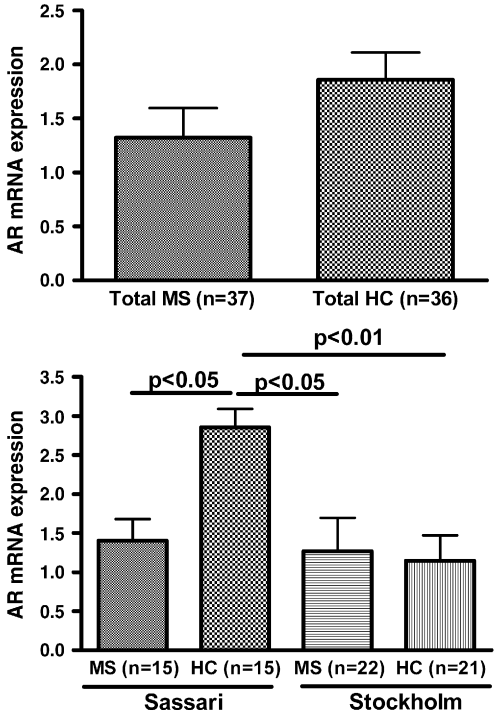

Fig. 3. Androgen receptor (AR) mRNA expression by MNC from MS and HC. Levels of AR mRNA do not differ between all MS patients and all HC. HC from Sassari have significantly higher AR mRNA levels as compared with other groups in the study.

No difference in mRNA expression profiles was observed for GR or PR between the whole group of MS patients and all HC, nor between the subgroups of MS patients and their corresponding HC from Sassari or Stockholm (data not shown).

Discussion

In a recent study comparing MS patients and HC from Sassari and Stockholm, we focused on pro- and anti-inflammatory cytokines involved in aggressive autoimmune responses, and reported the existence of a pro-inflammatory state in MS in general (Huang et al., 2005). In the present work, we aimed at identifying NHRs which are involved in innate immunity and inflammation in the two populations under study.

We found that MS patients from Stockholm showed lower mRNA expression of both LXR paralogues as compared with HC from the same area. In particular, LXRα mRNA expression was significantly lower as compared with all other groups including the MS patient group from Sassari. In contrast, LXR levels were not changed in MS from Sassari. LXRs have recently emerged as a new target for the treatment of multiple diseases such as inflammation, diabetes, and neurodegenerative diseases due to their control of lipid metabolism and inflammatory response (Whitney et al., 2003; Cao et al., 2004; Collins, 2004). However, the involvement of LXRs in inflammatory diseases has up to now not been verified. Recently, Tontonoz and colleagues used LXR single- or double-knockout mice and documented that the animals devoid of LXRs are more susceptible to *Listeria monocytogenes*, developing higher bacterial burdens with higher demise following infection (Joseph et al., 2004).

Chronic inflammatory responses are often activated by exogenous lipids derived from the membranes of microbial pathogens as well as by endogenous lipids generated from cholesterol metabolism (Freeman and Moore, 2003). Lipid-induced inflammatory responses can lead to a pro-inflammatory state and to autoimmune responses which are thought to be involved in a wide variety of human chronic inflammatory diseases. Therefore, it has not come as a surprise that cholesterol-reducing statins are claimed to have desirable immunomodulatory and anti-inflammatory effects in MS (Stuve et al., 2003), although proof is still lacking for a linkage between lipid metabolism and inflammation in MS. Being a link gene of negative regulator of lipid metabolism and reduction of inflammation, LXR expression would be expected to be lower in MS than in HC. But this is not the case in MS from Sassari. The results thus raise several questions. First, does the deficit of LXRs in MS from Stockholm play an important role in the context of innate immunity and/or anti-inflammation? Are cholesterol metabolism and host defense related in MS from Stockholm but not in MS from Sassari? If so, do LXRs mediate a link between lipid metabolism and inflammation in MS from Stockholm? Does it correlate to the different patterns of pro-inflammatory cytokine expression as observed in MS from Stockholm vs. Sassari (Huang et al., 2005)? Could LXR ligands be helpful to treat MS with low LXR mRNA? Efforts to solve these questions might open up new fields of MS research.

The role of sex hormones in MS can be verified by the well-established clinical facts that two-thirds of MS patients are women, the average age of onset overlaps the childbearing years, and the frequency of relapses decreases in late pregnancy but increases in the post-partum period (Paty and Ebers, 1998). This protective effect is probably related to hormonal and immune response changes induced by pregnancy. Estrogens may be responsible, at least in part, for this protective effect (Devonshire et al., 2003). Estrogen is also known to suppress certain infectious diseases (Salem, 2004), as well as T cell-mediated EAE (Polanczyk et al., 2003; Garidou et al., 2004), an animal model for MS. Estrogen exerts its effects through two nuclear hormone receptors, ERα and β (Nilsson and Gustafsson, 2002). It has recently been documented that ERα is the main estrogen receptor in mouse adipose tissue, with ERα mRNA levels being several hundred times higher than levels of ERβ (Lundholm et al., 2004). In addition, the effects of estrogen in EAE are dependent on ERα but not ERβ (Polanczyk et al., 2003). Mice with deleted ERα develop systemic lupus erythematosus disseminatus (Shim et al., 2004a), and mice with deleted aromatase develop Sjögren's syndrome (Shim et al., 2004b). ERβcx, a nonligand binding splice variant of ERβ, shows preferential heterodimerization with ERα, acting as antagonist of ERα.

MS from Sassari showed significantly lower mRNA expression of ERα but not ERβ as compared with the HC from the same area. Individuals from Stockholm, both MS and HC, also showed lower levels of ERα mRNA as compared with HC from Sassari. Levels of ERβcx were also lower in HC from Sassari as well as in MS and HC from Stockholm, as compared with MS from Sassari. Most interestingly, HC from Stockholm had significantly lower ERβ-cx mRNA levels as compared with MS from the same area. These findings indicate a deficiency of ERα mRNA in MS from both areas, but in a different pattern. ERs and LXRs cross talk (at least in adipose tissue) (Lundholm et al., 2004). This fact suggests that both NHRs, in concert, might play a role in MS.

AR expression plays an important role in the proliferation of human prostate cancer and confers a better prognosis in breast cancer (Yeap et al., 2004). A role for androgen is not well documented in MS. But androgen is supposed to have protective effects in male EAE mice (Bebo et al., 1998; Palaszynski et al., 2004). The androgen receptor (AR) belongs to the classical steroid hormone receptors and acts as a ligand-activated transcription factor that binds DNA response elements as a homodimer (McEwan, 2004). AR and its ligands regulate male sexual development and body composition. AR is also involved in the metabolism of lipids and maintenance of immunological homeostasis. In addition, AR, GR, and PR are functionally related to the ERs as well as to LXRs (Robinson-Rechavi et al., 2003). Although all the individuals included in the present study are females, there is a distinct difference in AR mRNA expression in HC from Sassari, who showed remarkably high levels of AR mRNA as compared to the corresponding MS from Sassari, and to MS and HC from Stockholm. Considering its role in lipid metabolism, inflammation and functional linkage with ERs and LXRs, AR may serve as protective factor in MS from Sassari.

Taken together, these results indicate that the nuclear hormone receptors LXRs, ERs, and AR are altered in MS. All these NHRs are associated with lipid metabolism and inflammatory signalling, and have anti-inflammatory functions (Nagpal, 2003). The present study was performed in highly matched MS patients and HC from two different parts of the world with distinct populations and environments. There is a reduced mRNA expression of LXRs in MS from Stockholm but not from Sassari. Low levels of ERα mRNA are seen in MS from both Stockholm and Sassari but in a different pattern. High levels of AR mRNA are present only in HC from Sassari which may implicate AR as a protective factor. We conclude that anti-inflammatory NHRs are involved in MS, and their gene expression levels appear to reflect both ethnic and environmental influences.

Acknowledgments

Work performed at Division of Neuroimmunology, Neurotec Department, Karolinska Institute, Stockholm, Sweden, is supported by grants from the Swedish Research Council, the European Union (grant number QLG3-CT-2001-00225), Swedish Physicians Association (SLS), Magnus Bergvalls Stiftelse, Åke Wibergs Stiftelse, the Norwegian Research Council (KRS), and the Karolinska Institute. The work performed at the Department of Biosciences, Novum, Karolinska Institute, Stockholm, Sweden, is supported by grants from the Swedish Research Council and from KaroBio AB. We thank Mats Soderstrom for financial support and providing samples; Carolina Ciumas, Hong Lian, Asa Pettersson, Charlotte Palmquist, and Ajith Sominanda for technical assistance.

References

Bebo Jr., B.F., Zelinka-Vincent, E., Adamus, G., Amundson, D., Vandenbark, A.A., Offner, H., 1998. Gonadal hormones influence the immune response to PLP 139–151 and the clinical course of relapsing experimental autoimmmune encephalomyelitis. J. Neuroimmunol. 84, 122–130.

Cao, G., Liang, Y., Jiang, X.C., Eacho, P.I., 2004. Liver X receptors as potential therapeutic targets for multiple diseases. Drug News Perspect. 17, 35–41.

Chawla, A., Repa, J.J., Evans, R.M., Mangelsdorf, D.J., 2001. Nuclear receptors and lipid physiology: opening the X-files. Science 294, 1866–1870.

Chinetti, G., Zawadski, C., Fruchart, J.C., Staels, B., 2004. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. Biochem. Biophys. Res. Commun. 314, 151–158.

Collins, J.L., 2004. Therapeutic opportunities for liver X receptor modulators. Curr. Opin. Drug Discovery Devel. 7, 692–702.

Costet, P., Lalanne, F., Gerbod-Giannone, M.C., Molina, J.R., Fu, X., Lund, E.G., Gudas, L.J., Tall, A.R., 2003. Retinoic acid receptor-mediated induction of ABCA1 in macrophages. Mol. Cell. Biol. 23, 7756–7766.

Devonshire, V., Duquette, P., Dwosh, E., Guimond, C., 2003. The immune system and hormones: review and relevance to pregnancy and contraception in women with MS. Int. MS J. 10, 44–50.

Freeman, M.W., Moore, K.J., 2003. eLiXiRs for restraining inflammation. Nat. Med. 9, 168–169.

Garidou, L., Laffont, S., Douin-Echinard, V., Coureau, C., Krust, A., Chambon, P., Guery, J.C., 2004. Estrogen receptor alpha signaling in inflammatory leukocytes is dispensable for 17beta-estradiol-mediated inhibition of experimental autoimmune encephalomyelitis. J. Immunol. 173, 2435–2442.

Huang, Y.M., Liu, X., Steffensen, X., Sanna, A., Arru, G., Fois, M.L., Rosati, G., Sotgiu, S., Link, H., 2005. Immunological heterogeneity of multiple sclerosis in Sardinia and Sweden. Mult. Scler. 11, 16–23.

Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A., Chen, M., Noh, G., Goodman, J., Hagger, G.N., Tran, J., Tippin, T.K., Wang, X., Lusis, A.J., Hsueh, W.A., Law, R.E., Collins, J.L., Willson, T.M., Tontonoz, P., 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. U. S. A. 99, 7604–7609.

Joseph, S.B., Castrillo, A., Laffitte, B.A., Mangelsdorf, D.J., Tontonoz, P., 2003. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat. Med. 9, 213–219.

Joseph, S.B., Bradley, M.N., Castrillo, A., Bruhn, K.W., Mak, P.A., Pei, L., Hogenesch, J., O'connell, R.M., Cheng, G., Saez, E., Miller, J.F., Tontonoz, P., 2004. LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 119, 299–309.

Kramer, P.R., Wray, S., 2002. 17-Beta-estradiol regulates expression of genes that function in macrophage activation and cholesterol homeostasis. J. Steroid Biochem. Mol. Biol. 81, 203–216.

Kurtzke, J.F., 1997. The epidemiology of multiple sclerosis. In: Raine, C.S., McFarland, H.F., Tourtellotte, W.W. (Eds.), Multiple Sclerosis, Clinical and Pathogenetic Basis. Chapman and Hall, London, pp. 91–139.

Landis, M.S., Patel, H.V., Capone, J.P., 2002. Oxysterol activators of liver X receptor and 9-*cis*-retinoic acid promote sequential steps in the synthesis and secretion of tumor necrosis factor-alpha from human monocytes. J. Biol. Chem. 277, 4713–4721.

Lundholm, L., Moverare, S., Steffensen, K.R., Nilsson, M., Otsuki, M., Ohlsson, C., Gustafsson, J.-A., Dahlman-Wright, K., 2004. Gene expression profiling identifies liver X receptor alpha as an estrogen-regulated gene in mouse adipose tissue. J. Mol. Endocrinol. 32, 879–892.

McEwan, I.J., 2004. Molecular mechanisms of androgen receptor-mediated gene regulation: structure-function analysis of the AF-1 domain. Endocr. Relat. Cancer 11, 281–293.

Nagpal, S., 2003. An orphan meets family members in skin. J. Invest. Dermatol. 120, viii–x.

Nilsson, S., Gustafsson, J.-A., 2002. Biological role of estrogen and estrogen receptors. Crit. Rev. Biochem. Mol. Biol. 37, 1–28.

Offner, H., 2004. Neuroimmunoprotective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J. Neurosci. Res. 78, 603–624.

Palaszynski, K.M., Loo, K.K., Ashouri, J.F., Liu, H.B., Voskuhl, R.R., 2004. Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J. Neuroimmunol. 146, 144–152.

Paty, D.W., Ebers, W.C., 1998. Multiple Sclerosis. F.A. Davis Co, Philadelphia.

Peng, X., Maruo, T., Cao, Y., Punj, V., Mehta, R., Das Gupta, T.K., Christov, K., 2004. A novel RAR{beta} isoform directed by a distinct promoter P3 and mediated by retinoic acid in breast cancer cells. Cancer Res. 64, 8911–8918.

Polanczyk, M., Zamora, A., Subramanian, S., Matejuk, A., Hess, D.L., Blankenhorn, E.P., Teuscher, C., Vandenbark, A.A., Offner, H., 2003. The protective effect of 17beta-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-alpha. Am. J. Pathol. 163, 1599–1605.

Polanczyk, M., Yellayi, S., Zamora, A., Subramanian, S., Tovey, M., Vandenbark, A.A., Offner, H., Zachary, J.F., Fillmore, P.D., Blankenhorn, E.P., Gustafsson, J.-Å., Teuscher, C., 2004. Estrogen receptor-1 (Esr1) and -2 (Esr2) regulate the severity of clinical experimental allergic encephalomyelitis in male mice. Am. J. Pathol. 164, 1915–1924.

Ramos-Payan, R., Aguilar-Medina, M., Estrada-Parra, S., Gonzalez, Y.M.J., Favila-Castillo, L., Monroy-Ostria, A., Estrada-Garcia, I.C., 2003. Quantification of cytokine gene expression using an economical real-time polymerase chain reaction method based on SYBR Green I. Scand. J. Immunol. 57, 439–445.

Repa, J.J., Mangelsdorf, D.J., 2002. The liver X receptor gene team: potential new players in atherosclerosis. Nat. Med. 8, 1243–1248.

Robinson-Rechavi, M., Escriva-Garcia, H., Laudet, V., 2003. The nuclear receptor superfamily. J. Cell Sci. 116, 585–586.

Salem, M.L., 2004. Estrogen, a double-edged sword: modulation of TH1- and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Curr. Drug Targets Inflamm. Allergy 3, 97–104.

Schmitz, G., Drobnik, W., 2002. ATP-binding cassette transporters in macrophages: promising drug targets for treatment of cardiovascular disease. Curr. Opin. Invest. Drugs 3, 853–858.

Schuster, G.U., Parini, P., Wang, L., Alberti, S., Steffensen, K.R., Hansson, G.K., Angelin, B., Gustafsson, J.-A., 2002. Accumulation of foam cells in liver X receptor-deficient mice. Circulation 106, 1147–1153.

Shim, G.J., Kis, L.L., Warner, M., Gustafsson, J.-A., 2004a. Autoimmune glomerulonephritis with spontaneous formation of splenic germinal centers in mice lacking the estrogen receptor alpha gene. Proc. Natl. Acad. Sci. U. S. A. 101, 1720–1724.

Shim, G.J., Warner, M., Kim, H.J., Andersson, S., Liu, L., Ekman, J., Imamov, O., Jones, M.E., Simpson, E.R., Gustafsson, J.-A., 2004b. Aromatase-deficient mice spontaneously develop a lymphoproliferative autoimmune disease resembling Sjogren's syndrome. Proc. Natl. Acad. Sci. U. S. A. 101, 12628–12633.

Sotgiu, S., Pugliatti, M., Fois, M.L., Arru, G., Sanna, A., Sotgiu, M.A., Rosati, G., 2004. Genes, environment, and susceptibility to multiple sclerosis. Neurobiol. Dis. 17, 131–143.

Steffensen, K.R., Gustafsson, J.-A., 2004. Putative metabolic effects of the liver X receptor (LXR). Diabetes 53 (Suppl. 1), S36–S42.

Stuve, O., Youssef, S., Steinman, L., Zamvil, S.S., 2003. Statins as potential therapeutic agents in neuroinflammatory disorders. Curr. Opin. Neurol. 16, 393–401.

Underhill, P.A., Shen, P., Lin, A.A., Jin, L., Passarino, G., Yang, W.H., Kauffman, E., Bonne-Tamir, B., Bertranpetit, J., Francalacci, P., Ibrahim, M., Jenkins, T., Kidd, J.R., Mehdi, S.Q., Seielstad, M.T., Wells, R.S., Piazza, A., Davis, R.W., Feldman, M.W., Cavalli-Sforza, L.L., Oefner, P.J., 2000. Y chromosome sequence variation and the history of human population. Nat. Genet. 26, 358–361.

Wang, L., Schuster, G.U., Hultenby, K., Zhang, Q., Andersson, S., Gustafsson, J.-A., 2002. Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. Proc. Natl. Acad. Sci. U. S. A. 99, 13878–13883.

Wang, Y., Kurdi-Haidar, B., Oram, J.F., 2004. LXR-mediated activation of macrophage stearoyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1. J. Lipid. Res. 45, 972–980.

Whitney, K.D., Watson, M.A., Collins, J.L., Benson, W.G., Stone, T.M., Numerick, M.J., Tippin, T.K., Wilson, J.G., Winegar, D.A., Kliewer, S.A., 2003. Regulation of cholesterol homeostasis by the liver X receptors in the central nervous system. Mol. Endocrinol. 16, 1378–1385.

Yeap, B.B., Wilce, J.A., Leedman, P.J., 2004. The androgen receptor mRNA. BioEssays 26, 672–682.