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Presentation of Ag bound to MHC class II (MHC II) molecules to CD4+ T cells is a key event in adaptive immune responses. Genetic differences in MHC II expression in the rat CNS were recently positioned to allelic variability in the CIITA gene (Mhc2ta), located within the Vra4 locus on rat chromosome 10. In this study, we have examined reciprocal Vra4-congenic strains on the DA and PVG av1 backgrounds, respectively. After experimental nerve injury the strain-specific MHC II expression on microglia was reversed in the congenic strains. Similar findings were obtained after intraparenchymal injection of IFN-γ in the brain. Expression of MHC class II was also lower on B cells and dendritic cells from the DA.PVG av1 congenic strain compared with DA rats after in vitro stimulation with IFN-γ. We next explored whether Vra4 may affect the outcome of experimental autoimmune disease. In experimental autoimmune encephalomyelitis induced by immunization with myelin oligodendrocyte glycoprotein, DA.PVG av1–Vra4 rats displayed a lower disease incidence and milder disease course compared with DA, whereas both PVG av1 and PVG av1–DA–Vra4 rats were completely protected. These results demonstrate that naturally occurring allelic differences in Mhc2ta have profound effects on the quantity of MHC II expression in the CNS and on immune cells and that this genetic variability also modulates susceptibility to autoimmune neuroinflammation. The Journal of Immunology, 2008, 180: 3289–3296.

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2 Address correspondence and reprint requests to Dr. Maria Swanberg, Department of Clinical Neurosciences, Karolinska Institutet, Center for Molecular Medicine L8:04, Karolinska University Hospital, 171 76 Stockholm, Sweden. E-mail address: Maria.Swanberg@ki.se
3 Abbreviations used in this paper: MS, multiple sclerosis; RA, rheumatoid arthritis; MHC II, MHC class II; SNP, single nucleotide polymorphism; VRA, ventral root avulsion; EAE, experimental autoimmune encephalomyelitis; rMOG, recombinant myelin oligodendrocyte glycoprotein; Aif1, allograft inflammatory factor; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; hm, littermate control.

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PVG\textsuperscript{av1}.DA-Vra4 congenics, respectively. Congenic animals displayed a reversal of the strain-specific expression pattern of MHC II in the CNS both after nerve injury and intraparenchymal IFN-\(\gamma\) injections, demonstrating the overriding effect of allelic difference in the Vra4 locus on this particular phenotype. In contrast, there were no discernible effects on other markers of microglial activation. MHC II expression was also lower on B cells and bone marrow-derived dendritic cells from DA.PVG\textsuperscript{av1}.Vra4-congenic rats compared with DA rats upon IFN-\(\gamma\) stimulation in vitro. Furthermore, DA.PVG\textsuperscript{av1}.Vra4 congenics displayed a lower susceptibility to experimental autoimmune encephalomyelitis (EAE) induced by active immunization with rat recombinant myelin oligodendrocyte glycoprotein (rMOG) protein compared to DA rats, suggesting that Vra4 allele-dependent quantitative differences in MHC II expression modulate susceptibility to autoimmune neuroinflammation.

**Materials and Methods**

**Animals**

DA, PVG\textsuperscript{av1}, and PVG\textsuperscript{av1}.DA-Vra4 adult rats were used. The congenics were bred by reciprocal crossing of DA (provided by Prof. Hans Hedrich, Medizinische Hochschule, Hannover, Germany) and PVG\textsuperscript{av1} (Harlan Breeders) rats. The original Vra4 donors were males selected from the G8 generation of a DA \(\times\) PVG\textsuperscript{av1} advanced intercross line, chosen to transfer a relatively short and well-defined fragment harboring the Vra4 locus (RNO10: 0-D10Rat184). Repeated backcrossing to the respective recipient strain was performed for an additional nine generations to create congenics with theoretically \(<0.1\%\) of the donor genome outside the Vra4 locus. Littermate controls (Ibm) from the breeding step leading to homozygous congenics were used to exclude effects from possible genetic contamination. Animals were kept in a barrier animal facility under specific pathogen-free and climate-controlled conditions with 12-h light/dark cycles, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water ad libitum. All experiments in this study were approved and performed in accordance with the guidelines from the Swedish National Board for Laboratory Animals and the European Community Council Directive (86/609/EEC).

**Genotyping**

Genomic DNA was extracted from tail tips or ear clippings using a standard protocol. PCR primers for polymorphic simple sequence length polymorphisms were selected from available internet databases (Rat Genome Database [rgd.mcw.edu], Center for Genomic Research, Whitehead Institute/MIT [www.genome.wi.mit.edu/rao/public/], Ensembl [www.ensembl.org]), and UniSTS at NCBI ([www.ncbi.nlm.nih.gov]). The primers were purchased from PROLOGO. One primer in each pair was labeled with \([\gamma\textsuperscript{32P}]\text{ATP}\) (PerkinElmer), genomic DNA was amplified with a standard PCR protocol, and the amplified fragments were separated on 6% polyacrylamide gels. Genotypes were recorded manually from autoradiographic films independently by two investigators. DNA from DA and PVG\textsuperscript{av1} rats were included for each marker.

**RT-PCR**

Total RNA was isolated from homogenized tissues using a RNeasy total RNA extraction kit (Qiagen). Each spinal cord sample consisted of the ipsilateral ventral quadrant from the L3 segment. RNA samples underwent 15 min on-column DNA digestion (27 Kunitz units; Qiagen) before cDNA synthesis to avoid amplification of genomic DNA. Reverse transcription was performed with 10 \(\mu\)l of total RNA, random hexamer primers (0.1 \(\mu\)g; Invitrogen Life Technologies), and superscript reverse transcriptase (200 U; Invitrogen Life Technologies). The following primers were used for RT-PCR: Aif1 (adipocyte inflammatory factor) forward 5’-GGAGGCGTCAAGCAAAGGATGC; reverse 5’-AGCATTTGCGCCACGAGAATA; B2m forward 5’-GTGTCGACATTGGAAGTAGAC; reverse 5’-ACCGTCCTCGAACTTCTGACAGCT; Cd74 forward 5’-GCCGTTAAATCT; reverse 5’-TTGGAGGTTGGTGAAGCTG; D10Rat184 forward 5’-CACTCTTCGGCTAAATTCTG; reverse 5’-TCTCGCTGCTGTACACACG; Hprt forward 5’-CTGGTATTCTCCACGAC; reverse 5’-TTCTTGTAGGTCTTCTGGATTC; Gapdh forward 5’-TCAACTCTAGTGGCTCAGTTACGCAG; reverse 5’-TCCAGTTTCTCAGCGCCGACTG; Hprt forward 5’-CTGTGACGTGATTGTTAGGAAG; reverse 5’-GAGGTCAGAAGAAGACCTTATG; Mhc2ta forward 5’-CATACTCTGCTGCTCAGCGGAC; reverse 5’-AGTTGTCAGCTGCTCGCCACCUCCA; for Cfdy real-time PCR detection system (Bio-Rad). All primers, except B2m, were designed with Primer Express software (PerkinElmer). B2m was designed using Beacon Designer software (Biorad). Primer specificity was assessed by analyzing amilpic sequence curves in each sample. Relative amounts of mRNA levels were calculated using the standard curve method, constructed by using serial dilutions of cDNA. All samples were analyzed in duplicates. The threshold level in each sample was calculated as the ratio between the relative amount of the specific marker investigated to the endogenous control, Hprt. Using Gapdh as control gave similar results for Mhc2ta and Cd74 (data not shown). For glial markers, the two reference genes were combined. Samples without template or with template where the reverse transcription step had been omitted served as controls for contamination and amplification of genomic DNA, respectively. The relative expression for each sample was normalized to the DA median value. Groups were compared using the Kruskal-Wallis test followed by Dunn’s post test with the following comparisons; DA vs DA lm, DA vs

**FIGURE 1**. Expression of Mhc2ta (A) and Cd74 (B) at 21 days after VRA. Congenic strains differ from their respective parental strains, while the lm retain the parental phenotype (*, \(p < 0.05\); **, \(p < 0.01\); ***, \(p < 0.001\)).

**Nerve lesion**

Five rats each from the DA.PVG\textsuperscript{av1}.Vra4 and PVG\textsuperscript{av1}.DA-Vra4 strains were subjected to unilateral avulsion of the left L3-L5 ventral roots under standardized conditions and in deep isoflurane anesthesia at an age of 8–10 wk, with a postoperative survival time of 21 days. Ten rats from the parental strains DA and PVG\textsuperscript{av1} and five littermates from each respective congenic strain were used as controls. Animals were sacrificed with CO\(_2\) and perfused with cold PBS. Spinal cords were carefully examined in a dissection microscope to verify the completeness of the lesion and to exclude signs of hemorrhage, necrotic zones, or direct damage to the spinal cord. The meninges were removed and the L3 segment was dissected. That is, after bisection at the midline, a horizontal section at the level of the central canal was made to isolate the ipsilateral ventral quadrant of the cord. The L4-L5 segments were kept intact for histological analysis. The tissue was subsequently snap frozen and stored at –80°C until further use.
Immunohistochemistry
The immunohistochemistry methods used have been described previously (17, 18). Cryosections (14 μm) were cut at the level of the L4-L5 segment of the spinal cord for VRA and surrounding the injection site for intraparenchymal injections, respectively. Sections were fixed in 80% ice-cold acetone and 20% methanol (5 min) and incubated overnight at 4°C in the following primary antisera/Abs diluted in PBS (pH 7.4), goat anti-rat CD3 (polyclonal IgG; 1/100; Santa Cruz Biotechnology), mouse anti-rat Cd11b (clone OX-42, 1/200; BD Pharmingen), mouse anti-rat macrophage Ag (clone ED-1, 1/200; Serotec), or mouse anti-rat Ia Ag (clone OX-6, 1/200; Jackson ImmunoResearch Laboratories). The biotin-labeled antiserum was visualized by subsequent incubation with Cy3-conjugated streptavidin (1/1000; Jackson ImmunoResearch Laboratories) for 45 min at room temperature. The sections were analyzed in a Leica DM RBE microscope. The specificity of the immunostainings was tested in control slides by omission of the primary Ab and incubation with unrelated isotype-matched Ab controls.

Intraparenchymal injections
Stereotactic intraparenchymal injections (coordinates anterior-posterior, −0.3; medial-lateral, +2.5; dorsoventral, +4.3) were given to isoflurane-anesthetized 8- to 10-wk-old male DA and DA.PVGav1−Vra4 rats using a Hamilton syringe (type 701 RN, gauge 26s). Total injection volume was 2 μl over 2 min, containing a total of 50 U of IFN-γ (BD Pharmingen) in PBS. Animals were sacrificed 3 days later and brains were removed and kept at −70°C until sectioned at 14 μm.

Splenocyte preparation and culture
Untreated female DA and DA.PVGav1−Vra4 rats (n = 5/group) were used for in vitro studies. Spleens were dissected and splenocytes were filtered through a 70-μm filter. Cells were cultured at a density of 2 × 10^6/ml in 12-well plates (Nunc) in DMEM supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 292 μg L-glutamine (DMEM complete, all from Invitrogen Life Technologies) with 0, 10, or 100 U/ml IFN-γ, respectively, for 8 h at 37°C in 5% CO₂.

Dendritic cell differentiation
Bone marrow from untreated female DA and DA.PVGav1−Vra4 rats (n = 5/group) was flushed from femur bones with DMEM complete using a 21-gauge needle and filtered through a 70-μm filter. Cells were cultured at a density of 1 × 10^6/ml in DMEM complete, as described above, in 24-well plates (Nunc) for 6 days. Cells were differentiated into myeloid dendritic cells (mDC) through exposure to 5 ng/ml GM-CSF and 25 ng/ml IL-4, or differentiated into plasmacytoid dendritic cells (pDC) through exposure to FIGURE 2. Micrographs displaying immunolabeling of MHC II (OX-6) in the left ventral horn of the lumbar spinal cord in parental strains (A and D) lm (B and E), and congenics (C and F) at 21 days after VRA. The MHC II expression follows the pattern of the Vra4 origin. Scale bar, 200 μm.

FIGURE 3. Expression of B2m (A), Aif1 (B), Cd86 (C), and Cd11b (D) at 21 days after VRA. No significant effect on expression could be attributed to the Vra4 region.
subset. Statistical analysis was performed using the Mann-Whitney U rank sum test.

Inoculum at the tail base containing 60 or 80 (each group) were immunized with 10 rMOG, aa 1–125 from the N terminus, was expressed in Escherichia coli and purified to homogeneity by chelate chromatography (19). Two separately titrated batches of rMOG were used in the two EAE experiments and thus doses are not directly comparable. In the first experiment, rats of both sexes (five in each group) from the DA.PVGav1-A and DA strains, respectively, were anesthetized with isoflurane and injected with a 200-μl inoculum at the tail base containing 60 or 80 μg of rMOG emulsified in PBS (Invitrogen Life Technologies) diluted (1/1) in IFA (Sigma-Aldrich). In the second experiment, female DA.PVGav1-A and DA rats (15 in each group) were immunized with 10 μg from the second rMOG batch as described above. Animals were weighed and scored daily from day 7 (exp 1) or 10 (exp 2) postimmunization. Clinical score was evaluated as follows: 1, limp tail; 2, hind leg paraparesis (wobbling gait); 3, hind leg paralysis; 4, tetraplegia, and 5, moribund state or death. Rats who suffered severe disease (score ≥4) for >1 day and/or lost >25% of their body weight were sacrificed. The score at sacrifice was used for the remaining days. The following clinical parameters were recorded: occurrence/incidence of EAE, i.e., signs of EAE present for more than 1 day; onset of EAE, i.e., first day of clinical signs; duration of EAE, i.e., number of days rats showed clinical signs; cumulative EAE score, i.e., sum of the EAE scores obtained from day 10 until the end of the experiment; and the weight loss index, calculated as (1–[minimum weight during the experiment]/[weight at day 10 postimmunization]) × 100. The statistical tests used were: Fischer’s exact test for incidence (number of affected animals), Mann-Whitney U rank sum test for cumulative score and median day of onset, and unpaired t test for weight loss (phenotype passed normality test).

Results

Ventral root avulsion

Quantitative differences in Mhc2ta expression 21 days after VRA were assessed by RT-PCR, which demonstrated a significant influence by Vra4 in the congenics (Fig. 1A). Furthermore, a similar expression pattern was present for transcript levels of Cd74, the MHC II-associated invariant chain (Fig. 1B). The lm rats were included for both congenics to assess the possible effect of contaminating background genome of the donor strain outside the human FMS-related tyrosine kinase ligand (all growth factors from R&D Systems). The medium was changed every 3 days. Cells were subsequently stimulated with 0, 10, or 100 U/ml IFN-γ (BD Pharmingen), respectively, for 8 h at 37°C in 5% CO2.

Flow cytometry

Cells were stained with the following FACS Abs: anti-CD11b-R-PE, anti-CD11c-FITC (both from Serotec), anti-B220-PE, and anti-OX6-PerCP (both from BD Biosciences). Splenocytes were stained with anti-CD11c-FITC, anti-B220-PE, and anti-OX6-PerCP to identify B cells. Bone marrow-derived mDC and pDC were stained with anti-CD11c-FITC, anti-B220-PE, and anti-OX6-PerCP. The stained cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Data were analyzed using Summit software (DakoCytomation). To quantify MHC II expression, we measured the mean fluorescence intensity of MHC II+ cells for each cell subset. Statistical analysis was performed using the Mann-Whitney U rank sum test.

Experimental autoimmune encephalomyelitis

rMOG, aa 1–125 from the N terminus, was expressed in Escherichia coli and purified to homogeneity by chelate chromatography (19). Two separately titrated batches of rMOG were used in the two EAE experiments and thus doses are not directly comparable. In the first experiment, rats of both sexes (five in each group) from the DA.PVGav1-A and DA strains, respectively, were anesthetized with isoflurane and injected with a 200-μl inoculum at the tail base containing 60 or 80 μg of rMOG emulsified in PBS (Invitrogen Life Technologies) diluted (1/1) in IFA (Sigma-Aldrich). In the second experiment, female DA.PVGav1-A and DA rats (15 in each group) were immunized with 10 μg from the second rMOG batch as described above. Animals were weighed and scored daily from day 7 (exp 1) or 10 (exp 2) postimmunization. Clinical score was evaluated as follows: 1, limp tail; 2, hind leg paraparesis (wobbling gait); 3, hind leg paralysis; 4, tetraplegia, and 5, moribund state or death. Rats who suffered severe disease (score ≥4) for >1 day and/or lost >25% of their body weight were sacrificed. The score at sacrifice was used for the remaining days. The following clinical parameters were recorded: occurrence/incidence of EAE, i.e., signs of EAE present for more than 1 day; onset of EAE, i.e., first day of clinical signs; duration of EAE, i.e., number of days rats showed clinical signs; cumulative EAE score, i.e., sum of the EAE scores obtained from day 10 until the end of the experiment; and the weight loss index, calculated as (1–[minimum weight during the experiment]/[weight at day 10 postimmunization]) × 100. The statistical tests used were: Fischer’s exact test for incidence (number of affected animals), Mann-Whitney U rank sum test for cumulative score and median day of onset, and unpaired t test for weight loss (phenotype passed normality test).

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MHC II expression was thus determined by the Vra4 origin.
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The up-regulation of MHC II was accompanied by a modest infiltration of T cells. DA rats display higher numbers of infiltrating T cells compared with PVG\textsuperscript{\textdollar Vra4\textdollar}, however, no discernible congenic phenotype was evident for this parameter (data not shown).

To study the Vra4 effect on the general inflammatory activation pattern in the congenics, transcript levels of B_{2m} and the microglial activation markers Aijl, B7-2 (Cd86), and integrin \alpha_{\text{m}} (Cd11b) were assessed in spinal cord by RT-PCR at 21 days after VRA. Significant differences in expression levels of Cd11b and B_{2m} (\(p < 0.05\)) could be detected between the parental strains, while there were no differences that could be attributed to Vra4 in the congenics for any of the markers (Fig. 3).

**Intraparenchymal injections of IFN\gamma**

To study the effect of Vra4 in a classic model for inducing MHC II expression, 50 U of IFN-\(\gamma\) was injected into the striatum of DA.PVG\textsuperscript{\textdollar Vra4\textdollar} and DA rats and OX-6 immunolabeling was analyzed at 3 days after injections. A strong up-regulation of MHC II expression on microglia was observed in DA rats, while a much weaker induction was present in DA.PVG\textsuperscript{\textdollar Vra4\textdollar} rats (Fig. 4). In adjacent sections, the morphology and distribution of OX-6-labeled cells corresponded to a subpopulation of OX-42-positive microglial cells (Fig. 5, A and B). In contrast, ED1 immunolabeling was very low at the site of injection, indicating absence of infiltrating macrophages (Fig. 5C). A difference was also evident adjacent to the needle tract through the cortex, where rounded OX-6-positive cells were more numerous in the DA rat compared with DA.PVG\textsuperscript{\textdollar Vra4\textdollar}. Some of these cells were stained by ED1, suggesting infiltrating macrophages (data not shown).

**Lower MHC II expression in APCs from DA.PVG\textsuperscript{\textdollar Vra4\textdollar} rats**

HMC II-mediated Ag presentation by APCs is essential for initiation of CD4\(^{+}\) T cell responses. Therefore, the expression of MHC II on APC subsets from DA.PVG\textsuperscript{\textdollar Vra4\textdollar} congenic rats compared with DA rats was investigated. Splenocytes derived from untreated DA.PVG\textsuperscript{\textdollar Vra4\textdollar}-congenic rats or DA rats were cultured with or without IFN-\(\gamma\) in vitro, after which surface MHC II expression in B220\(^{+}\)MHC II\(^{+}\)B cells and CD11b\(^{+}\)MHC II\(^{+}\)macrophages was determined by flow cytometry. The expression of

**FIGURE 6.** MHC II expression is lower in B cells from DA.PVG\textsuperscript{\textdollar Vra4\textdollar} rats than in B cells from DA rats. Expression of MHC II was measured by flow cytometry in splenic B220\(^{+}\)MHC II\(^{+}\)B cells (A), CD11b\(^{+}\)MHC II\(^{+}\)macrophages (B), bone marrow-derived CD11c\(^{+}\)B220\(^{+}\)MHC II\(^{+}\)pDC (C), and CD11c\(^{+}\)B220\(^{+}\)MHC II\(^{+}\)mDC (D) after stimulation for 8 h with 0, 10, or 100 U/ml IFN-\(\gamma\) in vitro. Data are represented as mean \pm SEM (\(n = 5\) group; *, \(p < 0.05\)).

**FIGURE 7.** Mean EAE score in male and female DA and DA.PVG\textsuperscript{\textdollar Vra4\textdollar} Vra4-congenic rats upon immunization with rMOG at different doses. A, Males, 60 \(\mu\)g of rMOG; B, males, 80 \(\mu\)g of rMOG; C, females, 60 \(\mu\)g of rMOG; and D, females, 80 \(\mu\)g of rMOG, p.i., Postimmunization.
MHC II in response to IFN-γ stimulation was significantly lower in B cells from DA.PVGαv1-Vra4-congenic rats compared with DA rats (Fig. 6A). In macrophages, there was a tendency toward lower MHC II levels in DA.PVGαv1-Vra4 rats, however, not achieving statistical significance (p = 0.06 at 10 U/ml IFN-γ; Fig. 6B).

To assess MHC II expression on dendritic cells, bone marrow cells from untreated DA.PVGαv1-Vra4-congenic rats and DA rats were isolated and cultured with FMS-related tyrosine kinase ligand to generate pDC or with IL-4 and GM-CSF to generate mDC. Levels of MHC II expression in pDC (CD11clowB220-+) and mDC (CD11clowB220+), respectively, were determined by flow cytometry. The expression of MHC II in response to IFN-γ stimulation tended to be lower and at the threshold level of statistical significance (p = 0.06 at 10 U/ml IFN-γ; Fig. 6B).

Taken together, these results suggest that allelic differences at the Vra4 locus regulate levels of MHC II also in APCs, but that differences were greater in the B cell subset.

Experimental autoimmune encephalomyelitis

To study the in vivo effects of the Vra4 locus in regulation of susceptibility to autoimmune neuroinflammation, we induced EAE in rats by immunizing with rMOG in IFA, a reproducible model of many similarities to MS (20). In a first experiment using a mild to moderate induction protocol, PVGαv1 and PVGαv1-DA-Vra4 rats were completely protected from EAE. In contrast, DA.PVGαv1-Vra4 congens of both sexes displayed a dose-dependent protection from EAE (Fig. 7). Thus, DA.PVGαv1-Vra4 males immunized with 80 μg of rMOG and females immunized with 60 μg of rMOG demonstrated a milder disease course compared with parental DA controls. This effect was, however, not seen in females immunized with the higher dose of rMOG, suggesting that the protective effect of Vra4 could be overcome by a stronger induction protocol. These findings were reproduced in a larger experiment using a relatively mild induction protocol, in which a protective effect of the Vra4 locus in female DA.PVGαv1-Vra4 rats compared with DA controls was confirmed (Fig. 8A).

Severity of disease, reflected by the cumulative EAE score and weight loss, was significantly lower in animals with PVGαv1 alleles at the Vra4 locus (Fig. 8B). There was also a significantly lower incidence rate in the congenic animals compared with the DA parental animals. However, there was a trend toward an earlier onset of disease in the DA.PVGαv1-Vra4 rats (Table I). In conclusion, the Vra4 locus affects both the susceptibility to EAE and the severity of disease.

Discussion

We demonstrate here that the Vra4 locus, comprising naturally occurring alternative alleles of the Mhc2ta gene, exerts a pivotal regulatory effect on the expression of MHC II in the CNS of congenic rats and that these allelic differences also affect MHC II expression on immune cells in the periphery. These findings corroborate and complement the mapping of differences in MHC II expression to the Vra4 locus obtained in previous intercross experiments (12, 16). By using congenics, that is, isolating Vra4 from a donor strain on a recipient background, we obtained the tools to study the effects in a well-controlled and reproducible model. Interestingly, the up-regulation of Mhc2ta and Cd74 in the CNS of PVGαv1-DA-Vra4-congenic strain was increased compared with the Vra4 donor strain DA. Possible explanations could be effects from the PVGαv1 genome acting upstream of Mhc2ta transcription or an interactive effect of the DA Mhc2ta allele with other gene regions present in the PVGαv1 background. In the original genome-wide scan of differences in MHC II expression in an F2 DA × PVG intercross, the Vra4 locus was the only region displaying genome-wide significance (16). However, after correction for genotype at the Vra4 locus, linkage to the MHC complex on chromosome 20 is also evident (O. Lidman, M. Swanberg, T. Olsson, and F. Piehl, unpublished observation). We have here used MHC-congenic rats and the discrepancy in expression of Cd74 between DA (RT1.1AV1) and PVGαv1-DA-Vra4-congenic animals can thus not be explained by an effect of the MHC complex. However, there is evidence for the existence of additional gene regions outside of the MHC complex that affect expression of MHC II. In the BN rat, a higher ratio of constitutive MHC II-positive microglia has been documented and the up-regulation of MHC II after VRA is more rapid compared with other strains despite the fact that BN carries the same Mhc2ta allele as the LEW and DA strains (12, 15, 21). Additional support for this hypothesis is provided by a whole genome scan in an F2 intercross between the BN (RT1.1N) and LEWα (LEW.BN-RT1N) strains revealing two quantitative trait loci outside of the MHC complex linked to differences in MHC II expression (M. Diez, N. Abdelmagid, K. Harnesk, M. Stro¨m, O. Lidman, M. Swanberg, T. Olsson, and F. Piehl, unpublished data). In one of these quantitative trait loci, the MHC II expression was driven by the allele originating from the LEWα strain, the original slow and
low responder. This exemplifies that such effects may go undetected in the original inbred strain, but become unmasked in an intercross or congenic experiment. However, it is strikingly clear from the findings obtained here that the Vra4 locus on its own is the main determinant for DA and PVG<sup>v1</sup> strain-specific differences in MHC II expression in the CNS.

In contrast, we were not able to detect any other glial expression phenotype in the congenic strains. This includes B<sub>r</sub>m as a marker of MHC I expression as well as Cd86, Cd11b, and Aif1, all markers of microglial activation. Other studies have demonstrated that the CIITA protein exerts a regulatory effect on the transcription of specific gene products other than MHC II, including MHC I (22), IL-4 (23), and cathepsin E (24) and have effects on global transcription as measured by microrarrays (11, 25). However, these studies were conducted in vitro, with cells deficient of MHC2TA and/or transfected with MHC2TA expression constructs. The results obtained here suggest that naturally occurring genetic variants of rat Mhc2ta studied in vivo under physiological conditions have limited effects on the VRA phenotype, except for MHC II-associated transcripts. This conclusion is also supported by in vivo findings in the mouse using transgenic animals (26, 27). In this context it is of interest that expression of MHC II is often used as a marker of microglial activation, although it can be dissociated from other markers of activation as judged from our results. This is supported by findings in the E3 rat that in the VRA model displays prominent microglial activation, but very sparse MHC II expression (15).

In this study, we provide the first experimental data showing that naturally occurring allelic variability in Mhc2ta modulates susceptibility to autoimmune disease. Previous studies using mouse knockouts with impaired MHC II expression demonstrate reduced susceptibility to EAE (26, 28, 29). Although these results are interesting mechanistically, knockout models do not provide information about the genetic influence in complex diseases such as MS. In the first set of experiments, rats of both sexes with the P<sup>v1</sup> Mhc2ta allele on the DA background displayed a milder disease course of rMOG-induced EAE than DA controls. However, the difference between congenics and DA female rats disappeared at the higher dose of immunogen, suggesting that the protective effect could be overcome by a stronger induction protocol. The disease modulating effect of Vra4 was subsequently reproduced in a larger experiment using a relatively mild induction protocol. As expected, DA rats displayed a mild, but chronic disease during the entire experiment. In contrast, DA.PVG<sup>v1</sup>-Vra4- congenic rats were largely protected and in the minority of immunized rats displaying clinical signs, the disease course was milder than in DA controls.

Lower expression of MHC II was demonstrated for peripheral APCs from the DA.PVG<sup>v1</sup>-Vra4- congenic compared with the DA strain when stimulated in vitro with increasing concentrations of IFN-γ. Our data may indicate that APCs from DA.PVG<sup>v1</sup>-Vra4- congenic rats have a reduced capacity to present autoantigens to encephalitogenic Th cells in the periphery, which could explain the altered susceptibility to EAE. It may also be that B cells, mDC, and/or pDC are involved in the priming of encephalitogenic Th cells in EAE. Further studies of the immune mechanisms involved are warranted and ongoing in the laboratory.

We previously found a weak, but significant association of a MHC2TA SNP (rs3087456) to risk of disease in three separate clinical materials, comprising patients with MS, RA, and myocardial infarction. This observation spurred a number of studies attempting to reproduce the original finding, where both negative (30–34) and positive (35, 36) results have been reported. Linkage to the 16p.13 region harboring the MHC2TA gene was also found in three RA linkage meta-analyses (37–39). In addition, positive association results have been reported for other autoimmune diseases (40, 41). A problem is that most studies published so far have been underpowered to detect a possible low to moderate effect on disease susceptibility. In some cases, the lack of proper population-based control materials may have interfered with the interpretation of the results. Of note, the largest study published so far reproduced the findings of an association between the rs3087456 SNP in the MHC2TA type III promoter to risk of mortality of cardiovascular disease and the metabolic syndrome (42). Confirmation of the candidate gene status of MHC2TA in human complex diseases thus awaits studies in larger clinical materials. In our view, the rationale for such studies is increased by the finding of a clinical effect on EAE in Vra4 congenics. However, it is intriguing that the lowered expression of MHC II in DA.PVG<sup>v1</sup>-Vra4 animals was associated with partial protection from EAE, while in the human the SNP genotype linked to lower MHC II expression was associated with increased risk of disease (12). This discrepancy may depend on a number of reasons. Most important, extrapolating data from acute animal models should be done cautiously when trying to understand lifelong, chronic diseases. In addition, the haplotypes detected in rats and humans, respectively, may be functionally different in other aspects than in the regulation of MHC II in microglia and peripheral blood cells. This underscores the need for further studies aimed at understanding the mechanisms of how allelic variations in MHC2TA modulate disease susceptibility. For example, it is possible that MHC2TA exerts effects on Th cell differentiation (43) or affects the response to environmental factors such as infectious agents (44). The congenic strains reported here can serve as valuable tools for such studies.

In conclusion, we demonstrate here that reciprocal Vra4 congenics on the DA and P<sup>v1</sup> background, respectively, display a reversed strain-specific pattern of expression of MHC II molecules on peripheral immune cells and on microglia, but not of other markers of microglial activation. Furthermore, Vra4 congenics on an EAE permissive background, i.e., DA, are partly protected from rMOG-induced EAE in the settings of a mild immunization protocol. These data support the notion that naturally occurring allelic variants of Mhc2ta are the main determinants for strain-specific expression of MHC II, in turn of importance for susceptibility to autoimmune neuroinflammation.

**Disclosures**

The authors have no financial conflict of interest.

**References**


