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Critical role of the major histocompatibility complex and IL-10 in matrilin-1-induced relapsing polychondritis in miceAnn-Sofie Hansson¹, Åsa CM Johansson² and Rikard Holmdahl²¹Department of Clinical Immunology, Göteborg University, Göteborg, Sweden²Medical Inflammation Research, BMC, Lund University, Lund, SwedenCorresponding author: Ann-Sofie Hansson, ann-sofie.hansson@vgregion.se

Received: 21 Oct 2003 Revisions requested: 26 Nov 2003 Revisions received: 3 Jun 2004 Accepted: 30 Jun 2004 Published: 12 Aug 2004

Arthritis Res Ther 2004, **6**:R484-R491 (DOI 10.1186/ar1218)© 2004 Hansson *et al.*; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.**Abstract**

Relapsing polychondritis (RP) is an autoimmune disease that affects extra-articular cartilage. Matrilin-1-induced relapsing polychondritis (MIRP) is a model for RP and is useful for studies of the pathogenic mechanisms in this disease. There are indications that the major histocompatibility complex (MHC) class II plays a major role in RP, since DR4⁺ patients are more commonly affected than controls. We have now addressed the role of the MHC region, as well as the non-MHC contribution, using congenic mouse strains. Of the MHC congenic strains, B10.Q (*H2^q*) was the most susceptible, the B10.P (*H2^p*) and B10.R (*H2^r*) strains developed mild disease, while B10 strains carrying the v, b, f, or u H2 haplotypes were resistant. A slight variation of susceptibility of *H2^q* strains (B10.Q>C3H.Q>DBA/

1) was observed and the (B10.Q × DBA/1)_{F₁} was the most susceptible of all strains. Furthermore, macrophages and CD4⁺ T cells were the most prominent cell types in inflammatory infiltrates of the tracheal cartilage. Macrophages are the major source of many cytokines, such as interleukin-10 (IL-10), which is currently being tested as a therapeutic agent in several autoimmune diseases. We therefore investigated B10.Q mice devoid of IL-10 through gene deletion and found that they developed a significantly more severe disease, with an earlier onset, than their heterozygous littermates. In conclusion, MHC genes, as well as non-MHC genes, are important for MIRP induction, and IL-10 plays a major suppressive role in cartilage inflammation of the respiratory tract.

Keywords: IL-10, matrilin-1, matrilin-1-induced relapsing polychondritis, major histocompatibility complex, relapsing polychondritis**Introduction**

Autoimmune diseases that affect cartilage tissue are widespread in the population. The most common one is rheumatoid arthritis (RA), in which joints are attacked by an erosive, relapsing inflammation. In a related human disorder, relapsing polychondritis (RP), mainly cartilage of the external ears, nose, and respiratory tract is involved in the disease process [1]. Joints are affected as a nonerosive, seronegative arthritis [2] and 20% of patients with RP develop nephritis, which is probably induced by the formation of immune complexes [3].

Similar pathogenic mechanisms are thought to be involved in RP and RA, partly because of the cartilage autoimmune inflammation but also because both diseases have been reported to be associated with the MHC allele HLA-DR4 [4-6]. Similarities, as well as differences, are also observed in animal models that mimic these human diseases. Colla-

gen-induced arthritis (CIA), in which animals are immunized with collagen type II (CII), is one of the most commonly used and best-characterized models for RA [7,8]. In this model, the *H2^q* haplotype has been found to be the one most strongly associated with CIA and the class II molecule A_q has been reported to explain this association. Interestingly, rheumatoid-associated class II molecules, such as DR4 (DRB1*0401), when expressed in the mouse, mimic the function of A_q. In one mouse strain, the human DQ6αβ/8αβ transgenic mouse, immunization with CII induces symptoms of arthritis as well as chondritis of the auricle that mimic RP [9].

A mouse and rat model for RP, matrilin-1-induced relapsing polychondritis (MIRP), was developed by our group to investigate the pathogenic pathways in RP [10]. Matrilin-1 is a cartilage-specific protein expressed in upper-airway cartilage [11], and consequently MIRP mimics the

inflammatory attack of the nose and respiratory tract, phenomena that are commonly seen in RP patients. There are also morphological similarities, such as infiltrations of macrophages and lymphocytes. In addition, a subgroup of patients with RP produces an antibody response to matrilin-1, and serum antibodies from these patients inhibit the binding of anti-matrilin-1-specific antibodies [12].

Surprisingly, when the MIRP and CIA models in rats are compared, major genetic differences are found regarding susceptibility to induction of disease symptoms. The DA rat is recognized as highly susceptible in most arthritis models, whereas it does not develop any sign of inflammation when immunized with matrilin-1 [10,13,14]. In contrast, the LEW.1F strain is a low responder to immunization with CII [15] but is highly susceptible to MIRP. On the other hand, the murine MIRP and CIA models are both dependent on B cells for the induction of clinical symptoms [16,17]. In addition, the complement system plays a major role in the pathogenesis of both diseases [16,18,19] and T cells are required in order to induce disease [10,20].

No data have been reported on the role of cytokines in RP, either in patients or in the corresponding animal models. In the CIA model, several cytokines have been shown to play major roles in the inflammatory process, anti-inflammatory mediators as well as proinflammatory ones. The cytokine interleukin-10 (IL-10) has been in focus for many years in autoimmune arthritis and in other autoimmune diseases. The human recombinant protein is currently being tested as a therapeutic agent in several human inflammatory diseases. Macrophages are the major source of IL-10 but this cytokine is also produced by B cells, T helper 2 cells, and monocytes [21-24]. IL-10 has an immunosuppressive effect on several proinflammatory cytokines, such as TNF- α and IL-1, both known as enhancers of the destructive inflammation in RA. It is also known that IL-10 down-regulates MHC class II on macrophages [25]. IL-10 was primarily considered to only suppress the inflammatory response in arthritis, but in recent years it has been shown to play a more complex and pleiotropic role [26]. Our group recently visualized this complexity. We showed that IL-10-deficient mice immunized with CII develop a more severe disease than their heterozygous littermates, while they are protected from antibody-transferred arthritis induced with CII-specific monoclonal antibodies [27]. In addition, we showed that IL-10 deficiency did not affect the proliferation to CII or IFN- γ production in comparison with their heterozygous littermates.

To further investigate the pathogenic pathways in RP, we used the mouse MIRP model. We immunized several strains of mice, including MHC congenic strains, to elucidate the role of MHC and non-MHC genes. We analyzed parameters reflecting activity of the cellular as well as the

humoral immune response, such as influx of cells and antibody production. In addition, to investigate the role of inflammatory mediators in MIRP, we immunized mice devoid of IL-10 in order to determine whether this cytokine, as in the CIA model, possesses significant effects on autoimmune chondritis in the extra-articular cartilage.

Materials and methods

Mice

Mice were bred and kept at the animal department at Medical Inflammation Research, Lund University. They were used at age 8–13 weeks and kept in a climate-controlled environment (temperature and humidity) with cycles of 12 hours light/dark and sound. IL-10-deficient mice were produced by a deletion in the IL-10 gene in a cross of C57BL/6 \times 129/Ola (originally provided by W Müller, Institute of Genetics, Cologne, Germany). They were further backcrossed into B10.Q ($H2^q$) mice (originally from J Klein, University of Tübingen, Tübingen, Germany, as were the B10.P mice [$H2^p$]) background for nine generations and intercrossed to provide homozygous littermates lacking IL-10 [27]. Additional strains were kindly provided by collaborators (C3H.Q [$H2^q$], from DSchreffler, St Louis, MO, USA) or purchased from Jackson Laboratories (Bar Harbor, ME, USA). Here we refer to (B10.Q \times DBA/1)F₁ mice as QD mice. Approval for the animal experiments was obtained from the ethical committee at Lund University.

Induction of disease

Mice were immunized at the base of the tail with 100 μ g of matrilin-1, purified as previously described [11], emulsified in complete Freund's adjuvant (Difco, Detroit, MI, USA). They were boosted at day 35 with 50 μ g of matrilin-1 in incomplete Freund's adjuvant (Difco). Control mice immunized in the same way but with matrilin-1 omitted were used in all experiments. Experimental mice were kept for 130 days. The severity of disease was scored using a modified version of a scale previously developed for the rat model [10]: 1, suspicion of respiratory distress; 2, discontinuous inspiratory stridor; 3, continuous inspiratory stridor; 4, continuous inspiratory stridor and abnormal breathing pattern; 5, cyanosis. Mice developing severe respiratory distress, indicated by score 5, were humanely killed at once.

Histology

Tissue samples were dissected in the acute phase at score 5 or at the end of the experiment at day 130. The tissue was immediately either snap-frozen at -70°C or fixed in 4% paraformaldehyde solution for 24 hours and further embedded in paraffin. Joints were decalcified for 2–3 weeks in EDTA solution. Sections 5–6 μ m thick were stained with hematoxylin and erythrosine. Immunohistochemical staining was performed in accordance with the standard protocol. Briefly, sections were incubated for 2 hours at room temperature with a primary antibody recognizing macrophages

(defined as CD11b⁺ cells), MHC II, CD4⁺ cells, and CD8 α ⁺ cells. A secondary biotinylated rabbit antirat Ig antibody (DAKO A/S, Glostrup, Denmark) was incubated for another 2 hours and binding was visualized with diaminobenzidine (Saveen Biotech, Malmö, Sweden). Immunohistochemical sections were scored by counting the mean number of positive cells in two areas of the same size from each section and were evaluated as follows: <5%, +; 5–25%, ++; 25–50%, +++; and >50%, ++++.

Antibody detection

Sera were collected and stored at -20°C until assay. ELISA was performed with sera diluted 1/10 and titrated in steps of 10. Plates (Costar; Corning Life Sciences, Oneonta, NY, USA) were coated with 1 μ g/ml of matrilin-1, 10 μ g/ml of CII, or 10 μ g/ml of cartilage oligomeric matrix protein (COMP) in PBS + 0.02% sodium azide overnight at 4°C. They were washed in washing buffer (0.1 M Tris/HCl+ 0.05% Tween 20) and incubated for 2 hours at room temperature in PBS buffer (PBS + 0.05% Tween 20 + 0.02% sodium azide). Washing was repeated and the plates were incubated for another 2 hours with conjugates detecting sheep antimouse IgG Fc γ (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The plates were developed with *p*-nitrophenol as the substrate and the amount of antibody was estimated as absorbency at 405 nm by using a Titertek Multiscan filter photometer. All plates detecting the same antigen were analyzed at the same time point. A

positive control, consisting of a mixture of sera from DBA/1 mice immunized with the protein in question, was used on all plates assayed. An established ELISA protocol was used for detection of anticollagen antibodies [28].

Statistical analysis

All assays were analyzed with the Mann–Whitney *U* test. Unless indicated otherwise, $P < 0.05$ was considered to indicate significance.

Results

MHC genes and non-MHC genes influence susceptibility to MIRP

To investigate the role of MHC in MIRP, we immunized several strains of male mice carrying different MHC class II molecules. The QD ($H2^q$) strain [F₁ of a cross between B10.Q ($H2^q$) and DBA/1 ($H2^q$)] was the most susceptible, developing severe, relapsing respiratory distress and with a significantly earlier onset of disease than any other strain (Table 1; Fig. 1a,1b). The B10.Q strain was also a high responder, as more than 50% of these mice were susceptible to disease. A few mice of the C3H.Q ($H2^q$) and DBA/1 strains developed respiratory distress in the acute phase, which in two mice had high scores. However, the symptoms in these strains did not proceed in relapses as they did in the QD and B10.Q mice, and therefore resulted in a lower mean score than for the other strains (Fig. 1c).

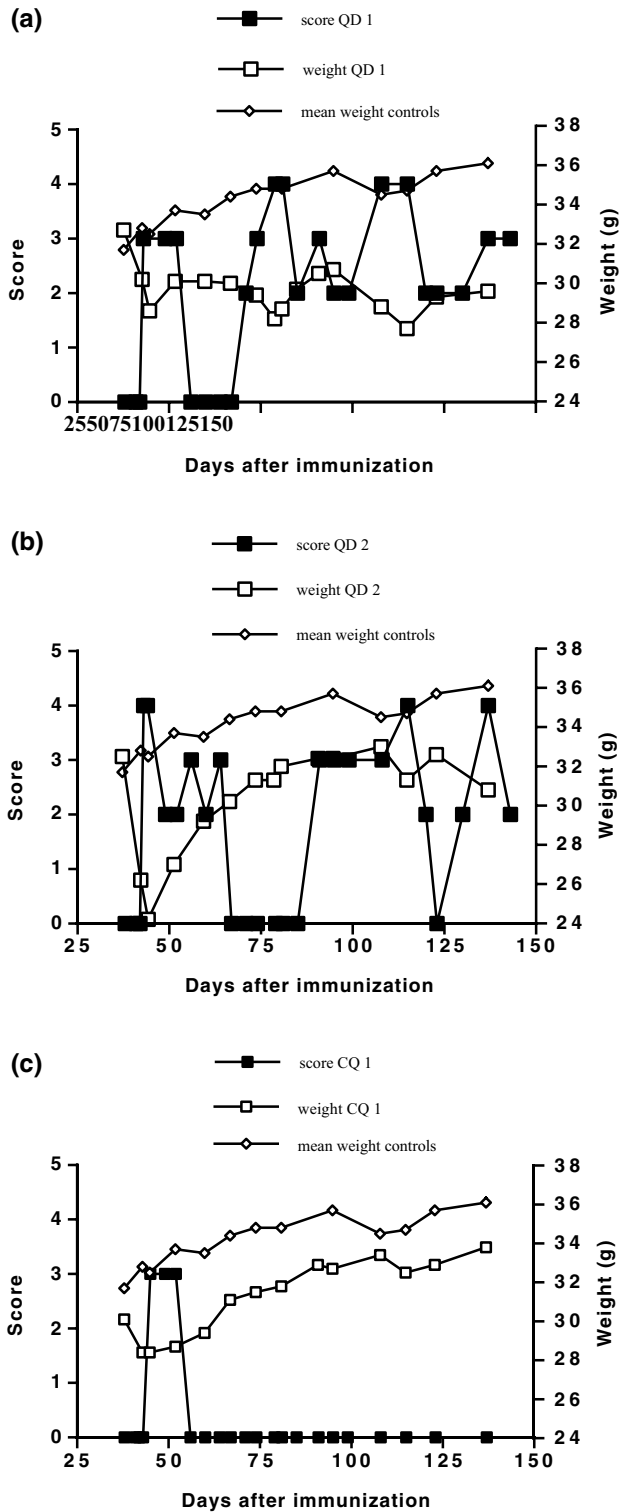
Table 1

Susceptibility of mouse strains to immunization with matrilin-1, as shown by their development of matrilin-1-induced relapsing polycondritis (MIRP)

Mouse strain	MHC	Gender	<i>n</i>	Incidence of disease (%)	Mean maximum disease score	Day of onset of symptoms
QD	$H2^q$	m	12	92	4.8 \pm 0.4 ^a	41 \pm 1 ^b
B10.Q	$H2^q$	m	16	56	2.6 \pm 0.7	46 \pm 4
B10.Q	$H2^q$	f	8	25	2.0 \pm 0	41 \pm 0
C3H.Q	$H2^q$	m	9	33	3.7 \pm 1.2	46 \pm 3
DBA/1	$H2^q$	m	12	50	3.2 \pm 0.5	55 \pm 8 ^c
Balb/c	$H2^d$	m	5	0		
NOD	$H2^g7$	m	10	0		
B10.P	$H2^p$	m	5	40	3.0 \pm 1.0	45 \pm 0
B10.RIII	$H2^r$	m	8	25	2.0 \pm 0	47 \pm 2
B10.V	$H2^v$	m	5	0		
B10	$H2^b$	m	5	0		
B10.F	$H2^f$	m	5	0		
B10.U	$H2^u$	m	1	0		

Only affected mice were included in the statistical analysis. ^aQD mice developed higher mean maximum disease scores than mice from the B10.Q, DBA/1, B10.P, and B10.RIII strains ($P < 0.05$). ^bQD mice developed disease symptoms earlier than all other strains ($P < 0.05$). ^cDBA/1 mice developed disease symptoms later than all other strains ($P < 0.05$). f, female; m, male; QD, (B10.Q \times DBA/1)F₁ mice.

Figure 1



Disease course and weight in individual mice immunized with matrilin-1 to induce relapsing polycondritis. (a, b) Two QD ([B10.Q × DBA/1JF₁]) mice (QD 1 and QD 2) and (c) a C3H.Q mouse (CQ 1) were scored for severity of disease on a scale from 0 to 5; see Materials and methods. All control mice (*n* = 4) were scored at the same time, and mean values of their weight are presented.

Inflammation and erosion of the cartilage were observed in sections from the nose, trachea, and larynx, and the degree of pathologic changes was correlated with clinical scores. The inflammatory infiltrates consisted of neutrophils, lymphocytes, and eosinophils. In addition, large numbers of macrophages were detected in the acute as well as in the chronic phase (Fig. 2). We did not detect any microscopic sign of inflammation in nonresponding mice or in control mice. In mice affected by respiratory distress, we observed a drop in body weight, which confirmed the clinical scores. Among mice of the QD strain, individuals that subsequently developed cyanosis lost as much as 25% of their body weight within a few days after the onset of respiratory symptoms (Fig. 1). Major weight loss was observed in several individual mice of other strains as well, but for strains analyzed as a group, only the QD mice lost significantly more body weight than the control group (data not shown).

In order to investigate the influence of gender, female mice on the B10.Q background were immunized at the same time as their male littermates. These females developed disease symptoms less severe than those of the males, with only mild respiratory distress for two or three days being observed (Table 1). However, the group of female mice produced levels of antibodies to matrilin-1 similar to those in the males.

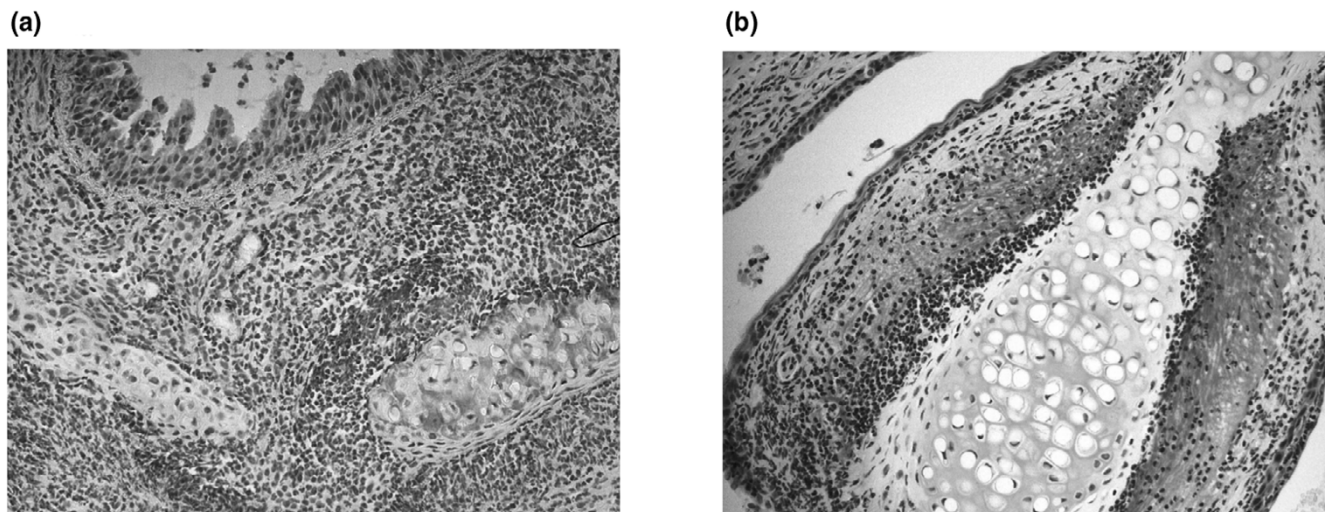
Antibodies to matrilin-1, CII, and COMP are produced equally in susceptible and resistant strains

All strains that were immunized with matrilin-1 produced antibodies to matrilin-1, and no difference in titers was detected in comparisons of two defined groups of susceptible and resistant strains (Fig. 3; Table 1). Balb/c (*H2^d*) mice produced the highest titers, while B10.P (*H2^p*) were low producers. However, when individual mice within each strain were considered, a tendency was seen for mice presenting severe respiratory distress, particularly those mice with the highest clinical score, to mount the highest levels of matrilin-1-specific antibodies. To investigate epitope spreading, we analyzed antibody responses to collagen type II (CII) and cartilage oligomeric matrix protein (COMP), two additional cartilage proteins involved in the autoimmune process [29,30]. QD mice produced low titers of antibodies to CII, and no CII-specific antibodies were detected in the other strains. While all of the QD mice responded to some degree to COMP, raised titers were seen in only some mice from the other strains and without any correlation with clinical score (data not shown).

Macrophages are important at the induction of MIRP

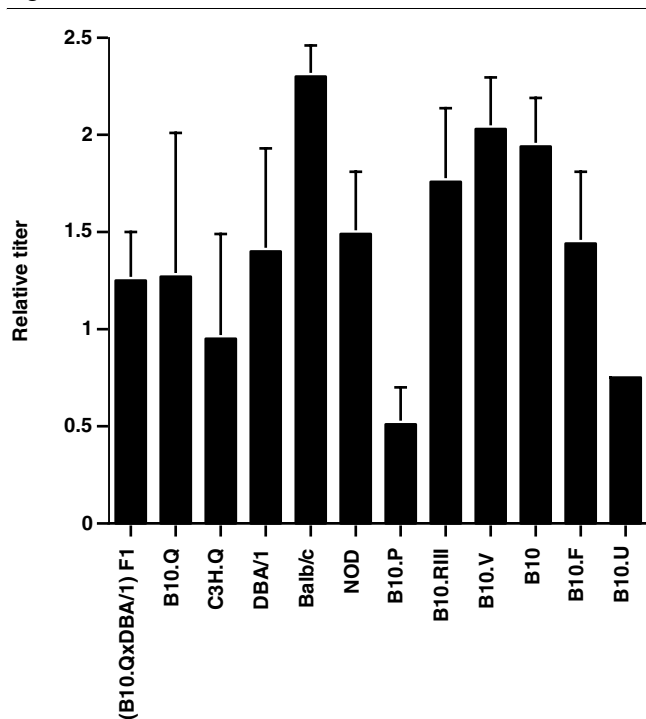
In order to define the infiltrating inflammatory cells in the acute and chronic phases of murine MIRP, we stained tissue sections dissected from cartilage of nasal, laryngeal, and tracheal specimens. Tissue samples were collected in the acute phase at the maximum of the clinical score

Figure 2



Tissue samples from a QD ([B10.Q × DBA/1]F₁) mouse immunized with matrilin-1 to induce relapsing polycondritis. **(a)** Section from the tracheal cartilage in the acute phase, showing inflammatory infiltrates and severe cartilage destruction. Cells detected in the infiltrates are macrophages, neutrophils, lymphocytes, and eosinophils. **(b)** Section from nasal septum, showing inflammatory infiltrates, fibrin deposition, and erosion of the cartilage. Staining with hematoxylin and erythrosine. Original magnification ×200.

Figure 3



Titers of antibodies to matrilin-1 in mice immunized with matrilin-1. Sera analyzed at day 35, with values expressed as relative titers in comparison with a positive control used on all plates assayed. For detailed information on the various strains and H2 haplotype, see Table 1.

(around the day of onset) and at the end of the experiment (on day 130). Two QD mice, two B10.Q mice, and two controls were analyzed at each time point. Macrophages, defined as CD11b⁺ cells, comprised more than 50% of the cells and were the most prominent cell type in the acute phase, whereas fewer, less than 25%, were detected in the chronic phase. In the chronic phase, there was a shift towards higher levels of macrophages in nasal and laryngeal cartilage than in the trachea. The control mice had less than 5% macrophages. T cells with a CD4⁺ phenotype comprised 5–25% of the cells in the acute phase and less than 5% in the chronic phase. Low numbers of cells (fewer than 5%) were positive for MHC class II or CD8, which were found only in the acute phase of disease. No CD4⁺, CD8⁺, or MHC-class-II-positive cells were detected in any phase in the control mice.

IL-10 has a protective effect in MIRP

Our finding that macrophages are prominent cells in MIRP led us to investigate the role of IL-10, an important product of macrophages. Mice devoid of IL-10 and their heterozygous littermates were immunized with matrilin-1 in accordance with the standard protocol. Respiratory distress was observed in 9 of the 11 IL-10-deficient mice but in only 4 of the 9 heterozygous littermates, indicating that IL-10 acts in a suppressive fashion (Table 2). The mean maximum score and the day of onset were significantly different in the homozygous group than in the heterozygous one (Table 2). No difference was detected between the two groups of mice in an analysis of the number of

Table 2**Susceptibility to immunization with matrilin-1 in mice heterozygous or homozygous for an IL-10 gene deletion**

Mice	<i>n</i>	Incidence (%)	Mean maximum score ^a	Day of onset of symptoms
IL-10 ^{+/-}	9	44	2.8 ± 0.8	68 ± 30
IL-10 ^{-/-}	11	82	3.5 ± 1.4*	41 ± 6*

All mice were bred on a C57BL/10 background carrying the *H2^q* haplotype in the MHC class II region. ^aScore of severity of matrilin-1-induced relapsing polycondritis, from a possible maximum of 5; see Materials and methods. **P* < 0.05.

macrophages or of cells positive for MHC class II, CD4, or CD8 in tests using immunohistochemical stainings of cartilage tissue from the nose, larynx, and trachea (two mice from the acute phase and two from the chronic phase). As was seen in the QD and B10.Q mice, more macrophages were observed in the acute than in the chronic stage.

All the mice produced antibodies to matrilin-1 and there was a tendency towards correlation between the titer of anti-matrilin-1 antibodies and clinical symptoms, in both the IL-10-deficient and the heterozygous mice. Surprisingly, several of the IL-10 knockout mice, all of which were taken off the experiment because of severe respiratory distress, produced higher levels of CII-specific antibodies than were detected in the QD mice (Fig. 4). Approximately half of the mice in both groups produced antibodies to COMP comparable with the levels found in the other strains, as described earlier (data not shown). No anticollagen or anti-COMP antibodies were detected in nonimmunized mice. Nor did we detect any inflammatory signs in joint sections from any mouse.

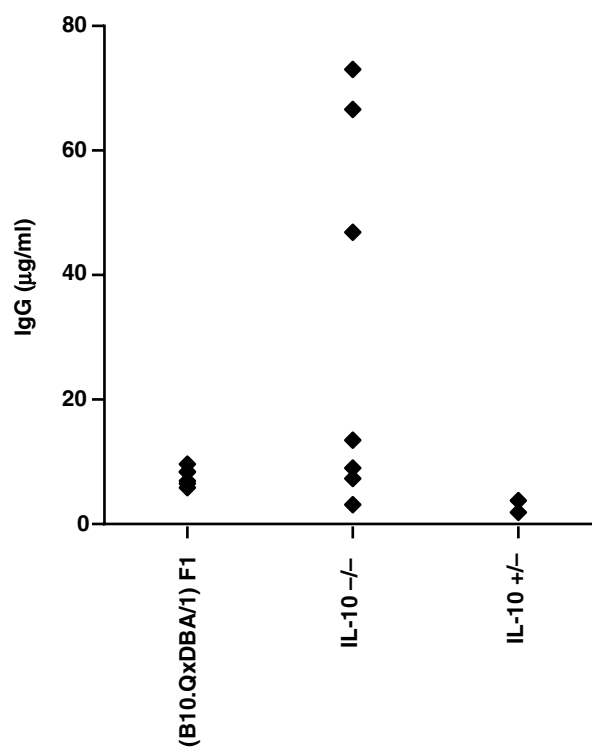
Discussion

The pathogenic pathways in relapsing polycondritis are largely unknown. In this paper we show that genes in the MHC region as well as genes outside that region are important for the induction of respiratory distress in murine MIRP. Strains that carried the *H2^q* haplotype were the most susceptible ones, and of these, the QD strain was the most sensitive. We found that males were more severely affected than females. All strains and both genders produced high titers of antibodies to matrilin-1, with no significant correlation to disease parameters at day 35. In addition, IL-10 was an important immunomodulator in the pathogenesis of MIRP.

The matrilin-1-induced symptoms appeared to be genetically controlled by the MHC region, as mice congenic at the *H2* region differed in susceptibility to disease. As in mouse models for arthritis, mice carrying the *H2^q* haplotype were the most susceptible ones: all strains tested that had this haplotype developed respiratory distress. However, the influence of non-MHC genes in MIRP differs from that in CIA, as the B10.Q mouse is relatively more resistant to MIRP. These data further strengthen several publications

that indicate similarities in the MHC genetic control of RP and RA, as both diseases are reported to be associated with HLA-DR4 [4-6], whereas differences in non-MHC genes contribute to the differing pathogeneses.

Surprisingly, we found no differences between strains in the anti-matrilin-1 antibody titers at day 35. However, all mice with clinical disease developed high levels of antibodies to matrilin-1. We have recently shown that B-cell-deficient mice are completely resistant to MIRP [16]. In addition, in these experiments we induced inflammation and erosion of the cartilage in the respiratory tract by injecting matrilin-1-specific monoclonal antibodies into B-cell-

Figure 4

Anticollagen type II antibody response after immunization with matrilin-1 in (B10.Q × DBA/1)F₁, B10.Q mice devoid of IL-10 through gene deletion, and their heterozygous littermates. Sera were analyzed for total IgG levels at day 35 after immunization. For detailed information on the experimental setup, see Materials and methods.

deficient mice. This indicates that the matrilin-1-specific humoral response plays an important role in the induction phase of disease. The discrepancies between our earlier results and the present findings of antibody titers could possibly be explained by the fact that titers at day 35 do not reflect the factors that are crucial for the initial triggering of the matrilin-1-induced symptoms. There are likely to be additional effector pathways of critical importance with regard to maintenance of disease, as for example epitope spreading. Unexpectedly, we found that some of the IL-10-deficient mice with high clinical scores developed high levels of anti-CII antibodies. We did not observe any clinical signs of inflammation from the articular cartilage, which indicated that these anti-CII specific antibodies were not arthritogenic but rather were a result of the cartilage-destructive inflammation in the trachea. However, the influence of IL-10 on immune reactivity to CII needs to be further investigated.

Macrophages were the dominating cell type in the inflammatory infiltrates of laryngeal and nasal cartilage tissue sections. Macrophages produce large amounts of several proinflammatory cytokines and are the major source of IL-10, a pleiotropic cytokine with a significant effect on several cell populations. Our finding that a lack of IL-10 increases susceptibility to MIRP indicates that IL-10 acts in a suppressive fashion in the MIRP model. This further highlights the potential of IL-10 as a target for intervention in patients with RP.

Conclusion

In conclusion, our results emphasize the contribution of MHC as well as non-MHC genes in the autoimmune chondritis model MIRP. We further show that macrophages and CD4⁺ T cells as well as IL-10 play major roles in the pathogenesis of cartilage inflammation of the respiratory tract. Additional investigations of the genetic control as well as the pathogenic pathways, particularly regarding inflammatory cytokines, are needed to elucidate the complexity of the autoimmune inflammation in cartilage tissue. Finally, we found major similarities between our MIRP model and the commonly used models for RA, indicating that pathogenesis and, as a consequence, therapeutic strategies similar to those for RA should be considered for RP.

Competing interests

None declared.

Acknowledgement

We would like to thank Prof Dick Heinegård at the section for Connective Tissue Biology at Lund University for contributing with the matrilin-1 production.

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