Positional cloning of the *Igl* genes controlling rheumatoid factor production and allergic bronchitis in rats

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Rheumatoid factors (RF), autoantibodies that bind the Fc region of IgG, are one of the major diagnostic marker in rheumatoid arthritis (RA) but occur with lower frequency also in other infectious and inflammatory conditions. Through positional cloning of the previously described quantitative trait locus (QTL) *Rf1* in congenic and advanced intercrossed rats, we identified the Ig lambda light chain locus as a locus that regulates the production of RF in rats. The congenic rats produce RF-Igλ and have significant higher levels of RF-IgG and RF-IgM in serum, while the DA rat has an impaired RF production and does not produces RF-Igλ. Thus, we could investigate the role of RF in pristane-induced arthritis (PIA) as well as ovalbumin-induced airway inflammation. We show that there was no difference in the development and severity of PIA between congenic and parental DA rats, suggesting that RF using lambda light chains have no impact on PIA. However, the RF producing congenic rats developed a more severe airway inflammation as indicated in the significantly increased number of eosinophils in bronchoalveolar lavage fluid as well as total IgE in serum. In addition, RF congenic rats had a significantly enhanced immune response toward OVA due to increased OVA-Igk but not OVA-Igl antibodies, suggesting a possible involvement of RF in the regulation of the humoral immune response.

OVA-induced airway inflammation | quantitative trait locus | rheumatoid arthritis | congenic animals

Rheumatoid factors (RF) were the first autoantibodies to be identified and their occurrence is still used as one of the classification criteria for rheumatoid arthritis (RA) (1). RF show a high sensitivity for RA, occurring in 70–90% of all patients, but have a lower specificity because they are also found in many other chronic inflammatory conditions such as systemic lupus erythematosus, hepatitis C-associated mixed cryoglobulinemia, and bronchial asthma (2, 3).

Since their discovery, the biological and pathological roles of RF have been the subject of extensive investigation. RF, which are most often of the IgM isotype, bind to the Fc region of IgG and therefore may contribute to inflammation by the formation of immune complexes, resulting in complement activation and recruitment of neutrophils and macrophages and sustaining inflammation (4–6). RF use a variety of *V* genes that can be both germ-line encoded and somatic mutated (7–10).

However, little is known about the genetic control of rheumatoid factors. To identify genomic regions affecting the production of RF in pristane-induced arthritis in rats, we used genetic segregation analyses (11).

We found 3 loci regulating RF production (12). Only 1 of the loci (*Rf2*) had previously been associated with arthritis severity (*Pia5*) while the other 2 (*Rf1*, *Rf3*) harbor genes specific for RF production and had no significant role in the disease course of arthritis induced with pristane (13).

Here we report the physical mapping of the most prominent QTL for the production of RF (*Rf1*) to the Ig lambda light chain region at chromosome 11. By inserting a genetic fragment from the RF high level producing E3 rat strain, into the DA strain, we generated a congenic rat, producing high levels of RF due to a polymorphism in the lambda genes. Thus, we could use this RF producing congenic DA to investigate the role of RF in different inflammatory diseases, such as ovalbumin-induced airway inflammation or pristane-induced arthritis.

Results

Congenic Rats Produce RF That Uses the Lambda Light Chain. Arthritis resistant E3 rats have an elevated level of rheumatoid factors compared with the arthritis susceptible DA rats but the RF production is nevertheless correlated with the development of pristane-induced arthritis (PIA) in DA rats. After performing a linkage study of animals from the F2 intercross between E3 and DA rats, this autoantibody difference could be partly explained by the QTL *Rf1* found at rat chromosome 11. To isolate the genetic fragment harboring the *Rf1* locus, we identified a 6.7-megabase (Mb) fragment containing the linkage peak associated with RF production. This chromosomal fragment was introgressed onto the DA background through backcrossing to avoid interference from other E3 loci. The phenotype, which was co-dominantly inherited, was confirmed in each backcross generation. After 10 generations backcrossing, rats were intercrossed. We observed significantly higher levels of RF of the IgM class (RF-IgM, $P = 0.0001$) as well as of the IgG class (RF-IgG, $P = 0.001$) in naïve homozygous congenic rats compared to DA littermate controls (Fig. 1*A*). When analyzing the level of RF using the different Ig lambda light chains (RF-Igλ and RF-Igκ) it became clear that the elevated level of RF in the congenic rats is mostly due to Ig lambda light chain expressing RF ($P < 0.0001$), as DA rats were found to be lacking these specific antibodies. In addition, there was only a minor difference between congenic rats and DA in RF that uses the kappa light chain ($P < 0.05$) and this difference was not highly reproducible. Therefore the *Igl* locus was a strong candidate region as it was included in the congenic fragment that was inserted into the DA strain. Thus, it was possible that the absence of RF-Igλ in the DA

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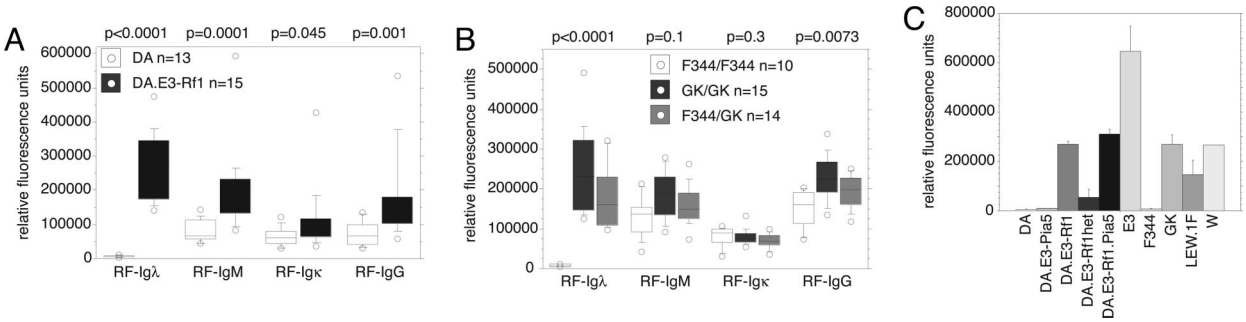

Fig. 1. Eu^{3+}LISA results for RF expressing the kappa or lambda light chain or the IgM or IgG heavy chain. (*A*) Comparison between naïve DA.E3-*Rf1* (F3) homozygous animals and naïve DA rats. A significant difference in RF-Igλ, RF-IgM, RF-Igk, and RF-IgG was observed. (*B*) RF in AIL (F344xGK) F19 rats with the full length fragment (D11Got79-D11Rat50). Comparison of RF levels between the different genotypes at marker D11Mir24; F344/F344, GK/GK, F344/GK. All rats homozygous for the F344 allele are negative for RF-Igλ and have a significant decreased level of RF-IgG. (*C*) RF-Igλ in different parental and congenic strains. All *p*-values between DA and congenic rats are indicated.

strain is because of the allotypic specificity of the monoclonal detection antibodies used in our assay. To exclude this possibility, we analyzed the level of total Igλ in europium^{3+}-linked immunosorbent assays (Eu^{3+}LISA) as well as the number of Igλ expressing B cells in FACS with the same detection antibodies that were used in the RF-Igλ Eu^{3+}LISA. In both tests, the DA was found to be positive for Igλ antibodies as well as Igλ-expressing B cells and there was no difference between DA and the congenic rats (data not shown). Therefore we conclude that the detection antibody is able to bind Ig lambda derived from the DA strain and the negative result from the DA rats in the RF-Igλ Eu^{3+}LISA is due to the lack of RF using the Igλ light chain in this strain.

Physical Mapping of the RF Locus and Identification of the *V* Lambda Genes. The Ig lambda light chain locus was thus a strong candidate for the RF production. However, the original congenic strain had a 6.7-Mb fragment from the E3 strain containing a number of genes that could contribute to the observed phenotype. To localize the gene we intercrossed heterozygous congenic rats to obtain recombinations within the *Rf1* fragment. After genotyping 600 rats using microsatellite markers, only 2 recombinations were found. One <4.6-Mb-long fragment (F5) covering the centromeric part of the locus and another overlapping, <3.2-Mb-long fragment (F4) covering the telomeric part (Fig. 2). Only the centromeric fragment (F5) showed the RF phenotypes and was isolated in the congenic DA.E3-*Rf1* strain. Because we obtained far less recombinations than expected in a genomic fragment of this size, we screened several rat strains for RF-Igλ production and also used an advanced intercross line between the GK and the F344 rat strain. While the F344 rats similar to the DA rats do not produce RF with lambda light chains, the GK rats produce these RF in a similar fashion as the DA.E3-*Rf1* congenic rat (Fig. 1*C*). After 19 to 21 generations of intercrossing these 2 strains, a high recombination rate in the *Rf1* locus was expected. We analyzed RF levels in 97 AIL rats that had either the full length fragment (Fig. 1*B*) or several recombinations between the typed markers and compared them with the parental strains. As it is shown in Fig. 2 we could confirm that the production of RF-Igλ is limited to the small region that only contains the genes for the Ig light chain.

To identify the location of all new marker and the Ig lambda light chain genes, we built a physical map of the fragment. Although the *Igl* locus for the mouse and human were well described, information about the number and arrangement of genes in the *Igl* locus of the rat was very limited. By using the published DNA sequences of Igλ producing B cells from rat, mouse, and human and aligning these to the rat genome in the University of California, Santa Cruz genomic browser with BLAT we have built a physical map of the *Igl* locus (Fig. 3*A*). The genomic sequence for this part of chromosome 11 (base pairs +83528330–84483930) was 90.25% complete, the sequence from the first *Igl-V* gene (base pair +83848599) to the last *Igl-C* gene (base pair +84240214) was 96.24% complete. Because the presence of some gaps and the genetic variability between different rat strains, it cannot be excluded that there are in fact more *V*, *J*, and *C* genes belonging to the *Igl* gene family. Until now 4 different *Igl-V* gene subfamilies, 3 different *Igl-J* genes, as well as 5 different *Igl-C* genes have been identified. No antibody using the *Igl-C5* gene could be found either in the published sequences or by our own sequencing, and the genomic distance to the *Igl* locus makes it likely that *Igl-C5* is, in fact, a pseudogene. From

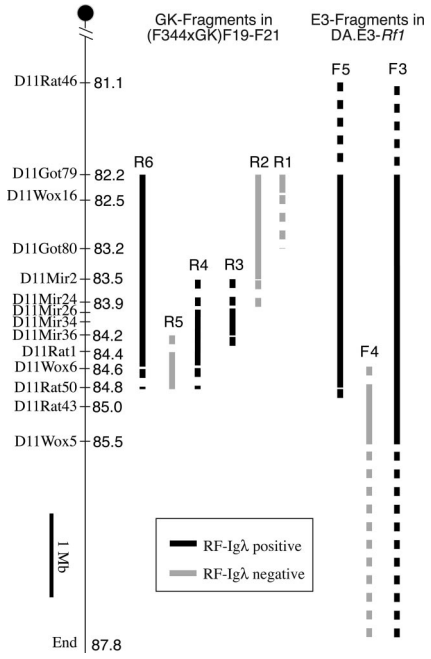

Fig. 2. Genomic map of fragments of DA.E3-*Rf1* congenic rats and AIL (F344xGK) F19–21 rats and their correlation with RF-Igλ in serum. The E3 fragments F3 and F5 in the congenic strain that correspond with the production of RF-Igλ antibodies (black lines), while the telomeric E3 fragment F4 that has no effect on the RF-Igλ production (gray lines). Animals from the AIL (F344xGK) were genotyped for all indicated markers between D11Got79 and D11Rat50. The recombinant fragments R3, R4, and R6 correlate to the RF-Igλ production (black), the non-RF-Igλ producing fragments R1, R2, R5 are indicated in gray. Dotted lines indicate area of uncertain genotype.

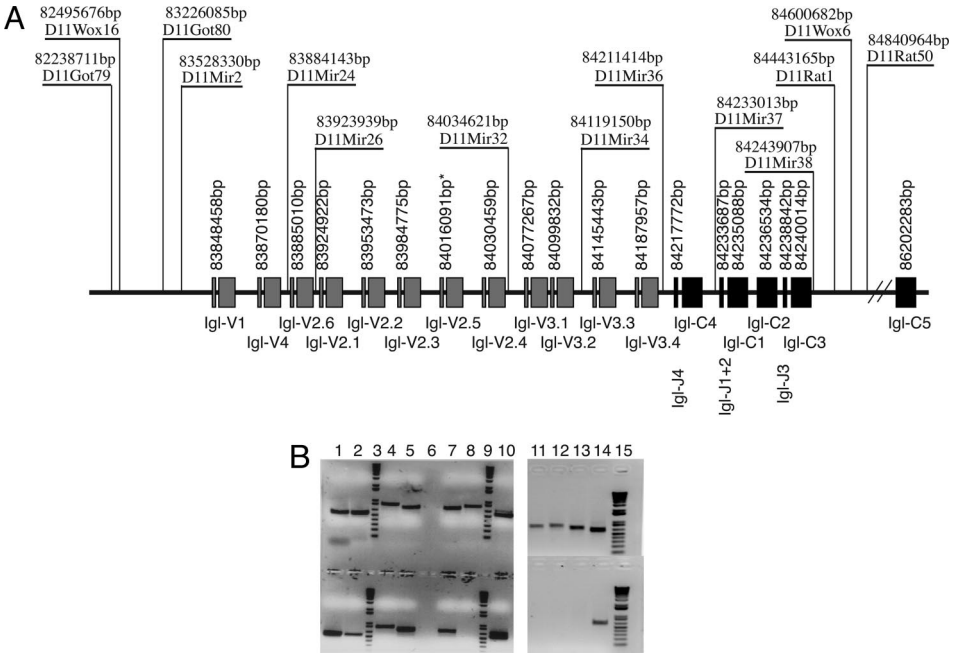

Fig. 3. Igλ light chain genes in the rat. (*A*) Physical map of the *Igl*-light chain locus on rat chromosome 11. The genomic starting position for each *Igl-V* gene as well as the *Igl-J* and *Igl-C* genes are presented in base pairs (bp). For the *Igl-V2.5* gene, the position of the second exon is shown because there is a region of ambiguous sequence where an exon one should be. The physical positions of microsatellite markers are indicated. All bp positions are derived from University of California, Santa Cruz BLAT Search Rat Genome Assembly Nov. 2004. (*B*) *Igl-V* genes in DA and E3 rats. Agarose gels from PCRs with *Igl-V* gene-specific primers show the difference between RF-Igλ positive E3 strain (*Upper*) and the RF-Igλ negative DA strain (*Lower*). Slots: 1, *Igl-V1*; 2, *Igl-V4*; 3, 1-kb DNA ladder; 4, *Igl-V2.1*; 5, *Igl-V2.2*; 6, free; 7, *Igl-V2.3*; 8, *Igl-V2.4*; 9, 1-kb DNA ladder; 10, *Igl-V2.5*; 11, *Igl-V3.1*; 12, *Igl-V3.2*; 13: *Igl-V3.3*; 14, *Igl-V3.4*; 15, 1-kb DNA ladder. The DA strain is missing the *Igl-V2.4*, *Igl-V3.1*, *Igl-V3.2*, and *Igl-V3.3* genes.

the *Igl-V1* and *Igl-V4* gene subfamily only 1 member exists, while the *Igl-V2* subfamily consists of 6 members (*Igl-V2.1*, *Igl-V2.2*, etc.) and the *Igl-V3* subfamily consists of 4 members (*Igl-V3.1*, *Igl-V3.2*, etc.) of which *Igl-V3.1* is a pseudogene containing a stop codon in the second exon. Despite the high recombination rate in the AIL no recombination was found inside the *Igl* locus, thus none of the *Igl* genes could be excluded.

Publicly available data from a whole genome scan of about 20,000 SNPs in 137 rat strains, among those DA and E3, were used to search for possible candidate SNPs (14). However, none of the SNPs were located in the *Igl* locus and in fact all 17 SNPs that were in close proximity to the *Igl* locus (megabase pairs 82.5–84.8) were non-polymorphic between DA and E3 rats.

Nevertheless, the combined data from the congenic and AIL rats conclusively show that the *Igl* locus is responsible for the RF phenotype.

RF-Igλ Use Multiple *V*, *J*, and *C* Genes. To investigate if the production of RF in DA.E3-*Rf1* congenic rats is due to one single gene that differs between DA and E3 rats, we performed PCR specific for the different *Igl-V* and *Igl-C* genes. Aguilar *et al.* (15) have shown that there is an allelic difference in the number of *Igl-V2* and *Igl-V3* genes between different rat strains. Indeed, we also found a difference between the DA strain and the E3 strain. While the E3 rat showed a PCR band for all investigated *V* genes, the DA rat appeared to lack the *Igl-V2.4*, *Igl-V3.1*, *Igl-V3.2*, and *Igl-V3.3* genes (Fig. 3*B*). There was no difference in the *C* genes (data not shown).

To determine if RF produced in parental E3 rats use one of the missing *Igl-V* genes, we established B cell hybridomas specific for rabbit IgG and sequenced the *Igl* genes in 10 of them. All monoclonal antibodies were of the IgM isotype and used germline encoded *Igl-V* genes. Surprisingly, only 3 of the clones used the *Igl-V* genes that were missing in the DA strain (Table 1). The rest of them used *Igl-V* genes that were present in both DA and E3 rats. Therefore, we could not exclude any *Igl-V* gene and conclude that not only the missing *Igl-V* genes (*Igl-V2.4*, *Igl-V3.1*, *Igl-V3.2*, and *Igl-V3.3*) but several *Igl-V* genes together may contribute to the RF phenotype.

RF-Igλ Have No Influence in PIA. With the DA and DA.E3-*Rf1* congenic strain, it was possible to assess the role that naturally occurring RF play in inflammatory disease models. First we immunized rats with pristane to investigate the influence of RF on the development of PIA. As expected, there was no significant difference between the DA and DA.E3-*Rf1* congenic rats in arthritis severity in the acute phase of arthritis [supporting information (SI) Fig. S1]. However, when we compared the RF level from naïve and arthritis induced rats we could see a significant increase in all RF types in the DA.E3-*Rf1* strain and all but the RF-Igλ in the DA strain, illustrating the relevance of the PIA model for the study of rheumatoid factors (data not shown).

Table 1. *V*, *J* and *C* gene usage of the λ light chain in RF-Hybridomas

Clone name	V gene	J gene	C gene
LuMo27.1	V3.2	J1 + 2	C1
LuMo33.1	V3.3	J1 + 2	C1
LuMo8.2	V2.1	J1 + 2	C1 or C2
LuMo8.6	V2.1	J5	C4
LuMo18.1	V2.2	J5	C4
LuMo47.3	V2.6	J1 + 2	C1 or C2
LuMo3	V2.2	J1 + 2	C1 or C2
LuMo10	V2.1	J3	C3
LuMo19	V2.5	J5	C4
LuMo35	V3.2	J1 + 2	C1

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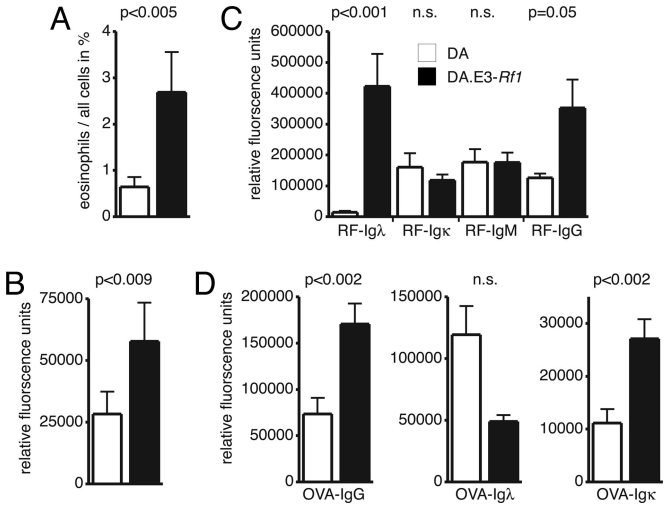

Fig. 4. OVA-induced airway inflammation in DA.E3-*Rf1* congenic rats. Data from 2 independent experiments were pooled and are shown as mean ± SEM. (*A*) The relative number of eosinophils in BAL is significantly elevated in RF-Igλ producing DA.E3-*Rf1* congenic rats (F3, $n = 23$, black bars) compared to DA rats ($n = 21$, white bars). (*B*) The total IgE level in serum is significantly increased in DA.E3-*Rf1* congenic rats. (*C*) The serum levels of RF-Igλ, RF-Igκ, RF-IgG, and RF-IgM from DA and DA.E3-*Rf1* congenic rats after induction of disease were compared. All significant *p* values between DA and congenic rats are indicated. (*D*) The levels of OVA specific antibodies in serum. The OVA-IgG and OVA-Igκ levels in serum were significantly increased in DA.E3-*Rf1* congenic rats. Congenic rats had a tendency toward a reduced Igλ response against OVA, this was, however, not significant.

RF-Congenic Rats Develop More Severe Allergic Bronchitis. To study the role of RF in an inflammatory model with a different pathogenic mechanism we selected OVA-induced airway inflammation (OIAI), a model for allergic bronchitis or asthma. We immunized DA and DA.E3-*Rf1* congenic rats with OVA, and after they had been challenged twice with intranasal administration of OVA, the rats were killed and bronchoalveolar lavage (BAL) fluids were investigated. Two separate experiments with similar results were performed, the P values were <0.03 and <0.04, respectively. The results from both experiments were pooled and are shown in Fig. 4. One clinical parameter to address the severity of airway inflammation is the number of eosinophils found in blood and BAL fluid (16). Interestingly, there was a significant increase in the relative number of eosinophils in the BAL in the RF producing congenic rats compared to the DA parental strain ($P < 0.005$). In addition, the total level of IgE was significantly increased in the DA.E3-*Rf1* congenic rat ($P < 0.009$). Other parameters, like the total cell count and protein concentration in BAL, were elevated but not significantly different between the strains (data not shown). We also investigated the distribution of RF and anti-OVA antibodies in both strains (Fig. 4*C*, *D*). OVA-IgE and RF-IgE were below the detection limits in our assays and could not be analyzed. However, in the DA.E3-*Rf1* congenic rats, we observed significantly elevated levels of RF-IgG ($P = 0.05$) and RF-Igλ ($P < 0.001$). In addition, anti-OVA IgG ($P < 0.002$) were increased in congenic rats. The enhanced anti-OVA IgG levels could be due to a RF-mediated, enhanced immune response or directly due to increased OVA specific Igλ and therefore, we measured anti-OVA Igλ and anti-OVA Igκ levels. DA.E3-*Rf1* congenic rats showed elevated OVA-specific Igκ antibodies ($P < 0.002$) and a tendency toward decreased anti-OVA Igλ (not significant), excluding a direct impact of OVA-specific Igλ antibodies in the increased immune response toward OVA.

Discussion

In this study we successfully show that when we combined the congenic strain and the advanced intercross line approach we could positional clone the RF regulating genes in a genetic region with only sparsely occurring recombinations as well as study the biological role of the RF in inflammatory conditions. It shows that RF plays a role in some types of inflammation but not in others, which may be an important finding to understand the role of RF in subtypes of RA and asthma.

The original linkage analysis was performed in a rat intercross in which pristane was used to induce PIA and the data, that were confirmed in the congenic strains, showed that the *Rf1* locus was not associated with the type of arthritis developing in PIA (12, 13). PIA has been shown to be a primary T cell-mediated model for RA, in which the arthritis can be transferred with T cells without B cells (17). Although RF are increased during the arthritis process, the rats lack other important autoantibodies like antibodies to citrullinated proteins or type II collagen (unpublished observations). Therefore, it is likely that the type of RF associated with the lambda gene is not involved in the development of PIA, but this does not exclude a role in other types of arthritis or inflammatory diseases with more antibody dependent pathways, such as antigen- or antibody-induced arthritis. These are arthritis models that are strongly dependent on complement activation and the formation of immune complexes. RF are known to mediate immune complex formation and therefore these models might be significantly influenced by the level of RF at the induction of disease (18).

In contrast to RA, no one has yet tried to address the pathogenic role that RF play in inflammatory conditions of the lung. In our study, we could show that RF have a direct effect on the severity of ovalbumin-induced airway inflammation, an animal model for bronchial asthma. Like RA, bronchial asthma is also a chronic inflammatory disease with an unknown etiology where both environmental as well as genetic factors are thought to contribute to the disease (19, 20). Furthermore, by using recombinant mouse strains, Piavaux demonstrated that different pathways are linked to different genetic regions (21). In this study, we postulate an important role of RF in one pathway for asthma development in which elevated RF levels may not only reflect eosinophilia and asthma severity but, in fact, are the direct course of it. Our results are in agreement with data from a clinical study of Japanese asthma patients where patients with positive RF levels had significantly higher eosinophil counts as well as more asthma severity than those in a negative RF level group (22). We could also show that congenic rats had an increased antibody response against OVA and this was not due to increased OVA-Igλ antibodies *per se*, suggesting that RF-Igλ in the congenic strain could enhance the humoral immune response via formation of immune complexes (23). Taken together, these data suggest that RF may have an impact on the severity of airway inflammation. Therefore the RF producing congenic rat will be of great interest in the understanding of the different pathological pathways of the disease, in particular antibody- and maybe more Th2-dependent pathways.

It is well known that RF are not specific for RA but frequently occur in several other inflammatory diseases and reflect instead a certain type of persisting inflammation. In regards to this, RF are found in patients with active pulmonary tuberculosis, chronic hepatitis C infection, as well as in patients with primary Sjögren's syndrome, systemic sclerosis, polymyositis, mixed connective tissue disease, and SLE (24–26). In addition, Edwards at al. recently demonstrated that RF are found to be an independent risk factor for ischemic heart disease (IHD) in men (27), another piece of evidence that RF may be directly involved in the pathology of the inflammatory process.

RA is not a single disease entity, but rather a heterogeneous syndrome caused by different pathways involving B cells and autoantibodies, T cells, the cytokine network, as well as the fibroblast and many other cell types (28). Most recently, B cells came back into the spotlight. Various autoantibodies have been found in sera of RA patients, but most important was the discovery of antibodies against citrullinated proteins and their high specificity for RA (29), as well as the positive therapeutic results using a B cell depleting antibody. Surprisingly, many studies show that B cell depletion reduced the levels of RF more dramatically than the levels of anti-citrullinated protein antibodies (30, 31). Therefore, RF might have a direct cause for RA or subtypes of RA, and the presence of RF might reflect one type of disease pathway.

Subgrouping of RA patients according to their gender; clinical parameters, such as arthritis severity, occurrence of RF, or anti-citrullinated protein antibodies (anti-CCP); as well as known genetic components like the MHC-shared epitope help to classify the heterogeneity of RA. Many genetic associations were found only in subpopulations of RA patients, so was the association to the C5-*TRAF* haplotype only found in RF positive patients (32) and the association to PTPN22 was found only in RF positive patients but was not linked to the shared epitope (33).

In conclusion, we have positional cloned the Ig lambda light chain locus as one locus regulating RF production in rats. In addition, we have provided evidence suggesting that the *Igl* locus may account for the development of more severe airway inflammation as congenic rats with high level of RF suffer from severe eosinophilia and increased production of IgE. This will now allow a more precise dissection of the role of RF in specific inflammatory pathways occurring not only in asthma but also in subtypes of other inflammatory diseases, including RA.

Materials and Methods

Animals. Rats of the E3/ZtmRhd, DA/ZtmRhd, and LEW.1F/ZtmRhd strains (originating from Zentralinstitut für Versuchstierzucht, Hanover, Germany) and rats of the GK/Swe and F344/Swe strains were kept in animal facilities in Lund and Malmö respectively in a climate-controlled environment with 12 h light/dark cycles. Rats were housed in polystyrene cages containing wood shavings and fed standard rodent chow and water ad libitum. The rats were free from common pathogens including *Sendai virus*, *Hantaan virus*, *corona virus*, *reovirus*, *cytomegalo virus*, and *Mycoplasma pulmonis*. Breeding to produce DA.E3-*Rf1* congenic rats ($\geq$ N10) and advanced intercross line (AIL) rats (GKxF344) F19 to F21 was performed in the same facilities. For all experiments, DA.E3-*Rf1* heterozygous rats were first intercrossed, then homozygous DA.E3-*Rf1* congenic rats as well as DA littermates were bred to produce pure DA.E3-*Rf1* congenic rats and DA littermate controls. All experiments were approved by the local (Malmö and Lund, Sweden) ethical committee.

Genotyping. DNA was prepared from toe biopsies or ear biopsies by alkaline lysis, amplified with fluorescence labeled microsatellite markers by PCR according to the standard protocol and analyzed on MegaBACE 1000 (Amersham Pharmacia Biotech). The genomic sequence from rat chromosome 11 (base pair 83497830 to base pair 84600779) was retrieved from the publicly available rat genome sequence at http://www.genome.ucsc.edu/, microsatellite regions were found by visual inspection and new primers were designed. A complete list of all new microsatellite markers that are polymorphic between the DA and E3 strains as well as the GK and F344 strain are provided in Table S1.

Induction and Evaluation of Arthritis. PIA was induced by a single intradermal injection of 100 μl pristane (2,6,10,14-tetramethylpentadecane, ACROS Organics) at the base of the tail in age- and cage-matched rats at the age of 7–9 weeks and arthritis development was monitored in all 4 limbs using a macroscopic scoring system. Briefly, 1 point was given for each swollen and red toe, 1 point for each affected midfoot, digit, or knuckle, and 5 points for a swollen ankle (maximum score per limb 15 and 60 for a rat). The rats were examined every second to third day for 1 month after induction of the disease in a blinded fashion.

Induction and Evaluation of Ovalbumin-Induced Airway Inflammation (OIAI). Rats at the age of 7–9 weeks were injected intra peritoneal with 1 mg of ovalbumin (OVA) and 50 mg of alum (aluminum potassium sulfate) dissolved in 1 ml PBS. At day 19 and 20 after the injection, rats were challenged intranasally with 100 μg OVA in PBS. At day 21 after immunization rats were anesthetized and killed by bleeding through the heart, tracheotomized, and cannulated through the trachea. BAL was collected by repeated washing of the lung with 2 ml PBS to a total of 10 ml. Cells from BAL were centrifuged, and the number of leukocytes was determined with a cell counter (Sysmex). The eosinophil cell count was assessed on cytospins of 1×10^5 BAL cells centrifuged for 6 min at 1000 rpm. After a cyanide-resistant peroxidase activity (CRPA) straining and a counterstaining with hematoxylin, eosinophils were identified under the light microscope at 1000$\times$ magnification. The mean value of eosinophils and total cell number was calculated after counting 4 different areas on each slide in the microscope in a blinded fashion by 2 independent observers.

Blood Sampling and Detection of Antibodies. Peripheral blood was collected from naive (GKxF344) N19 AIL, naïve DA, and congenic rats as well as from all disease-induced rats at termination day from the tip of the tail or bleeding through the heart. All antibodies in serum were analyzed by Eu^{3+}LISA. The Eu^{3+}LISA detecting RF were made by coating ELISA plates with rabbit IgG for total IgE levels, or anti-OVA antibodies plates were coated with anti-rat IgE or OVA, respectively. After blocking with 2% BSA, serum samples were added and incubated overnight. Biotin-labeled mouse anti-rat mAb (PharMingen) or polyclonal mouse anti-rat IgG (Zymed) were added and incubated. Then Eu^{3+}-labeled streptavidin was added. For final detection, Enhancement Solution was added and fluorescence emissions were read using Victor/Wallac (Wallac).

Hybridoma Production. Three 14-week-old E3 rats were immunized with 500 μl of pristane i.d. at the base of the tail. At day 6 after injection, spleens were taken and Igλ-producing B cells were enriched by MACS-system using biotinylated mouse anti-rat Igλ (PharMingen) and streptavidin MicroBeads (MACS, Miltenyi Biotec). 5×10^7 spleen cells were fused with 2×10^7 cells of the non-secreting mouse myeloma cell line P3xAg8.653 (ATCC). After fusion cells were seeded in 96-well plates in complete D-MEM medium containing 10%FCS, β-Mercaptoethanol, penicillin, streptomycin, and the selection chemicals hypoxanthine, thymidine, and aminopterin (HAT, Sigma). Cells were incubated in 37 °C in 7.5% CO_2. The production of RF was tested 2–3 weeks later in Eu^{3+}LISA. Positive clones were subcloned once with limiting dilution.

***V* Gene Sequencing.** Twenty million hybridoma cells were used for mRNA purification in each preparation. The cDNA was directly PCR-amplified from cDNA reaction according to the manufacturer supplied protocol (First-Strand synthesis kit, Amersham Pharmacia Biotech) by using *V* gene and *C* gene specific primers for the Igλ light chains: V3-SeqF 5-CTCTACTATTCCTTGCCTTCCTTC-3, V4-SeqF 5-CAACCACCTTCAG-CATCAGTCA-3, V4-Seq-altF 5-TCTCTTCTTCACCTCTTCTCT-3, C1 + 2-SeqR 5-TCAGTGGGCAAGGGAGAAGG-3, C3-SeqR 5-AGCCATGTACTTGTTGTTCTGT-TT-3, V3-Seq-altF 5-ACTATTCCTTGCCTTCCTTCATCA-3, C1 + 2-Seq-altR 5-GTATTTGTTGCCCTGTTTGGTG-3. PCR products were cloned into TOPO4 vector (TOPO TA Cloning Kit For Sequencing, Invitrogen). Transformed cells were selected by ampicillin resistance and positive clones were identified by EcoRI-restriction enzyme cleavage of miniculture preparations. Plasmid DNA was used as template DNA in the sequencing reactions (DYEnamic ET terminator sequencing kit, Amersham Pharmacia Biotech). The sequence reactions were purified with Sephadex columns in a 96-well plate and run on the MegaBACE1000. The sequences were analyzed in the SeqMan program (DNA Star) and aligned to the publicly available rat genome sequence. To avoid and correct misincorporations of nucleotides, 4 different clones were sequenced with the M13-Forward as well as the M13-Reverse primer.

Statistical Analysis. The Statview software program was used for all statistical analyses. Frequency of arthritis was analyzed by Fisher's exact test, the correlation of RF was calculated by Spearman Rank Correlation test and the non-parametrical Mann–Whitney *U* test (comparison of two groups) or Kruskal–Wallis test (comparison of three groups) were used in all other statistical analyses. P-values <0.05 were considered significant.

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