Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY ORIGINAL ARTICLE

Effects of *C2ta* genetic polymorphisms on MHC class II expression and autoimmune diseases

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doi:10.1111/imm.12692
Received 21 September 2016; revised 3 November 2016; accepted 9 November 2016.
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Summary

Antigen presentation by the MHC-II to $CD4^+$ T cells is important in adaptive immune responses. The class II transactivator (CIITA in human and C2TA in mouse) is the master regulator of MHC-II gene expression. It coordinates the transcription factors necessary for the transcription of MHC-II molecules. In humans, genetic variations in *CIITA* have been associated with differential expression of MHC-II and susceptibility to autoimmune diseases. Here we made use of a *C2ta* congenic mouse strain (expressing MHC-II haplotype H-2^q) to investigate the effect of the natural genetic polymorphisms in type I promoter of *C2ta* on MHC-II expression and function. We demonstrate that an allelic variant in the type I promoter of *C2ta* resulted in an increased expression of MHC-II on macrophages (72–151% higher mean florescence intensity) and conventional dendritic cells (13–65% higher mean florescence intensity) in both spleen and peripheral blood. The increase in MHC-II expression resulted in an increase in antigen presentation to T cells *in vitro* and increased T-cell activation. The differential MHC-II expression in B6Q.*C2ta*, however, did not alter the disease development in models of rheumatoid arthritis (collagen-induced arthritis and human glucose-6-phosphate-isomerase$_{325–339}$-peptide-induced arthritis), or multiple sclerosis ($MOG_{1–125}$ protein-induced and $MOG_{79–96}$ peptide-induced experimental autoimmune encephalomyelitis). This is the first study to address the role of an allelic variant in type I promoter of *C2ta* in MHC-II expression and autoimmune diseases; and shows that *C2ta* polymorphisms regulate MHC-II expression and T-cell responses but do not necessarily have a strong impact on autoimmune diseases.

Keywords: antigen presentation; class II transactivator; collagen-induced arthritis; experimental autoimmune encephalomyelitis; major histocompatibility complex.

Introduction

The MHC region encodes peptide receptors that are the critical molecules for activation of antigen-specific T cells. The MHC region is in strong linkage disequilibrium and with a pronounced polymorphism of critical importance for the immune response. Importantly, it is strongly associated with autoimmune diseases, consistently shown in multiple genome-wide association studies (GWAS) analyses. Well-studied autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA) and type I diabetes (T1D) are all strongly associated with MHC-II DR alleles, encoding MHC class II (MHC-II) chains.[1–4] These diseases are characterized by a chronic inflammatory, autoimmune process directed towards certain tissues and organs such as central nervous system (MS), joints (RA), or insulin-producing beta cells (T1D). Over 100 risk loci have been identified in both MS and RA with the main contribution from the MHC genes and minor contribution from non-MHC genes.[1–3] Although the strong associations with MHC-II genes have been recognized for several decades,[5–8] the high gene density, extreme polymorphism and strong linkage disequilibrium of the MHC region have hampered the identification of variants driving these associations.

MHC-II expression is controlled mainly at the level of transcription, by a complex consisting of a regulatory module known as the SXY module and multiple transcription factors.[9] The class II transactivator (CIITA) interacts with this multiprotein complex and coordinates the recruitment of additional factors important for chromatin modification, remodelling, and also transcription initiation and elongation.[9] There are four *CIITA* isoforms in humans (pI, pII, pIII and pIV),[10] and three in mice and rats (pI, pIII and pIV with promoter type I, III and IV). Isoform pI mainly controls *C2ta* expression on cells of myeloid lineage, including macrophages and dendritic cells; pIII regulates *C2ta* expression on B cells and plasmacytoid dendritic cells;[11] and pIV regulates *C2ta* expression on thymic epithelial cells.[12]

Genetic variations in *C2TA* have been associated with susceptibility to multiple autoimmune diseases in both humans and rodents. It was first identified in a genetic analysis of an advanced intercross line in rats that polymorphisms in the 5′ flanking region of *C2ta* explain strain-dependent differences in expression of MHC-II molecules.[13] It was later shown in the congenic strains in rats that the *C2ta* polymorphisms alter susceptibility to experimental autoimmune encephalomyelitis (EAE), a chronic relapsing model of MS.[14] The experimental findings in rats prompted further investigation of *C2TA* in humans and it was demonstrated that a single nucleotide polymorphism in the 5′ flanking region of type III promoter of *C2TA* is associated with susceptibility to RA, MS and myocardial infarction.[13] *C2TA* disease association has since then been shown in different patient cohorts in not only RA[15,16] and MS,[17,18] but also T1D,[19] coeliac disease[20] and systemic lupus erythematosus,[21,22] although disease associations could not be reproduced in some populations.[23–25]

In this study, we generated a *C2ta* congenic mouse strain to investigate the effect of an allelic variant of *C2ta* promoter on MHC-II expression in different antigen-presenting cells (APCs). We then assessed the functional implications of the resulting MHC-II expression difference on antigen presentation of the immune cells in the congenic versus wild-type control (wt) mice. To understand if this *C2ta*-driven MHC-II expression difference modulates autoimmunity, we selected models that have previously been shown to be controlled by the MHC-II locus and in particular by the A^q gene;[26] i.e. EAE, collagen-induced arthritis (CIA) and human glucose-6-phosphate-isomerase (hG6PI) $_{325–339}$-peptide-induced arthritis in these mice.

Materials and methods

Animal experimentation

Mice were bred and maintained in a barrier facility at Scheele Laboratory, Karolinska Institutet, and were specific pathogen-free according to the Federation of European Animal Laboratory Science Associations guidelines. All animals were housed in a climate-controlled environment with 14 hr light/10 hr dark cycles, in individually ventilated microisolator-cages (Allentown Inc. Allentown, NJ) containing wood shavings (Tapvei, Scanbur, Sollentuna, Sweden), and fed standard rodent chow (R70, Lantmännen, Stockholm, Sweden) and had free access to water. Age- and sex-matched, 9–14 weeks old, congenic and wt control mice have been used in all experiments, unless otherwise specified. All experiments were carried out with littermate controls and mice of different groups were mixed in cages. All experiments were performed with the identity of the mice blinded for the investigator. All experiments were approved by the Stockholm Ethical Committee and performed in accordance with the guidelines of the Swedish National Board for Laboratory Animals and the European Community Council Directive (86/609/EEC).

Mice

Congenic fragment containing the *C2ta* locus originated from 129X1/SvJ mice and was first backcrossed to C57BL/6J (MHC-II haplotype $H\text{-}2^b$) for 12 generations. The congenic fragment was subsequently backcrossed to C57BL/6N.Q (short-named B6Q, MHC-II haplotype $H\text{-}2^q$)[27] to generate mice with the homozygous expression of the MHC-II A^q. The maximum size of the introgressed fragment is roughly 10·2 Mb, spanning from 5·68 Mb (marker D16Mit182) to 15·91 Mb (marker D16Mit34), congenic mice are denoted B6Q.*C2ta*. Female mice used in CIA experiments were ovariectomized before immunization to increase their susceptibility to arthritis.[28]

Genotyping

Genomic DNA was extracted from progeny biopsies for genotyping.[29] Forward primers were fluorescently labelled, and genomic DNA was amplified with PCR according to a standard protocol and analysed on a 48-capillary ABI 3730 DNA analyser (Applied Biosystems, Foster City, CA) as previously described.[29]

Harvest and culture of bone marrow-derived macrophages

Mouse bone marrow cells were obtained from both femurs in PBS, filtered through a 40-μm filter and resuspended in in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA) supplemented with 10% (vol/vol) fetal calf serum (Gibco), 50 μM β-mercaptoethanol (Gibco), 10 mM HEPES (Gibco), 10 U/ml penicillin and 100 μg/ml streptomycin (both from Invitrogen Life Technologies, Carlsbad, CA). Cells were cultured at a density

of 1×10^6/ml in 100 × 20 mm cell-culture plates with 20 ng/ml murine macrophage colony-stimulating factor (Peprotech, Rocky Hill, NJ) for 6 days at 37° in 5% CO_2. The medium was renewed on day 3. Cells were subsequently stimulated with 0, 1 and 10 ng/ml interferon-γ (Peprotech) for 24 hr at 37° in 5% CO_2.

Antigen presentation assay

Spleens were harvested from untreated mice and single-cell suspensions were prepared by mechanical disruption of spleen and filtered through a 40-μm filter. Either 1×10^5 or 2×10^5 splenocytes were plated per well of a 96-well plate (Nunc, Roskilde, Denmark) in Dulbecco's modified Eagle's medium supplemented with 5% (vol/vol) fetal calf serum, 50 μM β-mercaptoethanol, 10 mM HEPES, 10 U/ml penicillin, and 100 μg/ml streptomycin. HCQ.3 hybridoma T cells (5×10^4), specific for the CII256-270 (Gal-264) peptide bound to MHC-A^q,[30] as well as CII peptide, were added to each well. CII peptides, containing the 259–273 sequence of rat CII with a β-D-galactopyranosyl residue on L-hydroxylysine at position 264, were synthesized as previously described.[31] After 24 hr of culturing at 37° in 5% CO_2, the supernatants were analysed for interleukin-2 by sandwich ELISA.

Cytokine ELISA

To determine the concentration of interleukin-2 in the supernatant, 50 μl supernatant was removed from the plates after 24 hr of culture. JES6-IA12 (2 μg/ml, home-made) and JES6-5H4-biotin (1 μg/ml, Mabtech, Nacka Strand, Sweden) were used as capture antibody and detection antibody, respectively. Eu^{3+}-conjugated streptavidin (DELFIA) was used for detection, performed on a Synergy 2 multi-mode plate reader (BioTek, Winooski, VT).

EAE induction and evaluation

Experimental autoimmune encephalomyelitis was induced by intradermal injection of 20 μg myelin oligodendrocyte glycoprotein $(MOG)_{1–125}$ or 50 μg $MOG_{79–96}$ peptide emulsified in complete Freund's adjuvant (Difco, Detroit, MI) at the base of the tail. For EAE induction by $MOG_{79–96}$ peptide, 400 ng pertussin toxin was injected intravenously on day 0 and day 2. For EAE induction by $MOG_{1–125}$ protein, 200 ng pertussin toxin was injected intravenously on day 0 and day 2. Recombinant rat MOG (amino acids 1–125 from the N terminus) was expressed in *Escherichia coli* and purified to homogeneity by chelate chromatography.[32] EAE was monitored on a scoring scale as follows: 0 = normal; 1 = tail weakness; 2 = tail paralysis; 3 = mild waddling movement; 4 = heavy waddling movement, unsecure foothold; 5 = paralysis of a limb, crawling; 6 = paralysis of a pair of limbs; 7 = paralysis of all four limbs; 8 = death. Animals were euthanized when they reached score 7.

Arthritis induction and evaluation

Collagen-induced arthritis was induced by intradermal injection of 100 μg of rat type II collagen (CII), prepared as described earlier,[33] emulsified in complete Freund's adjuvant (Difco) at the base of the tail. After 35 days, mice were boosted with 50 μg of CII in incomplete Freund's adjuvant (Difco) at the same site. The hG6PI-induced arthritis was induced by intradermal injection of 10 μg $hG6PI_{325–339}$-peptide emulsified in complete Freund's adjuvant (Difco) at the base of the tail[39]. Arthritis development was quantified using a macroscopic scoring system; one point was given for each swollen or red toe or joint and five points for a swollen ankle, adding up to a maximum score of 60 points per mouse.

Flow cytometry

Single-cell suspensions were prepared in ice-cold FACS buffer (Ca^{2+}-free and Mg^{2+}-free Dulbecco's PBS supplemented with 1% fetal calf serum, 10 mM EDTA), with subsequent lysing of erythrocytes. Both splenocytes and heparinized peripheral blood were incubated with 2.4G2 antibody for FcγR-blockade and stained with a saturating concentration of monoclonal antibodies on 96-well V-bottom polypropylene plates (BD Biosciences, San Jose, CA). The following antibodies were used: CD90.2 (Thy1.2), CD45R (B220), I-A/I-E (2G9) and CD11c (N418), purchased from BD Biosciences. CD11b (M1/70), Gr-1 (RB6-8C5) and F4/80 (BM8) were purchased from (BioLegend, San Diego, CA). Both LIVE/DEAD (Invitrogen) and the forward-scatter versus side-scatter plot were used to include only non-necrotic cells. A SORP BDLSR II analytic flow cytometer (BD Biosciences, San Jose, CA) was used for acquisition, and the data were analysed with FLOWJO (Tree Star, Ashland, OR).

Statistical analysis

All statistical analyses were evaluated by Mann–Whitney *U*-test using GRAPHPAD PRISM 6.0 software (San Diego, CA). Results are considered significant for $P < 0{\cdot}05$.

Bioinformatics

The sequence of the type I promoter of *C2ta*, 1000 bp upstream from the transcriptional start site, was retrieved from UCSC Genome Browser (GRCm38/mm10) Assembly. Regulatory factor binding prediction was performed by CONREAL.[34]

Results

Effect of genetic polymorphisms in *C2ta* on MHC-II expression in B6NQ.C2ta congenic mice

It has previously been shown[35] that there are three single-nucleotide polymorphisms (SNPs) in the type I promoter of *C2ta* (C57BL/6 versus 129X1/SvJ), which are located at −715, −712 and −402 relative to the transcriptional start site. A bioinformatic analysis of this region using CONREAL[34] showed that these polymorphic sites could potentially be the binding sites of various regulatory factors, such as nuclear transcription factor and cAMP response element-binding protein (CREB) (see Supplementary material, Fig. S1). Binding of CREB to the regulatory region of *CIITA* has been suggested to play important roles in the activation of CIITA-PIII in B cells.[36]

Since C2TA acts as a master regulator of MHC class II, we next assessed the impact of these genetic polymorphisms in the *C2ta* promoter on MHC-II expression. The MHC-II expression differs between the APCs (Fig. 1) and depends on the immune conditions, for example, MHC-II expression on macrophages can be stimulated by the presence of interferon-γ.[37] By flow cytometric analysis of splenocytes and peripheral blood from the B6Q.*C2ta* congenic and B6Q wt mice, we determined the surface MHC-II expression on monocytes/macrophages ($CD11b^{hi}$ $Gr\text{-}1^{-}F4/80^{+}$ and $CD11b^{hi}$ $Gr\text{-}1^{int}F4/80^{+}$), conventional dendritic cells ($CD11c^{+}$ $CD11b^{-}$ $CD45R^{-}$), plasmacytoid dendritic cells ($CD11c^{+}$ $CD11b^{-}$ $CD45R^{+}$) and B cells ($CD45R^{+}$ $CD11c^{-}$) (Fig. 1a). We found that, in the spleen, the B6Q.*C2ta* congenic mice showed a significantly increased MHC-II expression on monocytes/macrophages (72–110% higher), conventional dendritic cells (65% higher) and plasmacytoid dendritic cells (38% higher) compared with the B6Q wt mice (Fig. 1b–e). In the blood, elevated MHC-II expression was similarly found on the monocytes/macrophages (117–151% higher) and conventional dendritic cells (13% higher) from the B6Q.*C2ta* congenic mice (Fig. 1b–d). There was, however, no difference in the MHC-II expression on B cells obtained from spleen or blood (Fig. 1f). We further investigated if the increased MHC-II expression in spleen and blood was also observed in bone-marrow-derived macrophages. We found that the B6Q.*C2ta* congenic mice displayed an increased MHC-II expression on the bone-marrow-derived macrophages (29–37% higher) (Fig. 1g), indicating that the allelic differences in *C2ta* in the B6Q.*C2ta* congenic mice have a profound impact on MHC-II expression in both central and peripheral lymphoid organs.

Increased antigen presentation when co-cultured with HCQ.3 hybridoma T cells

Having shown higher MHC-II expression in B6Q.*C2ta* congenic mice, we investigated whether this could lead to different antigen presentation to T cells *in vitro*, resulting in altered T-cell activation. To achieve this, we performed an antigen presentation assay using rat type II collagen peptide ($CII_{259–273}$) with a β-D-galactopyranosyl residue on L-hydroxylysine at position 264 as the antigen.[31] We used splenocytes from naive B6Q.*C2ta* congenic and B6Q wt mice as a source of APCs and we cultured these splenocytes with HCQ.3 hybridoma T cells, which are specific for the glycosylated form of the $CII_{256–270}$ peptide bound to MHC-II A^{q}.[30] After 24 hr, the concentration of interleukin-2 in the supernatant was determined as a measure of T-cell activation. We found that APCs from B6Q.*C2ta* mice induced significantly higher levels of interleukin-2 production, compared with B6Q wt mice (Fig. 2a–b). These results indicate that the higher MHC-II expression on APCs in B6Q.*C2ta* congenic mice could lead to increased antigen presentation to T cells *in vitro* and that allelic differences in *C2ta* could lead to quantitative differences in antigen presentation.

No difference in CIA and hG6PI-induced arthritis

Considering the association between both CIITA and MHC-II with RA,[6,7,13] we investigated the effect of *C2ta* alleles on CIA, one of the most commonly used animal models for RA. To facilitate investigation of the effect of *C2ta* alleles in different models of autoimmune diseases, the congenic fragment C57BL/6J.129 was backcrossed to C57BL/6N.Q to generate a homozygous MHC-II $H\text{-}2^{q}$ haplotype, which is susceptible to both CIA, hG6PI-induced arthritis and EAE (C57BL/6N.Q.129, denoted as B6Q.*C2ta*).[31,38,39]

We found that B6Q.*C2ta* congenic and B6Q wt mice developed comparable CIA with no difference in both daily arthritis score and cumulative arthritis score(Fig. 3a, b); and similar results were obtained in both male and female mice (Table 1). As age is known to affect the susceptibility of mice to develop arthritis,[40] we performed the same experiment also in older mice (20–24 weeks old). We found that older mice developed more severe arthritis. However, similar to the younger mice (9–14 weeks old), both B6Q.*C2ta* congenic and B6Q wt mice developed comparable CIA (Table 1). We next investigated if *C2ta* polymorphisms regulate $hG6PI_{325–339}$-peptide-induced arthritis, a new arthritis model dependent on MHC-II, T and B cells, as well as complement factor C5.[39] Similarly, the B6Q.*C2ta* congenic mice developed arthritis as severe as the B6Q wt mice with no difference in both daily arthritis score and cumulative arthritis score (Fig. 3c–d).

Comparable disease development in EAE

We investigated whether MHC-II expression difference on macrophages and dendritic cells and the subsequent

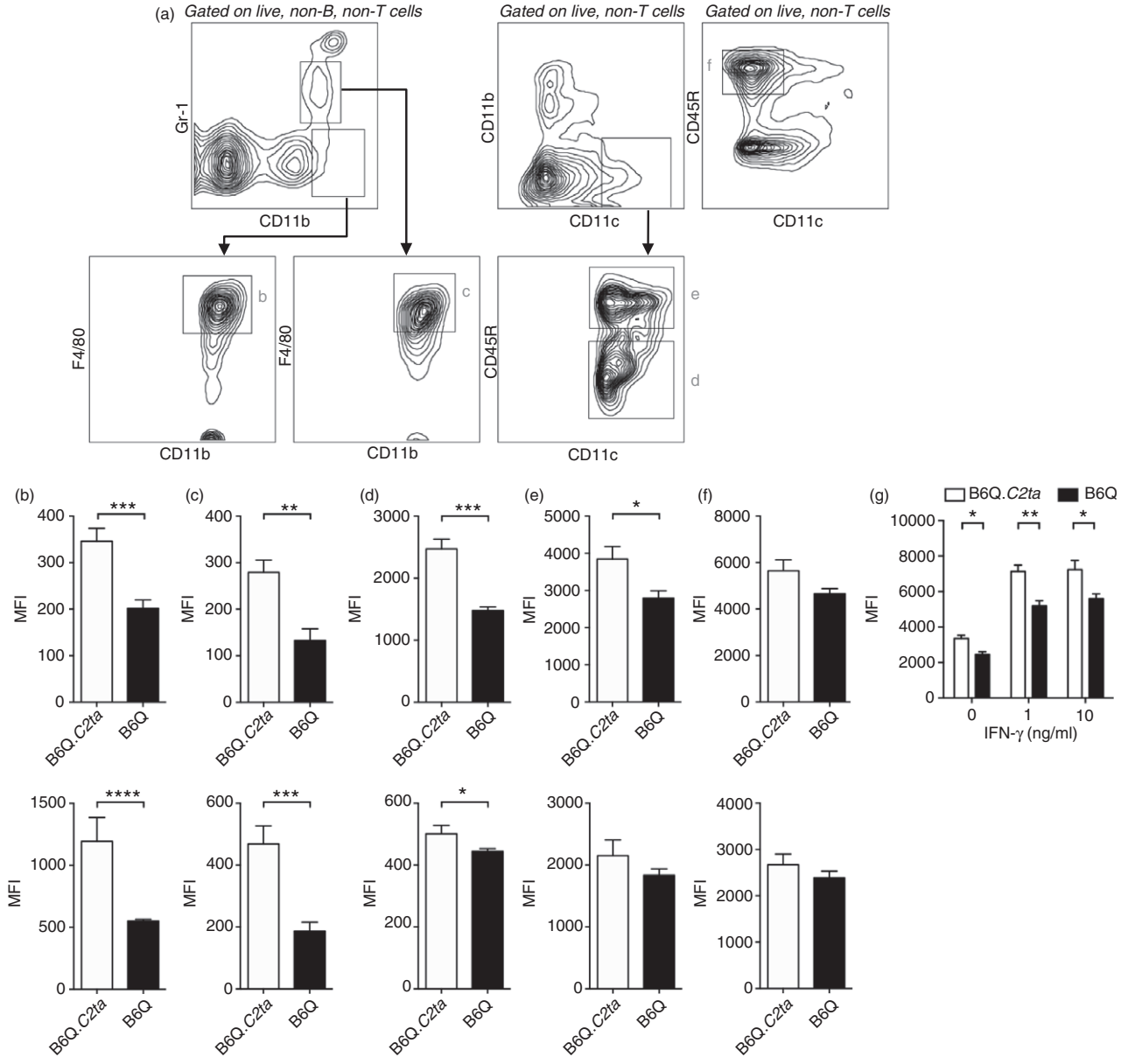

Figure 1. Increased expression of MHC-II on different antigen-presenting cells (APCs) in spleen and blood of B6Q.*C2ta* congenic mice. (a) Identification of monocytes/macrophages as CD11b^{hi} Gr-1^{-}F4/80^{+} CD45R^{-} CD90.2^{-} and CD11b^{hi} Gr-1^{int} F4/80^{+} CD45R^{-} CD90.2^{-}, conventional dendritic cells as CD11c^{+} CD11b^{-} CD45R^{-} CD90.2^{-}, plasmacytoid dendritic cells as CD11c^{+} CD11b^{-} CD45R^{+} CD90.2^{-} and B cells as CD45R^{+} CD90.2^{-} CD11c^{-} in spleen. (b–f) Mean florescence intensity (MFI; geometric mean) of MHC-II expression in spleen (upper) and blood (lower), (b) Gr-1^{-} monocytes/macrophages, (c) Gr-1^{int} monocytes/macrophages, (d) conventional dendritic cells, (e) plasmacytoid dendritic cells, and (f) B cells. Mean with SEM of 11 mice/group are shown. (g) MFI of MHC-II expression on bone marrow-derived macrophages upon interferon-γ (IFN-γ) exposure for 24 hr. Mean with SEM of six mice/group are shown. One of two representative experiments is shown. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$. Statistics were determined with the Mann–Whitney *U*-test.

increase in antigen presentation regulate susceptibility to autoimmune neuroinflammation. This is interesting especially since naturally occurring allelic differences in *C2ta* have been shown to regulate susceptibility to EAE in rats.[14]

We induced EAE in both B6Q.*C2ta* congenic and B6Q wt mice by immunization of either $MOG_{1–125}$ protein or $MOG_{79–96}$ peptide, both known to induce EAE in H-2^{q} mice.[38] In contrast to the findings in the *Vra4* congenic rats,[14] there was no difference in both the disease score and weight change between the B6Q.*C2ta* congenic and B6Q wt mice in both $MOG_{1–125}$ protein-induced EAE (Fig. 4a–c) and $MOG_{79–96}$ peptide-induced EAE (Fig. 4d–f). Similar results were found in both male and female mice (Table 2).

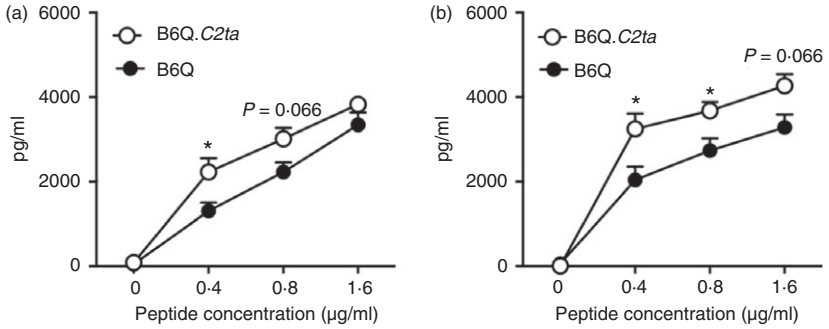

Figure 2. Higher MHC-II expression on antigen-presenting cells (APCs) in B6Q.*C2ta* led to increased antigen presentation to T cells. Splenocytes from naive B6Q.*C2ta* congenic ($n = 6$) and B6Q wild-type (wt) ($n = 9$) mice were cultured with HCQ.3 hybridoma T cells and after 24 hr, concentration of interleukin-2 (IL-2) in the supernatant was determined as a measure of T-cell activation. (a) 1×10^5 spleen cells and (b) 2×10^5 spleen cells were seeded per well together with 5×10^4 HCQ3 hybridoma T cells. One of two representative experiments is shown. $*P < 0{\cdot}05$ statistics were determined with the Mann–Whitney *U*-test.

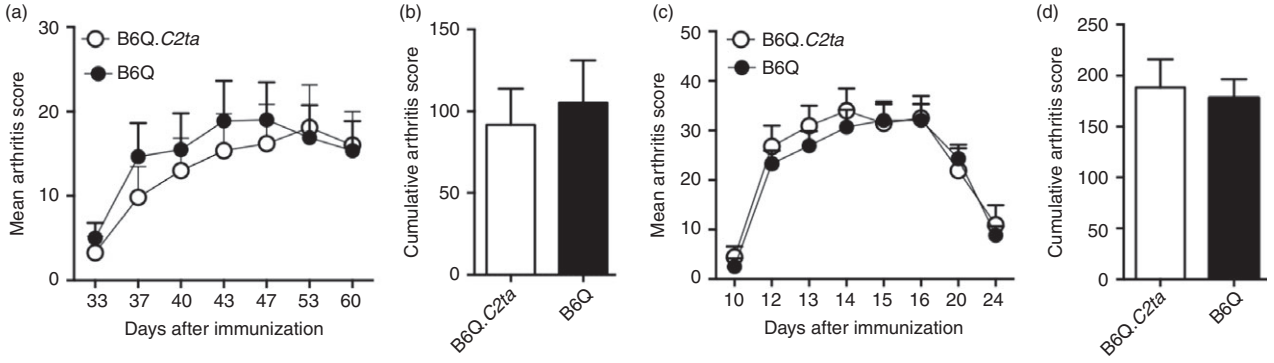

Figure 3. Increased MHC-II expression has no impact on collagen-induced arthritis (CIA) and human glucose-6-phosphate-isomerase (hG6PI)$_{325–339}$ peptide-induced arthritis in B6Q.*C2ta* mice. (a) Mean arthritis score and (b) cumulative arthritis score of CIA in male B6Q.*C2ta* ($n = 13$) and B6Q ($n = 23$) mice. (c) Mean arthritis score and (d) cumulative arthritis score of hG6PI$_{325–339}$ peptide-induced arthritis in male B6Q.*C2ta* ($n = 11$) and B6Q ($n = 17$) mice. Means with SEM of all the mice are shown. For CIA, one of two representative experiments is shown. For hG6PI$_{325–339}$ peptide-induced arthritis, data from two identical experiments with similar results are pooled together.

Table 1. Collagen-induced arthritis disease summary

Animals	Gender	Age (weeks)	Maximum arthritis score[1]	Cumulative arthritis score[1]	Disease incidence (%)[2]	No.[3]
B6Q.*C2ta*	M	9–14	18·1 ± 5·1	91·7 ± 22·1	85	13
B6Q	M	9–14	19·0 ± 4·5	105·2 ± 25·8	74	23
B6Q.*C2ta*	M	18–22	26·4 ± 2·3	133·7 ± 11·6	100	11
B6Q	M	18–22	31·8 ± 5·2	163·0 ± 34·0	100	8
B6Q.*C2ta*	F[4]	9–14	32·2 ± 4·5	90·3 ± 11·4	94	16
B6Q	F[4]	9–14	30·4 ± 5·6	100·3 ± 19·8	83	12

[1]Disease score is shown in mean values ± SEM.
[2]Denotes the percentage of animals which develop arthritis.
[3]Denotes the number of animals in the group.
[4]Female mice were ovariectomized before immunization to increase susceptibility to arthritis.[28.]

Discussion

We demonstrate here that an allelic difference in *C2ta* gene exerts an important regulatory effect on MHC-II expression of the B6Q.*C2ta* congenic mice. These findings were achieved by using congenics, isolating a *C2ta* congenic fragment from the donor strain 129X1/SvJ onto a recipient C57BL/6N.Q background. The mice also

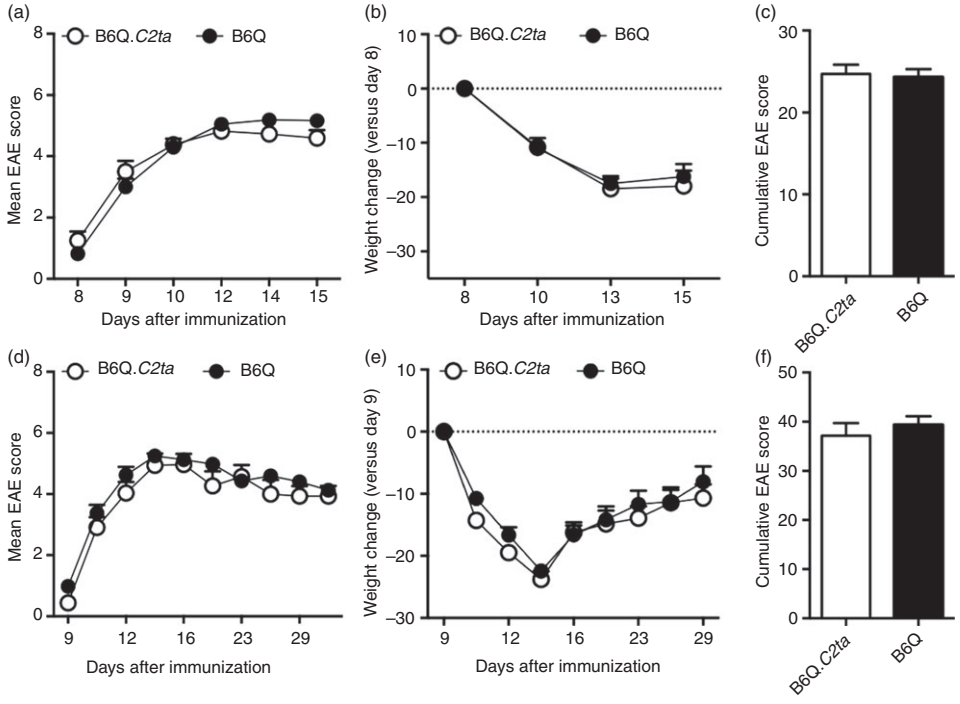

Figure 4. Increased MHC-II expression does not alter experimental autoimmune encephalomyelitis (EAE) susceptibility of B6Q.*C2ta* mice. (a) Mean EAE score, (b) weight change (versus day 8) and (c) cumulative EAE score of $MOG_{1–125}$ protein-induced EAE in female B6Q. *C2ta* ($n = 12$) and B6Q wild-type (wt) ($n = 22$) mice. (d) Mean EAE score, (e) weight change (versus day 9) and (f) cumulative EAE score of $MOG_{79–96}$ peptide-induced EAE in male B6Q. *C2ta* ($n = 16$) and B6Q wt ($n = 24$) mice. Means with SEM of all the mice are shown.

Table 2. MOG-induced experimental autoimmune encephalomyelitis (EAE) disease summary

Animals	Gender	Antigen	Age (weeks)	Maximum EAE score[1]	Cumulative EAE score[1]	Disease incidence (%)[2]	No.[3]
B6Q.*C2ta*	M	$MOG_{1–125}$ protein	9–14	5·8 ± 0·2	22·3 ± 1·4	100	9
B6Q	M	$MOG_{1–125}$ protein	9–14	5·5 ± 0·3	19·2 ± 1·6	100	9
B6Q.*C2ta*	F	$MOG_{1–125}$ protein	9–14	4·7 ± 0·2	24·7 ± 1·2	100	12
B6Q	F	$MOG_{1–125}$ protein	9–14	5·2 ± 0·1	24·3 ± 1·0	100	22
B6Q.*C2ta*	M	$MOG_{79–96}$ peptide	9–14	4·9 ± 0·4	37·1 ± 2·9	94	16
B6Q	M	$MOG_{79–96}$ peptide	9–14	5·3 ± 0·2	39·7 ± 1·3	100	24
B6Q.*C2ta*	F	$MOG_{79–96}$ peptide	9–14	4·6 ± 0·3	30·1 ± 1·7	100	20
B6Q	F	$MOG_{79–96}$ peptide	9–14	4·8 ± 0·3	33·9. ± 1·5	100	21

[1]Disease score is shown in mean values ± SEM.
[2]Denotes the percentage of animals which develop EAE.
[3]Denotes the number of animals in the group.

expressed the MHCII A^q gene, allowing studies of the effect of *C2ta* in different experimental models of MS and RA.[31,38,39]

The *CIITA* gene is of particular importance since the encoded protein functions as an assembler of transcription factors necessary for the transcription of MHC-II molecules. There are three *C2ta* isoforms in mouse (pI, pIII and pIV with promoters type I, III and IV) and the regulatory region of the *C2ta* gene was previously sequenced in multiple inbred strains.[35] Only type I promoter, but not type III and type IV promoters, is polymorphic between C57BL/6 and 129X1/SvJ.[35] These polymorphisms resulted in significantly higher *C2ta*, *Cd74* and *pI* expression in the congenic strains carrying the 129X1/SvJ *C2ta* allele on a B6 background, compared with parental controls.[35] Here we showed that an allelic variant in the type I promoter of *C2ta* led to differential MHC-II expression in both macrophages and conventional dendritic cells in both spleen and peripheral blood, consistent with the known function of type I promoter in controlling *C2ta* expression in cells of myeloid lineage. We further showed that an increased MHC-II expression

in the B6Q.*C2ta* congenic mice has a regulatory effect on the antigen presentation to T cells and resulted in an increased T-cell activation *in vitro*.

The importance of *CIITA* gene is shown by the fact that loss of function of the *CIITA* gene due to splicing mutation can result in bare lymphocyte syndrome, leading to a severely compromised immune system and inability to fight infection.[41] Chromosomal translocation of *CIITA* has been linked to primary mediastinal B-cell lymphoma and classical Hodgkin lymphoma.[42] Genetic variations in the *CIITA* gene have also been associated with several autoimmune disorders in humans, including RA,[13,15,16] MS,[13,17,18] myocardial infarction,[13] T1D,[19] coeliac disease[20] and systemic lupus erythematosus.[20,21] Different SNPs have been associated with these diseases, including rs3087456 in the type III promoter of *CIITA,* an intronic SNP rs8048002, and rs4774, which is a missense mutation resulting in an amino acid change from glycine to alanine.[13,15,18] The -168A/G polymorphism (rs3087456) in the type III promoter of *CIITA* is one of the most investigated for disease association in humans. The pIII *C2ta* has also been investigated in rats in a congenic setting and has been previously shown to be associated with EAE.[14] Mice with a knockout in pIV *C2ta*, however, were found to not influence the development of CIA.[43] To our knowledge, genetic variants in the type I promoter of *CIITA* have not been shown for association with autoimmune disorders in humans and this is the first time a natural genetic variant in the type I promoter of *C2ta* has been evaluated for regulatory roles in immune response and in autoimmune diseases. Our data showed that increased MHC-II expression on macrophages and conventional dendritic cells as a result of polymorphisms in the type I promoter of *C2ta* influence T-cell activation. Surprisingly, however, we could not show any effect on T-cell-dependent autoimmune disease models. Abrogation of MHC-II expression on fibroblast-like synoviocytes in $pIV^{-/-}$ K14 CIITA-transgenic mice likewise also has no impact on experimental arthritis.[43] However, quantitative difference in MHC-II expression on B cells may have a small but significant impact on autoimmunity, as shown in the *Vra4* congenic rats, which displayed significantly lower MHC-II expression on B cells and are protected from EAE.[14] This is interesting considering that recent evidence has suggested that B cells may play more important pathogenic roles than previously anticipated, both as precursors of antibody-producing plasma cells and as APCs for the activation of T cells in MS;[44] and in the success of B-cell depletion therapy such as anti-CD20 treatment in rapidly reducing the formation of new inflammatory central nervous system lesions in MS.[45]

As the locus with the strongest association to the susceptibility of many autoimmune diseases, the genetic association of the MHC has been extensively studied in both MS[1] and RA.[46,47] In both RA and MS, the *HLA-DRB1* gene contributes the largest genetic variance among all risk genes,[46,48] although the pathogenic mechanisms of MHC-II in MS and RA are not the same: *HLA-DRB1*15:01* has the strongest effect in MS;[1] *HLA-DRB1*04:01*, **04:04* and **01:01* are the most frequent RA risk alleles in European populations. Whereas the effects of MHC-II coding variants on MS and RA have been studied in more depth, the impact of quantitative differences in MHC-II expression on autoimmune diseases has not been as extensively studied. We recently investigated this using a panel of MHC-II *Tcs2* congenic rat strains that displayed variation in severity in pristane-induced arthritis.[49] We found that variation in arthritis severity between the *Tcs2* congenic rat strains could not simply be explained by the MHC-II expression difference on APCs. The disease variation was in fact mainly explained by the coding variants in the P1 pocket of the MHC-II molecules, which subsequently influenced the organization and occupancy of the peptide-binding groove.[49] Hence, both the current and other studies[43,49] seem to suggest that the level of MHC-II expression plays a relatively minor overall role in the regulation of autoimmune diseases triggered by an exogenous immunization, like EAE and CIA. *CIITA* is regarded as master regulator of *MHC-II* genes and induces *MHC-II* gene transcription in a cell-specific manner. *CIITA* itself is in fact tightly regulated at a transcriptional level by not only genetic but also epigenetic mechanisms, including methylation of DNA and histone acetylation and methylation modifications. Long-range chromatin interactions are also involved in the transcriptional activation of *CIITA*.[50] Therefore, there are multiple levels of regulation on *CIITA* and *MHC-II* expression which subsequently may have different effects on autoimmunity.

In conclusion, we demonstrate in a congenic mouse setting that genetic polymorphisms in the type I promoter of *C2ta* regulate MHC-II expression on professional APCs including macrophages and conventional dendritic cells. We showed that the increase in MHC-II expression on APCs led to increased antigen presentation *in vitro* and increased T-cell activation. These MHC-II expression differences and increased antigen presentation capacity, however, did not have a strong impact on the susceptibility to EAE and experimental arthritis. We believe our findings provide important information on the complex regulation of MHC-II in autoimmunity.

Authors' contributions

ACYY, FP, TO and RH designed the research; ACYY performed the experiments and drafted the manuscript. ACYY, FP, TO and RH analysed the data and revised the

manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

We are grateful to Carlos Palestro, Kristina Palestro, Tomasz Klaczkowski and Evelina Wernersson for taking care of the animals. This work was supported by grants from the Knut and Alice Wallenberg Foundation, the Swedish Association against Rheumatism, the Swedish Medical Research Council, the Swedish Foundation for Strategic Research and the European Union Innovative Medicine Initiative project BeTheCure.

Disclosure

The authors declare that they have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The DNA sequence of the type I promoter of *C2ta* in C57BL/6N.Q mice.