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Genetic control of antibody production during collagen induced arthritis development in heterogeneous stock mice.

Michael Förster[1,3], Bruno Raposo[1,3], Diana Ekman[1], Dorota Klaczkowska[1], Marjan Popovic[1], Kutty S. Nandakumar[1], Therese Lindvall[2], Malin Hultqvist[2], Ivanka Teneva[2], Martina Johannesson[1], Emma Ahlqvist[2], Rikard Holmdahl[1]

[1] Section for Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

[2] Medical Inflammation Research, Department of Experimental Medical Sciences, Lund University, Lund, Sweden

[3] authors contributed equally to this work

Correspondence: Rikard Holmdahl, Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm 17177, Sweden. E-mail: Rikard.Holmdahl@ki.se

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Abstract:

Objective:

The aim of this study was to identify genetic factors driving pathogenic autoantibody formation in collagen induced arthritis (CIA), a mouse model for rheumatoid arthritis (RA) in order to understand the etiology of the disease and improve possibilities for therapeutic intervention. To this end we made a genome wide analysis of quantitative trait loci (QTL) controlling autoantibodies towards type II collagen (AC2A), anti-citrullinated protein antibodies (ACPA) and rheumatoid factors (RF).

Methods:

To identify loci controlling autoantibody production, we induced CIA in a heterogeneous stock (HS) derived mouse cohort, with contribution of 8 inbred mouse strains backcrossed to C57BL/10.Q (BQ). Serum samples of 1640 mice were collected before onset and at peak of the disease. Antibody concentrations were measured by standard ELISA and linkage analysis was performed using a linear regression based method.

Results:

We identified loci controlling formation of AC2A of different IgG isotypes (IgG1, IgG3), antibodies to major type II collagen (CII) epitopes (C1, J1, U1), to a citrullinated CII peptide (CitC1) and RF. The AC2A, ACPA and RF responses were all found to be controlled by distinct genes, one of the most important loci being the immunoglobulin heavy chain (IgH) locus.

Conclusion:

Here, we provide a comprehensive genetic analysis of autoantibody formation in CIA. Our study demonstrates that not only AC2A, but interestingly also ACPA and RF are associated with arthritis development in mice. These results underscore the importance of non-MHC genes controlling the formation of clinically relevant autoantibodies.

Introduction

RA is a complex autoimmune disease of unknown aetiology involving chronic inflammation of the peripheral joints leading to cartilage destruction and bone erosion. The major genetic factor contributing to susceptibility to RA across species is the major histocompatibility complex (MHC), in humans designated human leukocyte antigen (HLA). CIA is an animal model resembling human RA, which is in mice strongly associated with the positionally identified MHC class II Aß gene (1). Interestingly, the RA associated MHC class II molecules expressed in the mouse system allow the development of CIA, thereby responding to the same CII peptide as Aq expressing mouse strains (2). A hallmark of human RA is the development of autoantibodies directed against the Fc portion of immunoglobulins (RF), antibodies directed against citrullinated peptide antigens (ACPA) and in a subset of patients anti-type II collagen antibodies (AC2A). In fact, the development of ACPA is a classification criterium for RA diagnosis and both ACPA and RF development have a high predictive value for later onset of RA (3). A close association between ACPA and certain MHC alleles strongly indicate specific T cell driven autoimmune responses preceding development of RA. However, despite the high value of RF and ACPAs as prognostic biomarkers for severe erosive

arthritis, it has been difficult to demonstrate a direct link between autoantibody development and RA pathogenesis (4). In turn, AC2A antibodies are less frequent and there is no evidence that they are occurring as early as ACPA or RF (5). These antibodies can be detected around disease onset and several lines of evidence argue for their pathogenic importance. In CIA, AC2As are a driving force in disease pathogenesis, illustrated by the fact that passive transfer of AC2A from CIA immunised mice into naïve hosts elicits a similar disease (6, 7). Markedly, the same results have been obtained across species after transfer of the AC2A containing Ig fraction of a RA patient into mice (8). Moreover, in CII immunised mice, the B cell response is essentially directed towards conserved triple helical CII epitopes (9). Similar fine specificity has been observed in human arthritis patients strengthening the role of CIA as a relevant model for pathogenic B cell response in RA patients (10, 11). Antibodies specific for these major epitopes induce arthritis in mice upon single transfer or in combination further supporting a pathogenic role of anti-CII autoimmunity in RA (12). AC2A is also associated with the MHC class II region in humans, though not as strongly as either ACPA or RF. However, little is known about the contribution of non-MHC genes controlling autoantibody formation against CII in RA. So far, a candidate gene approach associated the R620W polymorphism in Ptpn22 in conjunction with HLA-DRB1 alleles with C1 directed humoral immune responses in RA patients (13).

Animal models like CIA provide a unique opportunity to study the genetics of antibody response systematically, as large cohorts of mice can be phenotyped under stable environmental conditions. Recently, heterogeneous stock mice have been successfully introduced to fine map known QTLs and high-resolution genome wide mapping of a

diverse range of quantitative phenotypes. Moreover, HS derived mouse cohorts have been used to map the genetic control of arthritis severity in an antigen dependent model as well as a mouse model based on passive transfer of arthritogenic autoantibodies (14, 15). So far the contribution of non-MHC alleles to genetic susceptibility of autoantibody formation towards CII in human RA, as well as its murine counterpart, is incompletely understood. However, resolution of QTL controlling autoantibody production in CIA and the identification of the underlying polymorphisms may result in identification of novel therapeutic targets for human RA. Here we report a detailed global genetic analysis of the antibody profile during CIA development using our previously described heterogeneous stock derived cohort of mice (14).

Materials and Methods

Animals and arthritis induction

The animals used for the CIA experiment, the experimental procedures regarding CIA induction as well as the details concerning production of the HSxBQ cohort including a detailed breeding scheme have been described in detail in Ahlqvist et al. (14). In brief, HS mice known as the Northport stock derived from 8 common inbred strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and LP/J originated in R. Hitzemann's lab and have been backcrossed in an F3 cross with C57BL/10.Q (hereafter BQ) and mice expressing the CIA susceptible MHC class II allele Aq were selected. Three batches comprising a total number of 1764 mice were produced, immunized and

monitored for CIA development. All mice were immunized with 100 μg rat type II collagen (rCII) emulsified in 50 μl IFA (Difco, Detroit, MI, USA) containing 25 μg *Mycobacterium butyricum* at day 0. Thereafter, they were followed for seven weeks for development of clinical arthritis. Details concerning incidence, onset and disease severity are included in the supplement. (supplementary table 1). For the verification experiment, Cia9 congenic mice were immunized with 100 μg rCII emulsified in 50 μl CFA (Difco, Detroit, MI, USA) at day 0 and subsequently boosted 35 days after initial immunization with 50 μg rCII emulsified in 25 μl IFA. HSxBQ mice were genotyped using a custom designed Illumina panel (for details see Ahlqvist et al. 2011) (14, 16). All experimental animal procedures were approved by local ethical committees (Lund, Malmö) (M107-07).

Serum sampling

Of 1764 animals 1640 have been bled from the retro-orbital venous sinus into heparin containing tubes (10 U/ml). Blood samples were collected prior to CIA onset (day 14), as well as at the peak of the disease seven weeks (day 50) post rCII immunization upon termination of the experiment. Cia9 congenic mice were bled 35 days after initial immunization. Plasma has been separated from erythrocytes after centrifugation for 20 minutes at 4000 rpm and was frozen at -20°C until it was assayed.

AC2A ELISA

For detection of AC2A NUNC Maxisorb immuno plates (NUNC, Roskilde, DEN) were coated with CII (10 μg/ml) in carbonate/bicarbonate buffer (pH = 9.6) over night at 4°C and subsequently blocked with 2% bovine serum albumin (BSA) (Sigma, St. Louis, MO, USA). Diluted serum samples were added and incubated at 4°C over night. Immunoglobulin Ig, IgG1 and IgG3 antibodies were measured using polyclonal goat derived horseradish peroxidase (HRP) conjugated secondary antibodies anti-Ig, -IgG1, and -IgG3 (Southern Biotechnologies Associates, Birmingham, AL, USA).

AC2A and ACPA peptide ELISA

Synthesis of collagen peptides was previously described elsewhere (10). The citC1 peptide was obtained from (GL Biochem, Shanghai, China). For measurement of peptide directed antibody responses Maxisorb immuno plates (NUNC, Roskilde, DEN) were coated with triple helical peptides (U1, J1, C1, citC1) at 5 μg/ml in PBS over night at 4°C and subsequently blocked with 2% BSA (Sigma, St. Louis, MO, USA). Plates were washed and diluted serum samples were added and incubated at 4°C over night. All antibody titers have been measured using HRP conjugated anti-κ light-chain specific secondary antibody (clone 187.1 - Southern Biotechnologies Associates, Birmingham, AL, USA). All peptides were investigated at both time points (day 14, day 50) except the J1 peptide, which was only assessed at day 50.

RF ELISA

RF detection using anti-κ light-chain specific antibodies has been outlined elsewhere (17). Briefly, all plates were coated with rabbit IgG (10 μg/ml) (Sigma, St. Louis, MO, USA). After blocking with 2% BSA all plates were washed and diluted serum samples added and incubated at 4°C over night. All antibody titers have been measured using HRP conjugated anti-κ light-chain specific secondary antibody (clone 187.1 - Southern Biotechnologies Associates, Birmingham, AL, USA).

Plate development

All ELISA plates were developed after addition of ABTS: 2,2'-azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid] (Roche, Mannheim, Germany). A pool of serum samples has been used as standard and all antibody titers were measured as arbitrary concentrations. Anti-CII IgG isotype (IgG1, IgG3) and anti CII-peptide antibody titers (C1, U1, J1) were quantified using defined affinity purified antibodies from CIA immunised DBA/1J mouse sera. RF and citC1 titers were not quantified due to the lack of a similarly defined standard.

Statistical Analysis:

The statistical analysis of the HS population for CIA has been extensively outlined earlier. Before analysis all antibody titers were normalized using Box-Cox transformations.

Single locus mapping – The haplotype structure of the mice was inferred using the haplotype reconstruction method HAPPY, thereby utilizing founder strain genotypes to calculate the probability $F_{Li}(s,t)$ that a locus interval originates from founder strains s,t. (18). Evidence of a QTL was calculated for each interval by fitting the regression model $E(y_i) = \sum_{st} F_{Li}(s,t)TL(s,t)$ where $T_L(s,t)$ is the phenotype effect due to strains s,t at the locus T. A 5% genome-wide significance threshold was determined by 200 permutations of the dataset. Age, sex and batch were used as covariates in all analyses.

Resample model inclusion probability (RMIP) – The complex family structure of heterogeneous stock derived mouse populations has been shown to increase the risk of false positive findings. To address this issue a resampling based multi-locus modeling method has been developed (19). RMIP for loci were calculated by subsampling (N=60, subsample size 80%) with forward selection, adding loci to the model as long as the addition generated a p-value less than 5% genome-wide significance threshold (adjusted to the subsample size) as described previously (19). All loci were included in the model selection. Closely spaced inclusion signals were assumed to originate from the same locus and similarly to Ahlqvist et al. inclusion signals within a 5 Mbp window were added to give a range probability (range RMIP). Single marker associations were calculated with PLINK v1.07 http://pngu.mgh.harvard.edu/~purcell/plink/ (20). Correlation between antibody titers and disease parameters was performed by logistic or linear regression. The antibody titers were ln-transformed before analysis and sex, age and experiment batch were included as covariates.

Results

When we investigated the Ig response against CII and against CII peptides we found for all time points that sick animals display significantly higher antibody titers than healthy ones, regardless of which serological parameter was assessed (figure 2A, supplementary figure 1). We also observed a significant increase in antibody titers over time during CIA for all serological parameters except AC2A of IgG3 isotype, which showed significantly higher titers on day 14 than on day 50 (figure 2A, supplementary figure 1).

QTL mapping of anti-collagen IgG antibody response

As in our previously published study concerning QTL mapping of CIA in HSxBQ mice, we started out using the HAPPY algorithm inferring the founder haplotype structure in any given interval based on the genotype of the adjacent markers. We investigated the genetic control of anti-CII -Ig, -IgG1, which represents a T cell dependent response against protein antigens constituting the major component of the Ig fraction and -IgG3, representing a T cell independent response against sugar moieties of polysaccharide antigens. We identified 58 sometimes overlapping loci for these three phenotypes (AC2A Ig, IgG1, IgG3) that reached a range RMIP > 0.25. Out of these, 15 loci displayed a range RMIP > 0.50 and are included in figure 2B (for the 43 remaining loci see supplementary table 5). The most strongly associated locus controls total Ig response on day 50 and maps to the telomeric part of chromosome 12 to the IgH locus in the mouse at around 119 Mb with a -LogP of 29.3 and a range RMIP of 0.80 (figure 2B). This locus also

associates with anti-CII response of IgG1 isotype, during the late phase of CIA displaying a range RMIP of 0.81 and a -LogP of 10.7 (figure 2B). When we investigated the shared genetic component controlling the T cell dependent and independent anti-CII IgG isotype responses, we found loci that are uniquely associated with one isoform (e.g. IgG1 or IgG3) as well as loci that simultaneously associate with both phenotypes (figure 2B, supplementary table 5). Interestingly, anti-CII IgG1 levels on day 50 uniquely associate with a locus on chromosome 1 at approximately 173 Mb with the peak marker located near the Fc receptor (FcR) gene cluster (-LogP = 12.6, range RMIP = 0.68). On the other hand, IgG3 associates with loci distinct from the FcR locus with the peak markers located at 155 Mb (-LogP = 7.3, range RMIP = 1.0) and 169 Mb (-LogP = 8.1 range RMIP = 0.30) on the same chromosome (figure 2B, supplementary table 5).

QTL mapping of anti-collagen peptide antibody and RF response

Furthermore, we mapped the antibody response towards the most relevant CII epitopes (C1, J1, U1), ACPA (citC1) and RF. For these five phenotypes, a total of 57 loci reached a range RMIP > 0.25, of which 20 showed a range RMIP > 0.50 (figure 2B, supplementary table 6). Thereby, a locus on chr. 4 at 136 – 140 Mb associates strongly with RF production and displays a range RMIP = 1.0 at both time points (figure 2B, 4A). When we examined the genes in the associated interval, we identified C1q as a likely candidate. This notion was supported by the strain distribution of coding non-synonymous SNPs within the C1q gene cluster that follow the same pattern as the haplotypes that correlate with low RF levels (figure 4B and supplementary table 3). For RF and CII-specific epitope responses (C1, U1, J1) we found an association with the IgH

locus on chr. 12 between 109 Mb and 120 Mb for both time points during CIA development (-LogP: 6.2-156.3; range RMIP: 0.50-1.0) (figure 2B). In fact, the IgH locus is the only locus significantly associated with J1 response on day 50 in HSxBQ mice. Next, we compared the genetic control of antibody response against the major B cell epitope C1 ($CII_{(359-370)}$) and its citrullinated form citC1 (ACPA), which are both also recognized in human RA (21). Here, we found a distinct genetic regulation pattern for the citC1 (ACPA) response compared with C1. This is shown both by the low correlation antibody responses against the C1 and citC1 epitope (r^2= 0.06 (day 14); r^2=0.15 day 50 linear regression) and by the genetic mapping (figure 1C, 1D and figure 2B). In fact we detected several loci uniquely controlling either C1 or citC1 response at both time points. For example, the C1 response maps to the IgH locus, whereas only a weak association (range RMIP < 0.25) was detected for citC1 (figure 2B).

Correlation between CIA and anti-collagen antibody levels

All antibody isotypes measured at day 14 were associated with an increased risk of developing CIA (figure 5A). Of the epitope specific antibody titers, antibodies towards the C1 epitope were the strongest predictor of disease. ACPAs directed towards the citC1 epitope also showed a significant association with incidence of the disease, but the increase in risk was comparatively small (figure 5A and 5B). This was also the case for RF (figure 5A and supplementary table 4). Time of onset was significantly associated with all antibody isotypes as were antibodies against the C1 and the U1 peptide. Because RF, ACPA and AC2A are all predictors of disease severity in human RA patients, we analyzed the association between day 14 antibody titers and CIA score at day 49

(adjusted for time of onset). In contrast to the human situation we found that only total Ig, IgG1 and IgG3 response against CII were associated with increased disease severity at day 49 (supplementary table 4). No correlation between RF concentrations on day 14 and disease severity was detectable in our experiment. At peak of the disease (day 50) all antibody isotypes and epitope specificities as well as RF correlated positively with disease score day 49 (supplementary table 4).

Comparison between QTL mapping of antibody response and CIA

Finally, we assessed the shared genetic control of anti-CII antibody response and CIA development, by comparing the loci that were most strongly associated with CIA in our previous study (14). We used C1 and IgG1 levels, which are the best predictors of disease and found that two CIA loci overlap directly with loci controlling these phenotypes (figure 1A and 1B). One of these loci maps to the telomeric region of murine chromosome 1 to a locus previously described as *Cia9*, with the peak marker for CIA severity, onset and incidence being located close to the Fc receptor (FcR) gene cluster (figure 2B, figure 3A) (22). In order to confirm these findings we performed a CIA experiment in a 10 Mb NOD derived congenic fragment, that includes the previously described Cia9 region (supplementary figure 2) (23). We found that upon immunization with rCII CIA severity as well as AC2A of IgG1 and IgG3 isotype are strongly increased in these mice compared to BQ controls thus providing further evidence of the usefulness of heterogeneous stock mice for fine mapping of QTLs (figure 3A, 3C and 3D).

The other disease locus is located on chromosome 12 at around 77 Mb with the peak marker mapping close to Esr1 gene (estrogen receptor alpha) (table 3). The strongest CIA

locus, which was found on chromosome 2 mapping to the Hc gene (complement component 5) did not show an association with neither IgG1 nor C1 response (figure 5B).

Discussion

With our study we provide a comprehensive genetic analysis of clinically relevant autoantibody responses during CIA development using a mixed inbred-outbred HS derived mouse cohort.

Rheumatoid factor

Antibodies against the Fc portion of Ig were already described 60 years ago and have been used as a serological marker to classify human RA according to the ACR (24). Their role in RA susceptibility has long been debated and despite the fact that RF can be detected prior to disease onset, evidence for a clear role in RA pathogenesis is lacking. We found that circulating levels of RF prior to disease onset, slightly increase the risk of developing CIA. The locus with the highest association for RF was detected on chromosome 4. We examined the genes in the associated interval and identified C1q as a likely candidate gene, which is supported by single marker association of closely located SNPs. The importance of the classical pathway of complement activation for antibody production is illustrated by the fact that mice deficient in either one of its components (C1q, C2, C3) or its receptors (CR1/2) display severely impaired primary and secondary antibody responses upon immunization (25-27). Experiments with CR1/2 knock-out animals demonstrated a critical requirement for IgM/antigen complexes for efficient

primary antibody responses (28). Furthermore, C1q deficiency in mice indicated that this molecule plays a role in the negative selection of B cells, as they exhibit higher levels of B1 cells and an increased secretion of low affinity IgM autoantibodies (29). On the other hand conventional B cells, in these mice, which generate autoantibodies of IgG isotype and require T cell dependent maturation, are anergized in the periphery (30).

ACPAs

In addition to the RF response we investigated the ACPA response in our mice. ACPAs directed against cyclic citrullinated antigens (CCP) are a highly RA specific biomarker and have been successfully introduced into the clinic within the last decade (31). They are also part of the revised ACR and EULAR criteria for RA diagnosis (32). However, opposite to human RA, specific anti-CCP antibodies are not present in murine CIA (33, 34). When we analyzed ACPA response against the citC1 epitope, which is also present in RA patients, we found that in CIA similarly to the human situation these antibodies predict disease. Moreover, we showed that at the peak of the disease citC1 antibody levels correlate with disease severity. This is in line with our previous observations concerning the ACC4 monoclonal antibody specific for the citC1 epitope, which is not arthritogenic itself, but aggravates arthritis severity after co-administration with the M2139 antibody in subarthritogenic dose in the collagen antibody induced arthritis model, which mimicks the effector phase of RA (35). Nevertheless, C1 antibodies have a much higher predictive value for CIA development and correlate better with disease severity during the late stage of CIA than citC1 specific ACPAs, probably reflecting the

fact that CII autoimmunity is the driving factor in CIA following immunization, whereas in human RA it occurs only in a subset of patients reacting to modified cartilage CII (2).

Immunoglobulin heavy chain locus

Mapping autoantibody responses against CII in HSxBQ mice strongly highlighted the critical importance of the IgH locus. Not surprisingly, we found strong associations for all AC2A isotypes during early and late CIA with IgH indicating that in certain mouse strains B cells preferably use a certain CH gene or are differentially responsive to T cell aided IgG class switch recombination. More interestingly, we found that CII epitope responses against U1, C1 and J1 also map to the IgH locus in HSxBQ mice. It has been shown that these responses vary greatly during chronic CIA development, with both genetic and environmental factors contributing (36). Studies on genetic control of CII epitope specific autoantibody development in BQ x (BALB/c x BQ) N2 mice previously showed association of late upcoming epitopes J1 and U1 and IgH during chronic CIA (37). Our study underscores the importance of IgH indicating that certain mouse strains preferably use certain V_H-genes to mount a response against specific pathogenic CII epitopes. It is thus easy to imagine that SNPs in a particular V_H gene segment in this locus select for certain CII epitope specificities. As an example serves the response against J1, predominantly controlled by the IgH locus and its production being strongly limited to a pair of V_H genes in the IgH locus (manuscript in preparation).

Genetic link between AC2A response and clinical CIA

When we investigated the direct overlap of loci controlling CIA and antibody phenotypes we found that the most prominent locus linking both phenotypes is located on chromosome 1 mapping to the FcR locus. This locus controls CIA onset, incidence and severity as well as IgG1 and anti-C1 response, but surprisingly none of the other epitopes nor IgG3. Multiple studies using genetically targeted mice indicated Fc receptor genes in susceptibility to different models of arthritis in mice (38, 39). Besides, a SNP in the promoter of the Fc receptor like 3 gene has been associated with human RA in Asians (40, 41). In addition, we reproduced our findings in the HS concerning CIA and IgG1 in a NOD derived congenic fragment that partially spans over the original Cia9 interval. By single marker association, we found that SNPs located between the *Fcgr4* and *Fcgr3* yielded the highest association for IgG1. The alteration of anti-CII IgG1 response by Fc locus argues for an altered Th1/Th2 differentiation, since it is well known that the IgG1 production strongly associates with skewing towards a Th2 response in mice (42). It has been demonstrated that Fcgr3 signaling is absolutely essential for an optimal Th2 T cell response (43). Previous experiments suggested that IgG, in an isotype-dependent manner, can enhance antibody responses towards large protein antigens like collagen by shutteling immune-complexes to APCs via activating Fc receptors subsequently leading to more efficient CD4 T cell responses (44). In addition, *Fcgr2b*, to date the only described inhibitory Fc receptor, has been implicated to indirectly participate in IgG isotype specific mediated enhancement of antibody responses, being a critical check-point allowing signals via the activating Fc receptors (45). Thereby, the T cell independent IgG3 response does not seem to be controlled by the FcR locus, probably due to the fact that this isotype binds only weakly if at all to *Fcgr1* (46, 47). On the other hand the

Traf1-C5 (complement component) locus, which is highly associated with arthritis development across species, was not associated with antibody development (22, 48). This finding supports experimental data from rodent animal models of arthritis, where C5 deficiency was associated not only with protection in CIA but different arthritis models based on passive transfer of arthritogenic antibodies outlining its importance for mediating effector mechanisms in murine arthritis pathogenesis (49, 50).

In conclusion, we report a comprehensive genetic analysis of clinically relevant autoantibody response during CIA development using a HS derived cohort of mice. Our data indicates complex genetic control of AC2A of different isotypes, ACPA and RF illustrating that non-MHC genes determine antibody specificity with particular importance of the IgH locus. Our study demonstrates the potential of HS stock mice for high resolution mapping of autoantibody controlling QTLs, thereby facilitating a more rapid identification of candidate genes as well as candidate polymorphisms that can be further exploited to define novel targets for therapeutic intervention. We also found that some CIA and anti-CII antibody controlling loci like the FcR or the Esr1 locus are overlapping and thereby co-segregate within the mouse genome, indicating that the underlying genes were co-selected during the evolution of murine inbred strains.

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Figure legends

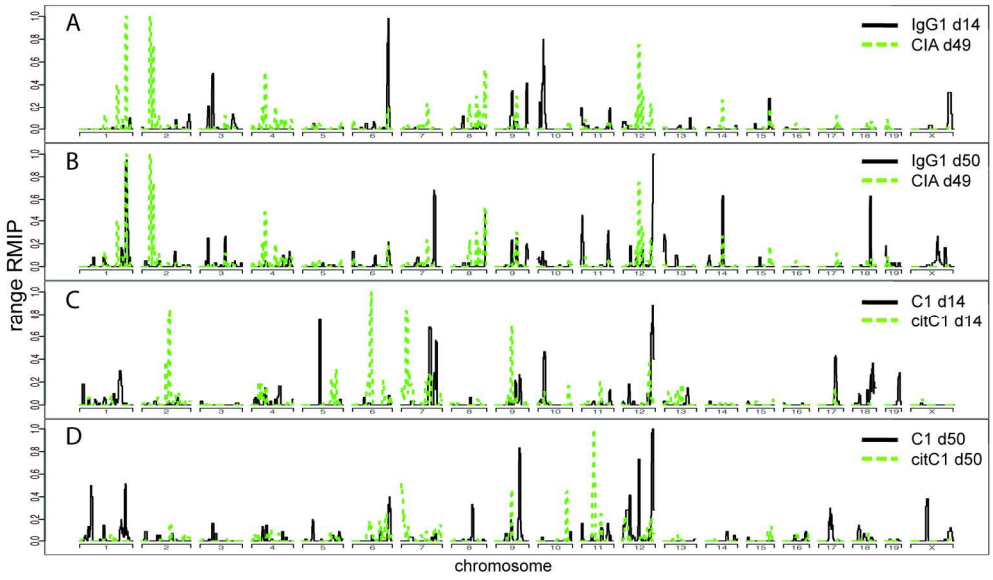
Figure 1 – Genetic regulation of anti-CII response in immunized HSxBQ mice. Genome-wide marker-trait association to: clinical score day 49 and anti-CII IgG1 response at A) day 14 and B) day 50; anti-C1 and anti-citC1 epitopes response at C) day 14 and D) day 50.

Figure 2 – Distribution and genetic regulation of anti-CII responses in HS mice. A) Anti-collagen antibody titers correlate with clinical disease. Antibody titers between healthy and sick animals are always significantly different, with a $p<0.0001$. For the exception of IgG3, all titers are significantly higher on day 50, compared to day 14. Only male animals are represented, with a similar response being observed in females (suppl. figure 1) * for observed $p<0.05$ and # for $p<0.0001$. B) Genome-wide associated loci for anti-CII isotypes, anti-CII main epitopes, RF and citC1 responses (range RMIP>0.5). Represented are the potential immunological relevant genes/loci within the analyzed region. The peak range includes the positions with a $-\log P \geq 0.7*\max(-\log P)$. (f) - data obtained with the full model.

Figure 3 – Fine mapping of the main QTL regulating anti-CII IgG1 production. A) CIA and IgG1 associations with a locus on chromosome 1 (HAPPY). Genome wide significance levels between 4.8 and 5.0 for displayed phenotypes. B) Haplotype distribution of the anti-CII IgG1 response mapping to the Fcgr locus (detailed information on suppl. table 2). Black bars indicate the strains that differ from the reference C57BL/6J strain. C) and D) Disease and anti-CII antibody phenotypes regulated by the congenic fragment Cia9. * - $p<0.05$; ** - $p<0.01$; *** $p<0.001$. # - SNP also associated with CIA Sum Score phenotype.

Figure 4 – Fine mapping of the main QTL regulating RF production. A) RF association with a locus on chromosome 4; and B) haplotype reconstruction of the RF response mapping to the C1q locus. Black bars indicate the strain that differs from the C57BL/6J sequence.

Figure 5 – Titers of anti-CII antibodies correlate with disease incidence and severity. A) Odds ratio for the correlation of antibody titers at day 14 with observed incidence at day 50 (detailed information on supplementary table 4). OR ± 95% CI is represented. B) Identified loci that strongly correlate with disease, anti-CII IgG1 titers and anti-C1 response. * - locus identified for anti-CII IgG1 response at day 14; ** - locus identified for anti-C1 response at day 14. All other loci have been identified for CIA sum score. (f) - data obtained with the full model.Supplementary figure 1 – Distribution of the anti-CII responses in HS female mice. Anti-collagen antibody titers correlate with clinical disease. Antibody titers between healthy and sick animals are always significantly different, with a $p<0.0001$. For the exception of IgG3, all titers are significantly higher on day 50, compared to day 14. ** for observed $p<0.01$ and *** for $p<0.0001$.



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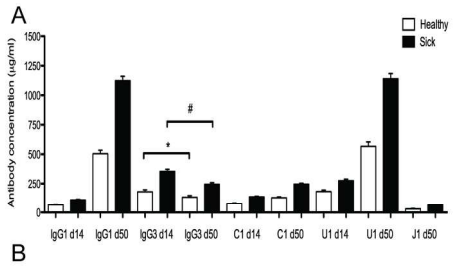
A



B

Chr	Phenotype	Peak range (Mb)	Candidate	-logP max	range RMIP
1	IgG3_d50	152.1–163.0	–	7.3	1.00
1	IgG1_d50; C1_d50	168.5–179.1	FcgR locus	12.6; 10.3	0.68; 0.51
2	citC1_d14	93.8–109.2	Cd59a	7.4	0.85
2	total_Ig_d50	154.8–156.9	–	7.3	0.65
4	RF_d14 (f)	41.4–51.7	–	6.9	0.53
4	RF_d14; RF_d50	136.0–140.5	C1q	17.2; 11.0	1.00; 1.00
5	total_Ig_d50	53.2–55.5	–	6.9	0.58
5	IgG3_d14; C1_d14	67.3–73.8	Tlr1	9.8; 5.9	0.61; 0.75
6	total_Ig_d50	6.5–8.3	–	9.4	0.70
6	citC1_d14	64.2–76.3	Igxv locus	12.0	1.00
6	IgG1_d14	134.3–138-4	Bcl2l14 / Igbp1b	9.4	0.98
7	citC1_d50	3.1–15.1	–	5.2	0.51
7	citC1_d14 (f)	12.8–31.5	–	9.7	0.83
7	C1_d14	105.4–110.8	–	6.7	0.68
7	C1_d14; IgG1_d50	119.0–133.6	–	8.3; 9.4	0.56; 0.68
8	total_Ig_d14; IgG3_d14	90.9–102.2	–	6.8; 5.7	0.55;0.55
9	citC1_d14	53.5–66.1	Map2k1 / Map2k5	9.3	0.70
9	U1_d14	73.7–80.5	–	5.3	0.60
9	C1_d50	89.9–95.9	–	6.4	0.83
10	IgG1_d14; IgG3_d14	22.0–39.8	Ptprk	5.7; 11.4	0.80; 0.73
11	citC1_d50	47.2–52-7	Col23a1	8.9	1.00
11	total_Ig_d14	55.7–62.9	–	10.1	0.81
11	U1_d50 (f)	72.9–82.5	–	7.7	0.58
11	U1_d50	98.2–103.2	–	6.5	0.88
12	total_Ig_d50; U1_d50	15.7–35.0	–	7.2; 7.7	0.80; 1.00
12	C1_d50	64.4–72.5	–	11.4	0.73
12	C1_d14; U1_d14; total_Ig_d50; IgG1_d50; IgG3_d50; C1_d50; J1_d50; U1_d50; RF_d50	108.3–120.4	IgHv locus	16.3; 7.2; 29.3; 10.7; 7.1; 19.6; 156.3; 7.7; 6.2	0.88; 0.80; 1.00; 1.00; 0.78; 1.00; 1.00; 1.00; 0.73
14	IgG1_d50	69.2–72.7	Tnfrsf10b / Bmp1	5.6	0.63
17	RF_d14	85.4–88.4	Socs5 / Prkce	9.3	0.66
18	IgG1_d50	69.2–70.2	Tcf4	7.3	0.66

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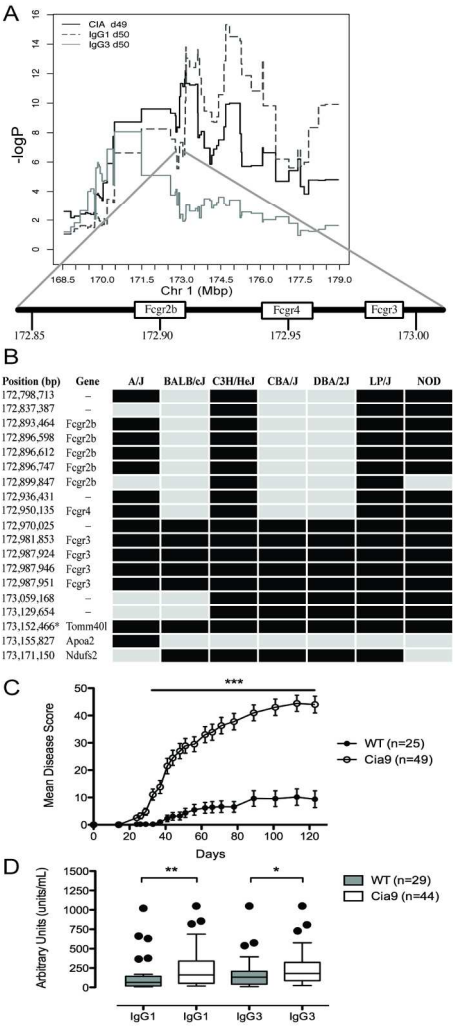

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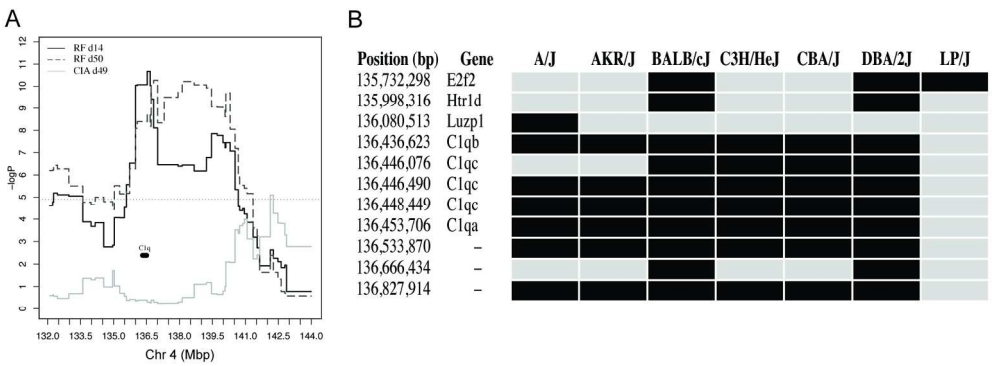

Figure 4 – Fine mapping of the main QTL regulating RF production. A) RF association with a locus on chromosome 4; and B) haplotype reconstruction of the RF response mapping to the C1q locus. Black bars indicate the strain that differs from the C57BL/6J sequence.

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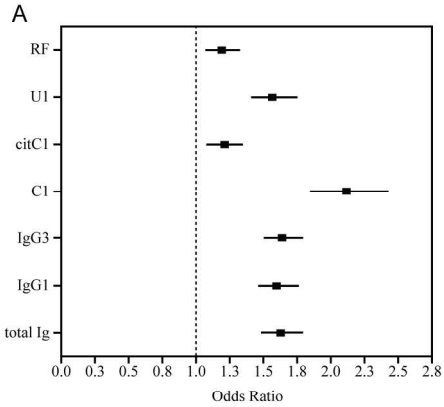

B

Position (chr_Mb)	Locus	Sum Score (-logP)	IgG1 day 50 (-logP)	C1 day 50 (-logP)
1_173	FcgR	12.3	13.8	6.7
2_34	Traf1/C5	13.4	1.5	2.7
8_122	–	14.3	3.8	1.7
8_130	–	11.8	6.4	1.8
12_77	Esr	10.6 (f)	8.5 (f)	7.2 (f)
6_135 *	–	7.8	5.5	8.7
7_125 **	–	5	10.1	6.4

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