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Homologous collagen-induced arthritis in rats and mice are associated with structurally different major histocompatibility complex DQ-like molecules*

Collagen-induced arthritis (CIA) in rats, induced with homologous type II collagen (CII), is a genetically more restricted disease and has better resemblance to rheumatoid arthritis by its chronic disease course, than CIA induced with heterologous CII. The DA strain is highly susceptible to CIA induced with homologous CII, while the Lewis strain is resistant. (DAxLew)F_1 is susceptible and backcrossing to Lewis reveals a close, but not complete, association of both arthritis and CII responsiveness to the $RT1^a$ haplotype. Analyses of congenic strains on DA and Lewis backgrounds suggest that expression of a major histocompatibility complex class II B^a molecule, encoded from the $RT1B^a$ locus, is associated with arthritis susceptibility and CII responsiveness. The second exons coding for the first domains of the α and β chains of both the $RT1^a$ and $RT1^l$ haplotypes were sequenced and the deduced amino acid sequences compared with the corresponding molecule associated with susceptibility to CIA in the mouse (H-2 A^q). The sequences of the respective alleles revealed no obvious structural homology explaining the extensive similarities in the development of chronic autoimmune arthritis. Instead, this finding implies that different trimolecular constituents (i.e. class II, T cell receptor, and CII peptides) may yield an antigen presentation event that is able to trigger a similar autoaggressiveness in the two rodent species.

1 Introduction

Rheumatoid arthritis (RA) is a disease of uncertain etiology although recent data suggest a T cell-dependent autoimmune inflammatory attack on joints to be a crucial event in the disease. One of the most exciting recent findings is the genetic association of RA with certain MHC haplotypes. It was recently suggested that classical, severe, RA is closely associated with class II molecules sharing structures in the third and fourth hypervariable region of the DRB chain [1–3]. This is the hitherto best-defined genetic association of the disease and supports an important role of antigen specific recognition by autoreactive T cells. However, it has not yet been possible to demonstrate which self peptides, or which autoreactive T cells, are of importance. These interactions, and their importance for the development of arthritis, need to be studied in vivo and for this purpose animal models are useful. The only model

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Abbreviations: CIA: Collagen-induced arthritis CII: Type II collagen

for RA in which susceptibility to arthritis has reproducibly been shown to be MHC associated is the type II collagen-induced arthritis (CIA). In the mouse, immunization with type II collagen (CII) of heterologous [4–6] or homologous [7] origin leads to arthritis only in H-2^q and H-2^r haplotype strains. The ability to respond to homologous CII was found to be critical for arthritis susceptibility since immunization with heterologous CII induced a strong immune response to CII in mouse strains with other H-2 haplotypes, whereas immunization with homologous CII evoked an immune response to CII only in H-2^q and H-2^r strains. Similarly to RA, small structural differences between class II molecules determine arthritis susceptibility, as demonstrated by the finding that only four amino acid differences located in the 85, 86, 88 and 89 position of the A_β chain of q, as compared with p, determine CIA susceptibility and immune responsiveness to CII [8–9]. The rat MHC has been termed RT1. The rat homologues of the mouse H-2 class II genes, A_α, A_β, E_α and E_β (corresponding to the human DQA, DQB, DRA and DRB), have been termed RT1-B_α, B_β, D_α and D_β, respectively. In the rat, the MHC association has been less clear than in the mouse. Immunization with heterologous CII leads to the highest incidence of arthritis and the strongest anti-CII immune response in strains with RT1^u, RT1^l and RT1^a haplotypes, although no haplotype confers resistance to arthritis [10–11]. Analysis of the MHC association of CIA induced with homologous CII in rats is needed for three reasons. First, immunization with homologous CII may induce a disease more similar to RA with a chronic development of arthritis [12]. Second, the disease induced with homologous CII is critically dependent on the activation of autoreactive T cells, whereas in the disease induced with heterologous CII the disease is dependent on the activity of T cells reactive with heterologous CII [13]. Third, a structural comparison of the MHC association of

arthritis, induced with homologous CII, in the mouse and rat might tell us whether or not there are similarities in class II structures with disease association in these two closely related species.

2 Materials and methods

2.1 Induction and evaluation of arthritis

DA rats, originally obtained from Bantin and Kingman Ltd, and Lewis rats, originally obtained from Moellegaard Labs, Roskilde, Denmark, were bred and kept at the Biomedical Center in Uppsala. In a separate experiment, specific pathogen-free DA and Lewis congenic rats were obtained from Zentralinstitut für Versuchstierzucht (Hannover, FRG) and kept isolated. The rats were kept in a climate-controlled environment with 12-h light/dark cycles, housed in polystyrene cages containing wood shavings and fed standard rodent chow and water *ad libitum*. All experiments were performed on age- and sex-matched rats at an age of 8–14 weeks. The rats were found to be free from common pathogens including Sendai virus, Hantaan virus, coronavirus, reovirus, cytomegalovirus and *Mycoplasma pulmonis*. Native rat CII was prepared either from a rat chondrosarcoma by pepsin digestion or from lathyritic chondrosarcoma as described by Miller [14]. For induction of CIA, native CII were disolved in 0.1 M acetic acid at 4 °C and emulsified 1 : 1 on ice with IFA (Difco, Detroit, MI) to a final concentration of 0.5 mg/ml. Rats were injected intradermally in the base of the tail with 300 μl of the emulsion. Arthritis development was followed for approximately 4 months after immunization by inspections using a macroscopic scoring system for the four paws ranging from 0 to 4 (1 = swelling and/or redness of one toe or finger joint, 2 = two joints involved, 3 = more than two joints involved, and 4 = severe arthritis in the entire paw with functional gait and grip impairment). The maximal score obtained during the disease course for each rat was determined. To ascertain that only severity, and not incidence, is reflected, the mean value of the maximal scores of arthritic rats only are shown.

2.2 Quantification of antibodies in serum

Sera were collected individually and stored at − 20 °C until assayed. For the quantification of CII-reactive autoantibodies in serum, a modified ELISA was used [6]. Micro-ELISA plates (Dynatech, Plochingen, FRG) were coated overnight at 4 °C with 10 μg/ml of native rat CII in phosphate-buffered saline. All tests were carried out in duplicates, and the standard deviations did not exceed 10%. The amount of bound antibody was estimated after incubation with a goat anti-rat IgG (H+L) affinity-purified antibody conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, West Groove, PA). The subsequent quantification of bound enzyme was performed with a p-nitrophenol-containing substrate buffer in a Titertek Multiscan (Flow Labs., Irvine, Scotland) spectrophotometer. To estimate the amount of CII-reactive antibodies present in the serum samples, affinity-purified rat CII-reactive antibodies were used as standards.

2.3 DNA analyses

Genomic DNA was prepared from liver tissue of DA and Lewis rats as described earlier in detail [8, 9]. The following primers were synthesized on an Applied Biosystems 380A (Warrington, GB) oligonucleotide synthesizer and used for amplification of exon 2 encoding the B_β chain first domain; LA3 : 5′-ATGGATCCGTCCGTCCGCAGGGCATTT-3′, R1 : 5′-CCCGAATTCGCGCTCACCAAGCCGCCG-3′. The following primers were used for the amplification of exon 2 coding for the B_α chain first domain; R2 : 5′-CGCG-GATCCCACCTAAATTCCTCAGCC-3′, R3 : 5′-CCGG-AATTCAGGGTGGTGAGCACGTAC-3′. The polymerase chain reaction (PCR) amplifications were carried out using 1 μg of genomic DNA, oligonucleotide primers at a concentration of 1 μM and 2.5 units Taq polymerase (Perkin-Elmer, Cetus, Emeryville, CA) in a final volume of 100 μl. The amplified products were cloned into M13mp18 and/or M13mp19 and the nucleotide sequence determined using the chain termination method [15]. The sequence of at least three different M13 clones were determined, in order to detect PCR misincorporations.

2.4 Statistical analyses

Incidences of arthritis were analysed by their proportionate group frequencies (two-tailed χ^2 test with continuity correction) and the Mann Whitney U-test was used for analysis of arthritic scores. Antibody levels were analyzed by the two-tailed Student's *t-test*.

3 Results

3.1 Both arthritis susceptibility and CII autoimmune responsiveness are associated with the RT1ᵃ haplotype

The DA rat strain develops chronic arthritis after immunization with homologous rat CII emulsified in IFA (the so-called collagen-induced arthritis) [12]. If induced with lathyritic rat CII or essentially pepsin-free pepsin-modified rat CII, Lewis rats are resistant and (DAxLewis)F_1 hybrids susceptible even after immunization with high doses of CII (Holmdahl et al., to be published; Table 1). These findings suggest an influence of recessive or co-dominant gene loci. The DA and Lewis rat strains carry different MHC genes, RT1ᵃᵛˡ and RT1ˡ, respectively, and the data implicate an association of disease susceptibility to RT1ᵃᵛˡ but not to RT1ˡ, in contrast to CIA induced with heterologous CII which is associated with both haplotypes [10, 11]. To address directly this possibility, (DAxLewis)F_1 rats were backcrossed to Lewis and the offspring analyzed for arthritis susceptibility and CII autoantibody response and the results correlated with RT1 haplotype. In experiment 1 (Table 1) the rats were immunized with 500 μg lathyritic CII and in experiment 2 (Table 2) with 150 μg pepsin-digested CII giving similar results. No sex differences in arthritis susceptibility or CII responsiveness were found. Both arthritis susceptibility and CII autoimmune response were associated with the expression of RT1ᵃᵛˡ. Some homozygous RT1ˡ rats developed arthritis and a low immune response to CII showing that RT1ˡ may permit CII respon-

Table 1. Arthritis susceptibility of DAxLewis backcross rats immunized with 500 µg rat CII

Rats	RT1	*n*	Incidence of arthritis (%)	Mean day of onset	Mean maximal severity	Anti-CII antibody serum levels (µg/ml) (day 28)
Lewx(DAxLew)	a/l	10	90	20 ± 4	7.7 ± 3.8	533 ± 331
p value			= *0.001*			<*0.0001*
Lewx(DAxLew)	l/l	20	20	24 ± 3	7.3 ± 3.5	28 ± 29
(DAxLew)F₁	a/l	5	100	19 ± 6	9.5 ± 6.2	339 ± 158
Lewis	l/l	5	0			12 ± 12

Table 2. Arthritis susceptibility of DAxLewis backcross rats immunized with 150 µg rat CII

Rats	RT1	*n*	Incidence of arthritis (%)	Mean day of onset	Mean maximal severity	Anti-CII antibody serum levels (µg/ml) (day 28)
Lewx(DAxLew)	a/l	13	54	31 ± 8	6.3 ± 3.4	176 ± 142
p value			= *0.06*			<*0.0001*
Lewx(DAxLew)	l/l	13	13	26 ± 1	3.5 ± 3.5	6 ± 12

Table 3. MHC haplotypes of rat strains used[a]

RT1 haplotype	RT1.A	RT1.B	RT1.D	RT1.E
av1	a	a	a	–
a	a	a	a	–
i	n	a	a	u
l	l	l	l	–
n	n	n	n	–
c	c	c	a	–
d	d	d	d	–
f	f	f	f	–
u	u	u	u	–

a) The summary of the alleles in various rat MHC haplotypes are compiled from data reviewed by Gill III et al. [30] and Diamond et al. [17]. RT1.A and RT1.E are class I molecules and RT1.B and RT1.D are class II molecules. The RT1.B molecule corresponds to the H-2 A in the mouse and the HLA DQ molecule in humans, whereas the RT1.D molecule corresponds to the H-2 E in the mouse and the HLA DR in humans.

siveness and arthritis development. A comparison with the Lewis rats, which are resistant to arthritis but develop low levels of anti-CII autoantibody levels, shows an influence of non-MHC background genes. The development of arthritis in l/l rats did not correlate with development of an anti-CII autoantibody response, in fact, some of these rats had no detectable (<1 µg/ml) anti-CII antibodies in serum.

3.2 MHC-congenic strains

To investigate the role of the class II region and whether susceptibility to arthritis and CII responsiveness were permitted only in rats expressing the RT1[a] haplotype we analyzed a series of RT1-congenic rat strains on Lewis and DA backgrounds. Only female rats were used in the experiment and the rats were observed for a long time after immunization (4 months) in order to determine the character of the disease course. A summary of class I and class II alleles in the different RT1 haplotypes carried by rats in the experiment are depicted in Table 3. Only strains carrying a, av1 or i haplotypes were susceptible to arthritis and

Table 4. Susceptibility to arthritis induced with homologous CII in RT1-congenic Lewis rats

Strain	RT1	*n*	Incidence of arthritis (%)	Mean day of onset	Mean maximal severity	Anti-CII antibody serum levels (µg/ml) (day 21)
DA	av1	5	100	12 ± 0	14.0 ± 4.0	376 ± 138
DA11	i	4	100	18 ± 12	13.2 ± 5.2	195 ± 92
Lewis	l	6	17	45	1	0
LEW.1A	a	11	64	31 ± 15	10.3 ± 5.7	174 ± 125
LEW.1N	n	9	0			16 ± 24
LEW.1C	c	4	0			5 ± 6
LEW.1D	d	6	0			0
LEW.1F	f	6	17	61	3	15 ± 20
LEW.1W	u	6	0			278 ± 292

developed a strong autoimmune response (Table 4). Not unexpectedly, the association is with the class II region since the DA.1l strain, carrying non-a alleles flanking the class II region, was as highly susceptible as the DA rat. Furthermore, it is likely that the susceptibility is associated with the MHC class II molecule encoded from the B locus since the Lew1C rat, which was resistant to arthritis, carries the c haplotype known to express a D molecule indistinguishable from that of the a haplotype [16, 17]. The congenic strains carrying other RT1 haplotypes were low responders to CII except the u haplotype which developed a strong anti-CII autoantibody response but no arthritis. One rat of the f haplotype developed arthritis, although very late after immunization, and this single rat developed a relatively high antibody response to CII. A Lewis rat developed mild arthritis in a proximal interphalangeal joint, late after immunization, but did not develop a CII autoantibody response. Taken together, these findings suggest that self CII responsiveness and development of arthritis after immunization with homologous CII are associated with expression of a B^a molecule whereas other RT1 haplotypes confer weak and variable responses.

3.3 Structural analysis of class II genes

We have earlier shown that CIA in mice is associated with expression of an A^q molecule, both when induced with rat CII or with homologous mouse CII. Since the A molecule in the mouse corresponds to the B molecule in the rat, and since in both species chronic arthritis can be induced with homologous CII [12, 18], we wanted to compare the structures of these molecules. For this purpose we sequenced the second exons, encoding the first domains of

RT1Bα exon 2

```
a  gccgaccacgtaggctcctatggtatagagatgtatcaatattataaatccagaggccagtacac
l  ---+----------c-g----------a-t--------g------g---------------t---

a  atttgaatttgatggtgacgagaaattctatgtggacttggataagaaggagaccatctggagga
l  -ca-------------------g-------------------------------------------

a  tccccgagtttggacaactgacaagctttgacccccaaggtggacttcaagagatggctacagca
l  ---------------g-----------------------------------a-t--a----t-at-

a  aaacacaatttggaactcctgataaagaggtcaaattcaacccccagctgttaacaaggt
l  ---------------a--t----g--------------------a------c---
```

Figure 1. Nucleotide sequences of the second exons coding for the first domains of the A$_\alpha$ and A$_\beta$ chains of a and l alleles. Primer sites are indicated by squares. The obtained sequence is coding for the amino acid position 7 to 88 of the A$_\alpha$ chain and for 7 to 92 of the A$_\beta$ chain. A full cDNA sequence of the A$_\alpha$ first domain of the l allele has earlier been published [31] and the present sequence was found to be identical.

RT1Bβ exon 2

```
a  ttcgtgtaccagttcaagggccagtgctactacaccaccgggacgctgcgcatacgggtcgtga
l  --+-----------c------t-------------a--------a-----------at----

a  tcagatacatctacaaccgggaggagtacgtgcgctacgacagcgacgtgggcgagtaccgcgc
l  --------------------a----------c--------------------------------

a  gctgacccagctggggcggccggacgccgagtactataacaaacagtacctggagcagacgcgg
l  -------g--------------ctca----------t------g--------------------

a  gcccaggtggacacggtctgcagacacaactacgaggagacggaggtccccacctccctgcgg
l  ---g--c----------------------------g-t--------g----------+---
```

developed a strong autoimmune response ...

the B$_\alpha$ and B$_\beta$ molecules, of the susceptible RT1^a haplotype and the resistant RT1^l haplotype (Fig. 1). The deduced amino acid sequences showed a number of differences between the two haplotypes (Fig. 2). Several of these differences were found in the postulated antigen-binding site (ABS) [19]. Thus, of a total of 16 ABS residues in the β chains, 4 were different between the haplotypes. The corresponding figures for the α chains were 8 differences of a total of 20 ABS residues. An alignment of the respective rat α and β chain amino acid sequences with the corresponding mouse A molecule sequences showed no obvious structural homology which could explain the similarities in responsiveness to CII in the two species. Instead, of the 20 α chain ABS residues, 8 differed in all comparisons between the species. In the β chains, of the 16 ABS residues, 11 differed between the non-susceptible RT1 B^l and H-2 A^p, whereas 10 out of 16 residues differed, in the ABS, between the susceptible RT1 B^a and H-2 A^q haplotypes. In fact, the susceptible rat B^a molecule was found to share the sequence in the critical region, position 85–89 in the A$_\beta$ chain, with the mouse A^p molecule, which is associated with resistance to CIA in the mouse model [8].

4 Discussion

In this report we show that the development of CIA and an autoimmune response in rats, after immunization with homologous CII, are associated with the class II genes of the RT1^a haplotype. This is different from the MHC association of arthritis susceptibility after immunization with heterologous CII where also the l and u haplotypes have been found to be highly susceptible to arthritis. Arthritis induced with homologous CII provides several

Amino acid sequences of DQ-like α first domains

```
            10         15        20         25        30
RT1a  D H V G S Y G I E M Y Q Y K S R G Q Y T F E F D G D E
RT1l  -   - A A - - N - - - - E - - - - F - H - - - - - -
H-2q  -   - F - - V V - - S P G D I - - - H - - - - - -
H-2p  -   - F - - V V - - S P G D I - - - H - - - - - -

       35         40         45        50        55        60
RT1a  W F Y K D L D K K E T V W M I P R I G Q L T S F D P Q
RT1l  -   - E - - - - - - - - - - - - - - - - - - -
H-2q  -   - V - - - - - - L - Z F - - - - - - - -
H-2p  -   - V - - - - - - L - E F - - - - - - - -

       65         70        75         80        85
RT1a  G G L Q F M A T A K H N L E L L I K R S N S T P A V N
RT1l  -   - - N I - I I - - - I - M - - - - - Q - - -
H-2q  -   - N I - G - - - G G W T - - - F - - T -
H-2p  -   - N I - G - - - G G W T - - - F - - T -
```

Amino acid sequences of DQ-like β first domains

```
            10         15        20         25        30       35
RT1a  F V Y Q F K G Q C Y Y T G T L R I R V V I R Y I Y N R E
RT1l  -   - Q L - L - - - N - Q - - - D - - - - - Q -
H-2q  -   A - L - E - - F - N - Q - - S - N - - - - -
H-2p  -   A - L - E - - F - N - Q - - S - N - - - - -

       40         45        50         55        60
RT1a  E Y V R Y D S D V G E Y R A L T Q L G R P D A E Y Y N K Q
RT1l  -   L - - - - - - - - - E - - - S - - F - - -
H-2q  -   W - F - - - - - - V - E - - - - - W - S -
H-2p  -   W - F - - - - - - V - E - - - - - W - S -

       65         70        75         80        85         90
RT1a  * L E Q T R A Q V D T V C R H N Y E E T E V P T S L
RT1l  * -   * - - - - - L - - - - - - G S - R - - -
H-2q  P T I - - R - - E - - - - - - - G V - T H -
H-2p  P E I - - R - - E - - - - - - - - - -
```

Figure 2. Alignment of the deduced amino acid sequences of A$_\alpha$ and A$_\beta$ first domains from mice and rats. The A^q molecule has earlier been showed to be associated with development of CIA induced with heterologous or homologous CII while the A^p molecule is associated with resistance against disease [7]. Positions postulated to be involved in the ABS [19] are boxed.

advantages as a model for RA and for studies of autoimmune arthritis compared with arthritis induced with heterologous CII. It is a chronic disease [12] in which the autoimmune disease process has been shown to be driven by α/β receptor-expressing T cells [20] and in which a T cell-dependent anti-CII autoantibody production is induced. CIA induced with heterologous CII is a more self-limited disease dependent on anti-CII antibody production. Immunization with heterologous CII triggers predominantly T cells reactive with heterologous CII and a strong B cell activation leading to anti-CII antibody production. In fact, it has been suggested that the pathogenic effector mechanisms are antibody mediated and do not involve CII autoreactive T cells [21–24]. The earlier described association of several RT1 haplotypes may reflect their importance as restriction elements for heteroreactive T cells and does not necessarily imply a critical role for triggering of autoreactive T cells. We have analyzed earlier the immune response to heterologous and homologous CII in detail in the mouse and shown that immunization with heterologous CII induces CII-specific proliferation of T cells with no cross-reactivity to homologous CII [25]. In addition, a strong autoantibody response to CII, but no arthritis, develops after immunization with heterologous CII in mouse strains with certain H-2 haplotypes [7], parallelling the presently described situation in the rat. In both mice and rats, after immunization with homologous CII, the class II DQ-like molecule seems to be used as the restriction element for CII-reactive T cells of critical importance for the development of CIA and a CII autoantibody response. However, a structural comparison of the α and β first domains of the rat B^a and the mouse A^q molecules did not reveal any apparent homology which could argue for a shared structural interaction as an explanation for the development of chronic arthritis after immunization with homologous CII in the two species. This is in line with a structural comparison with class II genes associated with RA which yields even less similarities since the restriction element is the DR and not the DQ molecule. It follows that structural dissimilarities between mice and rats, or between rodents and humans, do not exclude similarities in the pathogenesis and etiology. Thus, our data indicate a situation where, in different species, different CII peptides along the highly conserved CII molecule, are recognized by different T cell receptors in the trimolecular antigen presentation event leading to similar disease development. However, existence of shared structural motifs in the class II molecules which allow binding and presentation of collagen peptides, as an explanation for the MHC association of RA andd CIA, cannot be excluded and structural similarities between the mouse A^q vs. DR4 and rat D^u vs. DR4 have earlier been highlighted [13, 26]. The latter cases are of interest since in the present investigation we found that the Lewis1W ($RT1^u$) developed a strong autoantibody response after immunization with homologous CII. However, we could not find any sign of arthritis in this strain. A possible explanation could be that a different functional subtype of T cells are preferentially activated by CII in conjunction with a class II molecule as has been shown for the immune response to type IV collagen in mice [27]. Another explanation might be that non-class II genes flanking the MHC or within the complex and differing between the congenic strains, influence the susceptibility to arthritis, but not the anti-CII autoimmune response, in

similarity with the role of TNF genes for susceptibility to murine lupus [28].

The MHC class II association of CIA susceptibility after immunization with homologous CII was surprisingly restricted, compared with the CIA induced with heterologous CII. However, in several other haplotypes a weak but significant autoantibody response developed, and in rats with $RT1^l$ and $RT1^f$ a few animals developed arthritis. This putative responsiveness is dependent also on the non-MHC background genes as is clearly seen in the DAxLewis backcross experiment and by the comparison of DA and Lew1A strains. In the backcross experiments several Lew(DAxLew) $RT1^l$ homozygous rats developed arthritis without detectable anti-CII autoantibodies in serum. We have obtained similar findings in the development of CIA in certain mouse strains induced with homologous CII and this supports the conclusion that CIA may develop without the participation of arthritogenic antibodies, although it does not exclude a critical role of B cell activation to CII as being important for the chronicity of the disease, as has been earlier discussed [13]. The above findings do also emphasize the influence of non-MHC genes in the development of arthritis. The same genes may be of importance also in the development of arthritis after injection of non-immunogenic adjuvant oils since DA, but not Lewis or (DAxLew)F_1 rats, are susceptible to this oil adjuvant-induced arthritis [29]. Thus, the rat provides us with disease models, induced without involvement of non-self immunogens, in which the genetic contribution of both MHC and non-MHC genes to the susceptibility for autoimmune arthritis can be analyzed and compared with analyses of the genetic contribution to RA.

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