Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene

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The Ncf1 gene was recently identified as a strong regulator of severe arthritis in rat. This finding was surprising, because the disease-promoting allele mediated a lower level of reactive oxygen species in NADPH oxidase-expressing cells. We have now investigated a splice mutation of the Ncf1 gene in B10.Q mice, causing a truncated and nonfunctional Ncf1 protein. We found that the mutated Ncf1 led to a more severe and chronic relapsing collagen-induced arthritis. Enhanced IgG and delayed-type hypersensitivity responses against type II collagen were seen, indicating increased activity of autoreactive T cells. Interestingly, female Ncf1-mutated mice spontaneously developed severe arthritis during the postpartum period. The arthritis was accompanied by an increased antibody response to type II collagen, with the same fine specificity as in collagen-induced arthritis. The enhancing effect of the mutated Ncf1 could also be shown to be more general in that it enhanced myelin oligodendrocyte glycoprotein protein-induced experimental autoimmune encephalomyelitis, a model for multiple sclerosis. These results show that Ncf1, a gene important for oxidative burst, regulates the susceptibility and severity of both arthritis and encephalomyelitis and modulates, directly or indirectly, the level of T cell-dependent autoimmune responses.

Rodent | T lymphocytes | NADPH

Rheumatoid arthritis (RA) is a common autoimmune disease that is partially genetically regulated. Like many other autoimmune diseases, a significant part of the genetic contribution is the association with the MHC region (1, 2). However, despite decades of research of linkage and association studies, including genetic dissection of the MHC region, successful molecular understanding of these complex diseases, including single mutations and polymorphisms, is rare (3–5). Recently, we identified a polymorphism of Ncf1 (alias p47phox) that regulates the severity of arthritis in rats (6). A surprising fact with the Ncf1 polymorphism in the rat is that the susceptibility allele of Ncf1 is accompanied by lower level of reactive oxygen species (ROS) than the resistant strain, contradictory to general dogma where high levels of radicals have been considered to promote inflammation (7). This association between ROS and autoimmunity is interesting, because it opens up the possibilities for new insight into the pathogenesis of complex autoimmune disorders. The Ncf1 protein is an essential component of the NADPH oxidase complex that catalyzes the transfer of a single electron from NADPH to oxygen, generating ROS. The release of ROS and its downstream products from phagocytic cells is known as the respiratory burst and is regarded as part of the protection against invading pathogens (7). The importance for the innate immune defense of having a functional phagocyte NADPH oxidase is clearly exemplified in chronic granulomatous disease (CGD), a rare genetic disorder characterized by severe recurrent infections due to the inability of neutrophils and macrophages to mount a respiratory burst and thereby kill the invading pathogens (8). The second most common genetic defect, responsible for ∼30% of CGD cases, is an autosomal recessive form caused by mutations in Ncf1 (9). Knockouts of Ncf1 (10) have been created through gene-targeting technologies, creating mouse models with a CGD phenotype. Targeted gene disruption in mice is the most common method to study the effects of gene defects. The selection of which genes to analyze by genetic manipulations, however, must be chosen through highly qualified predictions based on knowledge of the molecular pathology of the immune system. This approach is most feasible in mono- genic diseases like CGD. However, in polygenic diseases, which cause most common autoimmune disorders like RA, the underlying molecular mechanism is more complex due to the contributions and interactions of several genes. Hence, other ways to unravel the genetic regulation must first be considered. Through linkage analysis followed by positional cloning, naturally occurring polymorphisms that predispose susceptibility to diseases can be identified without prior determinations about regulating genes (3).

Given the identified linkage between arthritis and Ncf1 polymorphism in the rat, careful analysis of arthritis severity in NADPH oxidase-deficient animals is of interest. To this intent, we have used an earlier identified natural mutation in the Ncf1 gene in C57BL/6J mice (11). This mutation, located at a splicing site at position –2 before exon 8 of the Ncf1 gene, results in various forms of transcripts. One of these gave rise to low levels of protein but was truncated, lacking eight amino acids in the second SH3 domain of critical importance for interaction with the Ncf2 protein. Consequently, the mice had no detectable NADPH oxidase function, measured by ROS response of neutrophils. We have backcrossed this mutation to B10.Q strains, allowing study of its role in experimental models for RA and multiple sclerosis. These Ncf1-mutated mice showed augmented severity of both chronic experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA). An enhancing effect on T cell-dependent autoimmunity was observed. Interestingly, these mice spontaneously developed severe arthritis in the postpartum period, i.e., in a phase of life when both mice and humans are most susceptible to developing certain autoimmune diseases, such as arthritis (12–15).

Materials and Methods

Animals. Mice [B6.Cg-m+/- Lepr (db)], formerly known as C57BL/6J-m+/- Lepr(db) with a relative deficiency of Ncf1 caused by a point mutation in the splice site for exon 8 (11) were...

Abbreviations: RA, rheumatoid arthritis; ROS, reactive oxygen species; EAE, experimental autoimmune encephalomyelitis; CIA, collagen-induced arthritis; CFA, complete Freund’s adjuvant; CII, collagen II; COMP, cartilage oligomeric matrix protein; OVA, ovalbumin; DTH, delayed-type hypersensitivity; APC, antigen-presenting cells; MPO, myelin oligodendrocyte glycoprotein.

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purchased from The Jackson Laboratory. The mice were backcrossed to B10.Q (originally from Jan Klein, Tübingen University, Tübingen, Germany) for at least six generations to introduce the H2\textsuperscript{i} haplotype in the MHC locus and to breed out the leptin receptor (lepr\textsuperscript{6J}) defect present in these mice. Offspring from a B10.Q.Ncf1+/+/ (\(*\) denotes the mutation, and + denotes the wild type) intercross were used. All mice were kept and bred in a climate-controlled environment with a 12-h light/dark cycle, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water ad libitum in the animal house of Medical Inflammation Research. The experiments were approved by a local (Malmö/Lund, Sweden) ethical committee (license M7-01).

**Genotyping.** DNA was prepared from toe biopsies by heating the sample in 1 ml of 50 mM NaOH for 1 h (16). The DNA solution was neutralized with 100 \(\mu\)l of 1 M Tris buffer and used directly in PCR reactions. Single-nucleotide polymorphism markers that distinguish between the wild-type allele and the mutation in the splice site of exon 8, causing exclusion of this exon, were performed by using Ncf1-ex8-specific markers. The reaction was amplified by PCR using forward 5'-bio-TAG AAA GGG AAA GCC AGA AAG AAT-3' and reverse 5'-ACG CTT TGA TGG TTA CAT AC5 GT3'- primers yielding a DNA template of 365 bp that, after separation with streptavidin, can be detected in a sequencing reaction by the sequencing primer 5'-ACG CTT TGA TGG TTA CAT AC5 GT3'- using Pyrosequencing equipment according to protocols supplied by the manufacturer (Biotage, Uppsala).

**Induction and Evaluation of Arthritis.** The mice were age-matched (9–15 weeks) and given an intradermal injection at the base of the tail with 100 \(\mu\)g of rat collagen II (CII) purified from the Swarm rat chondrosarcoma, as described (17), emulsified in complete Freund's adjuvant (CFA; Difco). At day 35 after primary CII immunization, mice were boosted at the same location with 50 \(\mu\)g of rat CII in incomplete Freund's adjuvant. Arthritis development was monitored in all four limbs by using a macroscopic scoring system. Briefly, one point was given for each swollen or red toe, one point for each swollen joint (metatarsal phalangeal joints, metacarpal phalangeal joints, proximal interphalangeal joints, and distal interphalangeal joints), and five points for a swollen ankle (maximum score per limb 15, 60 per mouse). The mice were examined one to four times per week for 2 months after immunization. At days 40 and 102, serum was obtained through tail bleeding and kept at \(-20^\circ\)C until assayed. For histopathologic analyses, ankle joints were taken on day 31 after CII immunization. Paws were fixed in 4% formaldehyde, decalcified in EDTA, embedded in paraffin, sectioned, and stained with hematoxylin and erythrosine (18).

**Induction and Evaluation of EAE.** The mice were age-matched (12–15 weeks) and immunized at the base of the tail with 25 \(\mu\)g of recombinant rat MOG\textsubscript{1-125} protein or 25 \(\mu\)g of mouse myelin oligodendrocyte glycoprotein (MOG)\textsubscript{92-96} peptide emulsified in CFA (Difco), containing 50 \(\mu\)g of Mycobacterium tuberculosis. Clinical signs of EAE were assessed by using a standard scoring protocol (19). The recombinant MOG\textsubscript{1-125} protein was a generous gift from Robert Harris (Karolinska Institute, Stockholm). The MOG\textsubscript{92-96} peptide corresponds to amino acids of the mouse sequence (GKVTLRIONRFSDDEGGY) and was synthesized by Shafar-N, Copenhagen, with a purity of >97%.

**Determination of Serum Levels of Cartilage Oligomeric Matrix Protein (COMP).** Serum concentration of COMP was determined by a competitive enzyme-linked immunosorbent assay (ELISA) (20). Rat COMP was used for coating of the microtiter plates and for preparing the standard curve included in each plate. Plasma COMP was detected by using a polyclonal antiserum against rat COMP (generously provided by Dick Heinegård, Lund University, Lund, Sweden).

**Antibody Response Against CII and Ovalbumin (OVA).** Antibodies against CII or OVA in plasma were analyzed with ELISA in 96-well plates (Costar), coated overnight at 4°C with 50 \(\mu\)l of PBS per well containing 10 \(\mu\)g/ml rat CII, epitopes of CII [prepared as described (21)] or OVA (Sigma). All washings were performed by using Tris-buffered saline (NaCl 1.3 M/Tris 0.1 M, pH 7.4) containing 0.1% Tween 20 (Tris/Tween). The plasma was diluted in PBS/0.1% Tween and analyzed in duplicate. The amounts of bound IgM and IgG isotype antibodies (Southern Biotechnology Associates) were estimated after incubation with biotin-conjugated isotype-specific antibodies and 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt as substrate followed by detection in a Spectra Max (Molecular Devices). The relative amount of antibodies was determined in comparison with a positive control of pooled serum. Because different pools of serum were used for the different experiments, no comparisons should be made among the experiments.

**Delayed-Type Hypersensitivity (DTH) Response.** Mice, at age 9–15 weeks, received an intradermal injection at the base of the tail with either 100 \(\mu\)g of CII emulsified in CFA (Difco) or 100 \(\mu\)g of OVA (Sigma) emulsified in 50 \(\mu\)l of PBS and 50 \(\mu\)l of CFA. At day 13 after primary injection, the CII-immunized mice received an injection containing 20 \(\mu\)g of CII in 0.05 M acetic acid in the right ear. Acetic acid (0.05 M) was injected in the left ear as a control. The OVA-immunized mice were injected with 20 \(\mu\)g of OVA in PBS in the right ear. The control ear was injected with PBS. After 48 h, the DTH response was measured as the difference in percent in swelling of the ears between the right and left ears.

**Statistics.** Quantitative data are expressed as mean ± SEM, and significance analysis was performed by using Student's \(t\) test or the Mann–Whitney test. All results were compared to those from B10.Q.Ncf1+/+ mice.

**Results**

**An Ncf1 Splicing Mutation Backcrossed on B10.Q Mice Prohibits Ncf1 Expression and the ROS Response.** A mutation at position −2 before exon 8 generating a splice defect of the Ncf1 gene in a C57BL/6J colony also carrying a leptin receptor mutation has been reported to have a dramatically reduced expression of Ncf1 protein and an undetectable ROS response (11). To determine its effect on CIA and EAE, we backcrossed the mouse strain carrying the Ncf1 mutation to the B10.Q strain. The B10.Q mouse carries the C57BL/10 background and a DBA/1-derived congenic fragment containing the MHC class II gene A\textsuperscript{i} allowing an immune response to CII and MOG (19, 22). The experiments were performed on an intercrossed cohort obtained after four to six generations of backcrossing. Remaining differences in the genome background were excluded by littermate-controlled experiments. Differences in the linked fragment on chromosome 5 were unlikely to play a role, because none of the microsatellites defined on this chromosome differ in length between C57BL/6J and B10.Q (Mouse Genome Informatics, www.informatics.jax.org, data not shown). Consequently, no autoimmune quantitative trait loci have been found on chromosome 5 that differs between C57BL/6J and C57BL/10 (23). Analysis of spleen neutrophils from the backcrossed Ncf1-mutated B10.Q mice confirmed that the homozygous mutation led to a reduced Ncf1 expression and undetectable ROS response (see Supporting Text and Fig. 5, which are published as supporting information on the PNAS web site), as investigated in detail by Huang et al. (11).
Severe Chronic CIA in Ncf1 Mutated Mice. To investigate the effect of aberrant Ncf1 function and NADPH oxidase complex activity on arthritis, we investigated the development of CIA in Ncf1-mutated mice. Wild-type mice normally develop mild/medium severe arthritis with onset after booster (24). We observed more severe arthritis with earlier onset (P < 0.001) in B10.Q.Ncf1+/+ compared to wild-type mice (Fig. 1). Ncf1+/+ mice showed significantly milder arthritis than the homozygously mutated Ncf1+/+ mice, indicating that one fully functional allele of the Ncf1 gene is enough to protect from the severe form of the disease. Interestingly, the Ncf1-mutated mice showed an increasingly active chronic development of arthritis characterized by severe inflammation in a relapsing pattern.

The serum levels of COMP, which is regarded as a measure of ongoing cartilage destruction (20), were scored due to scarring for histology; B10.Q.Ncf1+/+ (n = 10), B10.Q.Ncf1+/− (n = 19), B10.Q.Ncf1−/− (n = 4). (A) Arthritis scores plotted against the different days after CII immunization. (B) Serum concentration of COMP used as a measurement of ongoing cartilage destruction days 40 and 102. Filled circles represent mice with arthritis, and lines represent the mean values [B10.Q.Ncf1+/+, n = 19, B10.Q.Ncf1−/+, n = 28, B10.Q.Ncf1+/−, n = 11]. #, few animals analyzed on day 102 (B10.Q.Ncf1+/+, n = 10, B10.Q.Ncf1−/+, n = 19, B10.Q.Ncf1+/−, n = 40), P > 0.001 for both wild-type and heterozygous mice in comparison with mutated mice on day 40. Day 102 P > 0.01 for wild-type and P < 0.5 for heterozygous mice. (C) Representative morphological sections of ankle joints taken at day 102 after CIA induction. Sections were stained with hematoxylin and erythrosine (×100). Severe arthritis with cartilage and bone destruction is seen in the B10.Q.Ncf1+/+ mice, whereas there was milder cartilage destruction in the B10.Q.Ncf1+/− and B10.Q.Ncf1+/− control mice.

Enhancement of MOG Protein-Induced EAE and Suppression of MOG Peptide-Induced EAE. Our results of an enhancement of CIA were surprising in light of earlier reported findings that EAE, induced with the MOG35-55 peptide, was completely protected in mice deleted for the Ncf1 gene (25). To clarify this, we immunized our Ncf1-mutated mice with MOG protein or MOG35-55 peptide, both known to induce EAE in H2a mice (19). MOG35-55 peptide-induced EAE was milder in the Ncf1-mutated mice than in the wild-type control (Fig. 3A), confirming the results reported by van der Veen et al. (25). However, induction of EAE with MOG protein induced a more severe chronic EAE in the Ncf1-mutated mice, corroborating our results in the CIA model (Fig. 3B). This finding shows that Ncf1 has a more general role in regulating autoimmune inflammation and also suggests that the effect of Ncf1 is related to the function of antigen-presenting cells (APC) in processing and presenting autoantigens to T cells.

Spontaneous Arthritis in B10.Q.Ncf1+/− Mice. The Ncf1-mutated mice showed no abnormalities apart from one consistent obser-
and U1 can be seen between wild-type and homozygously mutated mice. (arthritic mice compared to the healthy B10.Q. bodies against CII was detected in sera from the spontaneous

Histology showed a massive destruction of bone and cartilage in

so-called postpartum period), were observed to develop severe

T cell-dependent autoimmune response to CII, but not to OVA, is

enhanced in Ncf1-mutated mice. Antibody response directed against (A) CII and (B) epitopes of CII was measured in serum at day 40 after CIA induction in B10.Q.Ncf1+/+ (n = 19), B10.Q.Ncf1+/− (n = 28) and B10.Q.Ncf1−/− (n = 11) mice. A significant difference (P < 0.05) in levels of antibodies against C1, J1, and U1 can be seen between wild-type and homozygously mutated mice. (C) DTH response against CII in B10.Q.Ncf1+/+ (n = 17), B10.Q.Ncf1−/− (n = 20) and B10.Q.Ncf1−/− (n = 7) was evaluated for reactivity 48 h after stimulation with CII in the ear. DTH result is presented as percent volume increase of the stimulated ear in comparison to control injected left ear. A significantly lower response can be seen in B10.Q.Ncf1+/+ and B10.Q.Ncf1+/− compared with B10.Q.Ncf1−/− (P < 0.05). (D) DTH response presented as percent volume increase of the ear stimulated with OVA in comparison with control-injected left ear after 48 h and (E) antibody response measured in sera on day 18 after immunization with OVA in B10.Q.Ncf1+/+ (n = 7) and B10.Q.Ncf1−/− (n = 11).

Discussion

The identification of a single-nucleotide polymorphism in the rat Ncf1 gene leading to a reduced oxidative burst and severe arthritis was indeed a surprising but also challenging finding. We have now found similar effects in a mouse strain carrying another mutation in Ncf1, also leading to a reduced ROS response. We are now able to show that the Ncf1 polymorphism also enhanced autoreactive T cell functions and the development of chronic CIA and EAE. Interestingly, the mutated Ncf1 mice developed chronic severe arthritis spontaneously postpartum.

The oxidative burst, induced in phagocytic cells after exposure of bacterial components, is generally thought to be a local powerful component in the inflammatory response. It has also been widely anticipated that the ROS response promotes an autoimmune inflammation. Recently, however, further complexity of the redox status and the induced ROS response in particular has been revealed. Thus, the ROS response may occur not only in phagocytes but also in other cell types, including APC such as dendritic cells and class II-expressing macrophages (26). Ncf1 expression and functional activities in APC but not in T cells have earlier been reported (27, 28). Oxidation effects have also been shown to modulate T and B cell function, and interestingly on T cells a major effect is oxidation of cell membranes that are exposed for ROS from interacting cells (29). It is clear that oxidation effects on T cell receptor signaling pathways, such as in the linker for activation of T cells molecule (30), modulate T cell functions, and it is theoretically possible that ROS, in particular hydroxide radicals, could be transferred over the synapse from APC to T cells (31, 32). It is also possible that an oxidative burst response in an APC may change the antigen-presentation capacity of that cell. An interaction between APC and T cells occurs at several stages during T cell development in vivo; in the thymus, during migration into peripheral lymphoid organs, during priming, and maybe also during subsequent activation in the target tissue. The enhanced T cell autoimmune activities could be explained by an influence at any of these occasions in vivo. It also remains to be investigated whether the modulated T cell function by ROS leads to an enhanced regulatory function or a decreased activity of autoreactive T cells. Sakaguchi et al. (33) have recently reported a similar situation; the occurrence of spontaneous arthritis in mice was found to be caused by a structural polymorphism in the Zap70 gene. This mutation caused a decreased T cell receptor signaling, resulting in an enhanced T cell autoreactivity to joint antigens such as CII, and with the development of arthritis depending on an interactive effect with MHC class II. Thus, polymorphism in both the Zap70 and Ncf1 genes in rat and mouse argues for an important effect during T cell priming and selection as a cause for the enhancement of arthritis. The Ncf1

Fig. 2. T cell-dependent autoimmune response to CII, but not to OVA, is enhanced in Ncf1-mutated mice. Antibody response directed against (A) CII and (B) epitopes of CII was measured in serum at day 40 after CIA induction in B10.Q.Ncf1+/+ (n = 19), B10.Q.Ncf1+/− (n = 28) and B10.Q.Ncf1−/− (n = 11) mice. A significant difference (P < 0.05) in levels of antibodies against C1, J1, and U1 can be seen between wild-type and homozygously mutated mice. (C) DTH response against CII in B10.Q.Ncf1+/+ (n = 17), B10.Q.Ncf1−/− (n = 20) and B10.Q.Ncf1−/− (n = 7) was evaluated for reactivity 48 h after stimulation with CII in the ear. DTH result is presented as percent volume increase of the stimulated ear in comparison to control injected left ear. A significantly lower response can be seen in B10.Q.Ncf1+/+ and B10.Q.Ncf1+/− compared with B10.Q.Ncf1−/− (P < 0.05). (D) DTH response presented as percent volume increase of the ear stimulated with OVA in comparison with control-injected left ear after 48 h and (E) antibody response measured in sera on day 18 after immunization with OVA in B10.Q.Ncf1+/+ (n = 7) and B10.Q.Ncf1−/− (n = 11).

Fig. 3. Enhancement of EAE induced with native MOG but reduction of EAE induced with a MOG peptide in the Ncf1-mutated mice. Influence of the Ncf1 mutation on experimental EAE. (A) Development of EAE in mice immunized with the MOG peptide 79–96 in CFA B10.Q.Ncf1+/+ (n = 13), B10.Q.Ncf1+/− (n = 9) and B10.Q.Ncf1−/− (n = 12). (B) Development of EAE in mice immunized with native MOG recombinant protein in CFA B10.Q.Ncf1+/+ (n = 13), B10.Q.Ncf1+/− (n = 7) and B10.Q.Ncf1−/− (n = 4).

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which the effect could be transferred with observations in the pristane-induced arthritis model in the rat in the reduced Ncf1-mediated ROS response enhances T cell-reduce oxidative burst and promote arthritis severity. Nevertheless, both the rat and the mouse mutations Ncf1 reduced oxidative burst. The structural polymorphism in the rat hypothesis that increased arthritis severity is directly related to mouse increased arthritis severity. Our results using a mutation in the allele in the rat, which promotes a reduced ROS response and MOG-peptide-induced EAE (25). This finding is difficult to and a control mouse. The sections were stained with hematoxylin and erythrosine (×100). (D) The antibody response directed against CII and epitopes of CII in B10.Q.Ncf1+/− mice with spontaneous arthritis compared to healthy B10.Q.Ncf1 +/+ and B10.Q.Ncf1+/− mice (n = 3). The mutated mice with spontaneous arthritis have significantly higher levels of C1, J1, and U1 epitopes (P < 0.05).

gene, however, is likely to have divergent influence in various tissues and in different cell types. Thus, the local oxidative environment in the inflammatory focus as a drive for vascularization and cell toxicity and, on the other hand, its role in the APC–T cell interaction shifting the function of inflammatory cells and T cells may lead to contrasting outcomes of a ROS response. Recent results using mice unable to mount a ROS response, due to deficiency in a critical component in the NADPH oxidase complex, such as Ncf1, show a different effect on an inflammatory response. The observation that zymosan-induced arthritis is enhanced in Ncf1-knockout B6/129 mice (34) is not likely to be an effect on the selection of autoreactive T cells, because this is a local T cell-independent type of arthritis. Possibly a complete deficiency of the ROS response may also lead to a feedback regulation, increasing the number of circulating neutrophils. A contrasting effect is the observation that Ncf1-knockout B6/129 mice are completely protected from MOG-peptide-induced EAE (25). This finding is difficult to explain in light of our recent identification of a polymorphic Ncf1 allele in the rat, which promotes a reduced ROS response and increased arthritis severity. Our results using a mutation in the mouse Ncf1 gene may partly clarify these issues.

First, combining our results in rats and mice strengthens the hypothesis that increased arthritis severity is directly related to reduced oxidative burst. The structural polymorphism in the rat Ncf1, giving amino acid replacements at position 106 and 153, is different from the mutation in the mouse Ncf1, giving a splice defect. Nevertheless, both the rat and the mouse mutations reduce oxidative burst and promote arthritis severity.

Second, the results from the Ncf1-mutated mouse show that the reduced Ncf1-mediated ROS response enhances T cell-dependent autoimmunity. This is clearly compatible with the observations in the pristane-induced arthritis model in the rat in which the effect could be transferred with αβ T cells. After immunization with CII in the mouse, both a CII-specific DTH reaction and T cell-dependent IgG responses are enhanced. Interestingly, the immune response to the foreign antigen OVA is not affected. Taken together, our results suggest that Ncf1 plays a role in the selection of T cell autoreactivity.

Third, the finding that MOG protein-induced EAE is also enhanced in a similar way as CIA shows that the Ncf1 gene has a more general effect on T cell-dependent autoimmune disease. The finding is compatible with earlier results in the rat in which a strong linkage between EAE severity and the Pia4 locus (containing Ncf1) was identified (35). Interestingly, the enhancement occurred only if the disease was induced by immunization with the native MOG protein and not with the immunodominant MOG peptide. This indicates that immune recognition of native epitopes or uptake and processing of the protein is of importance for the Ncf1 function, because administration of the peptide will bypass such steps before binding to the MHC class II molecule on the APCs. This hypothesis may partly explain why Ncf1 knockout B6/129 mice are completely resistant to MOG peptide-induced EAE. Still, there are differences, and it is possible that embryonic stem cell-derived 129 genes linked to the deleted Ncf1 gene influence the stronger effect in the Ncf1 knockout mice as compared with the Ncf1-mutated mouse. There are clearly several linked genes in this particular gene region that could influence autoimmunity, as discussed concerning the diverse results obtained using various osteopontin-deficient mouse strains, possessing a 129 fragment in the same region (36). Another difference between the Ncf1 knockout and the mutated mouse is that the Ncf1 protein is still expressed in the mutated mouse, although at low levels and as truncated protein.

The last point to be made is the observation that spontaneous arthritis in fact develops in the Ncf1-mutated B10.Q mouse strain. Interestingly, this occurs only in the postpartum period. We have earlier observed a similar effect postpartum on the susceptibility to classical CIA in the DBA/1 mice in a study of the influence of various pregnancy factors (15). These mice developed relapses of arthritis postpartum, relapses that could be prevented by a continued estrogen replacement. Similarity to RA in humans (12, 13), mice are protected from arthritis during pregnancy (14) but are more vulnerable immediately after partus (15), and these effects seem to be mainly hormonally regulated through the estrogen receptor (37). In the case of the Ncf1-mutated mice, they develop severe arthritis spontaneously, and this arthritis continues along a chronic disease course with an erosive inflammatory arthritis. That this occurs only in a minority of the mice does not necessarily reflect remaining genetic heterogeneity of B6 and B10 genes, because the same reduced frequency of severe arthritis also occurs in inbred mice with an autologous CIA, i.e., mice induced with mouse CII (38) or mice expressing the immunodominant CII peptide in cartilage (39). The observation of a low frequency of spontaneous severe arthritis postpartum in fact confirms previous observations of DA rats, which carry the arthritis-promoting Ncf1 allele, also
occasionally developing arthritis postpartum (unpublished observations). Interestingly, the mice also develop a joint-specific autoimmune response, as seen with increased levels to CII, in fact to epitopes also recognized in human RA (21). Antibodies to these epitopes (C1, J1, and U1) do in fact induce arthritis upon passive transfer (40).

The Ncf1 polymorphism in the rat was found to be associated with an arthritis model, pristane-induced arthritis, with many features in common with RA, in particular during the chronic relapsing disease course (18). The CIA model in the mouse, despite being the most commonly used animal model for RA, usually develops only an acute disease without a subsequent chronic active disease course. In the present study, we show that Ncf1-mutated mice develop a chronic relapsing disease, thereby better resembling RA in humans. This, in fact, was achievable in a mouse strain that normally develops only mild arthritis with low frequency, but that tends to show a chronic disease course, as has been earlier shown in IL-4-deficient mice of the same genetic background (41).

Taken together, we show that the Ncf1 gene is associated with a previously undescribed pathway involving autoimmune T cells and modulating inflammatory diseases like arthritis and encephalomyelitis. It provides possibilities of generating immunotherapeutic drugs to target RA and multiple sclerosis, and the finding that it operates in both mice and rats increases the likelihood that similar genetics also operate in humans.

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