Rats made congenic for Oia3 on chromosome 10 become susceptible to squalene-induced arthritis

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Several quantitative trait loci (QTLs) regulating the risk of experimental arthritis have been identified by genome-wide linkage analyses, but only the MHC has thus far been reported to transfer arthritis susceptibility in congenic animals. We have produced a congenic strain for Oia3, a genetic factor originally identified as an oil-induced arthritis (OIA) QTL in arthritis-prone DA rats. A 46 cm telomeric region of chromosome 10 encompassing Oia3 was transferred from DA rats to MHC-identical but minutely arthritis-susceptible LEW.1AV1 rats by selective breeding. Arthritis development was provoked in Oia3-congenic rats by intradermal injection of different adjuvant oils. One successful arthritis trigger was squalene, which is approved for vaccinations in humans and has been implicated in Gulf War syndrome. The endogenous cholesterol precursor squalene induced T cell infiltration into joints and macroscopic arthritis in Oia3-congenic rats and DA rats, whereas LEW.1AV1 rats were almost resistant. Arthritis onset, ~14 days post-injection, coincided with arrested body-weight gain and increased plasma levels of the inflammation markers fibrinogen and α1-acid glycoprotein. Congenic rats displayed intermediate phenotypes compared with the two parental strains, and similar to rheumatoid arthritis in humans, female preponderance was observed in Oia3-congenic rats. Finally, recombinant rat strains were constructed and were used to map a susceptibility gene(s) in females to a telomeric 4–19 cm Oia3 subregion. The experimental system described allows transformation of multifactorial arthritis susceptibility into dichotomous phenotypes.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and destructive inflammatory joint disease with 1% prevalence in Western populations (1). Medical therapies have not been able to prevent or arrest this progressive autoimmune disease, which causes severe suffering of affected individuals and is a burden to the health care system (2,3).

The etiology of RA has been attributed to a combination of environmental and genetic factors, supported by the concordance rate in monoyzotic and dizygotic twins (12.3–15.4 and 3.5–3.6%, respectively) (4,5). Thus far, smoking is the only identified environmental factor. RA is polygenic, but up to now only MHC has been conclusively linked to the disease and is estimated to account for 30% of the genetic susceptibility (6,7). The polygenic nature and clinical heterogeneity of RA (8) complicate the identification of additional arthritis-related genes. Therefore, we have initiated genetic dissection of homogenous inbred rat models of RA, and have focused on T cell-mediated diseases that can be induced in arthritis-prone animals by molecules that activate the immune system nonspecifically, i.e. adjuvants. Arthritogenic adjuvants are structurally unrelated molecules of different origins (9), including specifically, i.e. adjuvants. Arthritogenic adjuvants are structurally unrelated molecules of different origins (9), including incomplete Freund’s adjuvant (IFA), which is used to trigger OIA (10–12). Another more potent adjuvant lipid is squalene (C30H50) (9,13), an intermediate in cholesterol biosynthesis. Squalene has been implicated as an etiological factor in Gulf War syndrome (14) and is used in a recently approved adjuvant formulation for vaccinations in humans (15). In genetically predisposed rats, squalene-induced arthritis (SIA) fulfils at least four out of seven criteria defined for the diagnosis of RA (8), i.e. long-lasting symmetric arthritis, involvement of at least three groups of joints including hand joints, and bone erosion (13). SIA can therefore be regarded as a satisfactory experimental model for RA.

Initial genetic analyses of oil-induced arthritis (OIA) in different rat strains revealed arthritis-susceptibility genes located both within and outside the MHC (Oia1) (12,16,17). A subsequent genome-wide scan of progeny from an F2 intercross between DA rats and MHC identical but virtually arthritis-resistant LEW.1AV1 identified new quantitative trait loci (QTL) determining susceptibility to OIA, including Oia3 on rat chromosome 10 (18). Recently, Oia3 was also linked to SIA in an F3 (DA × LEW.1AV1) intercross (13). In fact, this QTL and the homologous chromosome regions in the mouse and the
human have been linked to several inflammatory diseases (19–25), and were therefore suggested to harbor gene(s) of general importance in such disorders.

The aim of this study was to produce a congenic strain for Oia3 in order to determine whether transfer of this QTL can confer arthritis susceptibility. To date, such data has only been presented for one out of at least 12 QTLs previously reported in experimental arthritis, namely the MHC. The MHC influences susceptibility to most inflammatory diseases in animals and humans (26). A clear arthritis phenotype in an Oia3-congenic strain is an important step towards the identification of new disease pathways and an arthritis-susceptibility gene(s).

Consequently, we aimed at: (i) transfer of the arthritis permissive Oia3 haplotype from DA to LEW.1AV1 by selective breeding; (ii) identification of an Oia3-specific trigger of arthritis in the Oia3-congenic animals; (iii) characterization of the arthritis phenotype in Oia3-congenic rats in terms of macroscopic and microscopic signs of joint inflammation, arthritis-related phenotypes such as changes in body weight and plasma levels of the acute-phase reactants fibrinogen and α1-acid glycoprotein (AGP) as indicators of ongoing inflammation (27); and (iv) mapping of the arthritis-susceptibility gene(s) within the Oia3-region.

RESULTS

Arthritis triggers in the Oia3-congenic strain

IFA was first tested as an arthritis trigger in the newly established Oia3-congenic animals. LEW.1AV1 rats are completely resistant to OIA, Whereas the DA haplotype in Oia3 is linked to arthritis susceptibility. However, only low incidence and severity were observed in Oia3-congenics; 20% incidence (2/10 rats) and median arthritis max score of 3 in females and 0% incidence (0/5) in males (data not shown). The more potent arthritis inducer, squalene, triggers SIA in LEW.1AV1 rats, albeit at a low incidence (25% in females, 0% in males) (13). When tested in Oia3-congensics, squalene resulted in a high incidence of a more severe arthritis [incidences of 80% in females (4/5 rats) and 60% in males (3/5 rats) and median arthritis max scores of 7 and 4, respectively] and was therefore used in the following experiments.

Arthritis susceptibility in Oia3-congensics is mediated by Oia3

We investigated if the Oia3-congensics were devoid of contaminating DA genes in the three other major arthritis QTLs identified in crosses between DA and LEW.1AV1. No DA alleles were detected in Oia2 (18) or OiaW (18) on chromosome 4, or in Oia4 (Å.M. Jansson, H. Öhrström, H. Luthman and J.C. Lorentzen, unpublished data) on chromosome 10. Hence, arthritis susceptibility is mediated by DA alleles located in Oia3 (Fig. 1).

Arthritis development in Oia3-congensics and parental strains

After squalene injection, macroscopic joint inflammation developed in only 3/23 (13%) LEW.1AV1, whereas arthritis developed in 22/28 Oia3-congensics (79%, P < 0.0001) and 14/14 DA (Table 1). Only joints in paws were affected after squalene provocation. The first signs of arthritis were detected at day 14 post-injection (p.i.) (Fig. 2), and appeared symmetrically in ankles or meta-tarsal/-carpal joints, and progressed to include larger parts of the paws, including some finger joints. The median of maximum arthritis scores in Oia3-congensics were lower than those of DA (4 versus 11, P < 0.0001), but higher than those of LEW.1AV1 (4 versus 2, P < 0.0001). Arthritis maximum score differed between sexes in Oia3-congenic rats (P = 0.04), whereas there was a lack of gender dimorphism in DA rats. Arthritis was typically monophasic with occasional progression to ankylosis in Oia3-congensics. Incidence, ankylosis, day of onset and maximum score for DA, LEW.1AV1 and Oia3-congensics are summarized in Table 1.

Arthritis- and inflammation-related phenotypes in Oia3-congensics and parental strains

Paw sections from Oia3-congenic rats and DA rats were taken at day 16 (acute arthritis) and day 65 (ankyloletic stage of disease) post squalene injection and compared with normal rats. Arthritic paws from DA and Oia3-congenic rats were similarly heavily infiltrated with CD11b/c+ cells and moderately infiltrated with αβ+ T cells, whereas normal DA and Oia3-congenic paws had few CD11b/c+ cells and almost no αβ+ T cells (data not shown). Notably, infiltration of CD11b/c+ and αβ+ T cells was evident also in ankyloitic paws (day 65 p.i.). The paws were enlarged and bone-deformed but without clear signs of active inflammation, which was defined as swelling and redness. Besides assessment of macroscopic arthritis, the animals were repeatedly weighed. At the same time plasma was collected and analyzed for fibrinogen and AGP. LEW.1AV1 rats gained weight throughout the study and showed only minor fluctuations of fibrinogen and AGP, whereas DA rats experienced a marked weight loss and pronounced increases of fibrinogen and AGP. In Oia3-congensics, these arthritis-related phenotypes were intermediate between, and significantly different from, the parental DA and LEW.1AV1 rats (Fig. 3A–C). Thus, these disease sub-phenotypes appeared to co-variate with macroscopic arthritis, at least during arthritis development until 20 days after provocation. At this time-point, there was a strong correlation between arthritis score and body weight change (correlation coefficient r = -0.7; P < 0.001), plasma levels of fibrinogen (r = 0.5; P < 0.001) and AGP (r = 0.7; P < 0.001) (data not shown).

Arthritis development in Oia3-recombinant strains

Three Oia3-recombinant rat strains were constructed; Oia3a, Oia3b and Oia3c (see Materials and Methods). Each of these were DA-homozygous for an Oia3-subregion, as illustrated in Figure 1. When concomitantly challenged with squalene, two of these recombinants, Oia3b- and Oia3c-recombinant rats, were highly susceptible (73 and 59% arthritis incidences, with median arthritis maximum scores of 5 and 3, respectively), whereas the Oia3a-recombinant rats were more resistant (30% incidence and a median arthritis maximum score of 1) (Table 2 and Fig. 4A and B). Animals with DA alleles in Oia3b, covering the telomeric half of Oia3, were almost as susceptible to SIA as the Oia3-parental strain (no significant difference), but did not have a difference in susceptibility between sexes. In contrast, Oia3c-recombinant females, with DA alleles in a shorter telomeric segment of Oia3, developed a more severe
disease than their male littersmates ($P = 0.006$, Student’s $t$-test, unpaired). Incidences, day of onset and maximum scores for Oia3-recombinant strains are summarized in Table 2.

DISCUSSION

Genetic influence on the risk of RA is well documented, but MHC is thus far the only chromosome region that has been conclusively linked to disease susceptibility (5–7). MHC also stands out in experimental arthritis as the only QTL reported to transfer susceptibility in congenic animals. In this report, we demonstrate that the arthritis QTL, Oia3, from DA rats confers arthritis susceptibility when transferred to LEW.1AV1 rats, and that the macroscopic squalene-induced arthritis is accompanied by T lymphocyte infiltration into joints, arrest of body weight gain and increased plasma fibrinogen and AGP levels. Taken together, these data demonstrate that the transferred Oia3-region from DA rats (Fig. 1) harbors a gene(s) that confers arthritis susceptibility.

To reveal this genetic effect, we employed a gene–environment interaction approach, combining information on the different susceptibilities of rat strains (genetic factors) with the varying

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**Figure 1.** The Oia3 region on rat chromosome 10 (RNO10) and homologies to human chromosome 17 (HSA17) and mouse chromosome 11 (MMU11). Microsatellite markers on RNO10 correspond to tested polymorphic markers. Names written in italic indicate gene names. Candidate disease genes depicted are: CD7 (clusters of differentiation 7), TIMP-2 (tissue inhibitor of metalloproteinase-2), ILF1 (interleukin enhancer binding factor 1), STAT-3 (signal transducer and activator of transcription type 3), STAT 5a (signal transducer and activator of transcription type 5a), IGFBP4 (insulin like growth factor-binding protein 4), PKC-α (α-chain of protein kinase C), GH (growth hormone), MPO (myeloperoxidase), Grin2c (NMDA receptor 2c), TNFAP1 (TNF-a induced protein1), NOS2a (nitric oxide synthase 2a), ACLY (ATP citrate lyase), CNP (cyclic nucleotide phosphodiesterase), CSF3 (colony-stimulating factor 3), NGFR (nerve growth factor receptor), PRKAR1A (protein kinase, cAMP-dependent, regulatory, type 1 α), and ITGB4 (β4 integrin). Previously published radiation hybrid map positions (http://www.informatics.jax.org/, http://rgd.msw.edu/, http://ratmap.gen.gu.se and http://www.ncbi.nlm.nih.gov/genemap99/, data retrieved November 2000) are indicated for parallel markers on genetic maps. The heavy black bar indicates the congenized Oia3 region and the flanking regions where recombinations have occurred are illustrated as striped bars. The shorter thick black bars correspond to the Oia3-recombinant subregions, Oia3a, Oia3b and Oia3c, with their respective flanking regions similarly illustrated as striped bars.
disease-triggering potency of adjuvants (environmental factors). Thus, when IFA was used to provoke arthritis, the incidence was 0 and 20% (males and females, respectively) in Oia3-congenic rats, compared with 60 and 80% incidence of self-limiting arthritis following provocation with squalene. The observed gender dimorphism is interesting, especially considering the absence of sex effects in the parental DA strain. Presumably, this is a reflection of the high number of inflammation-promoting alleles in DA that override sex effects mediated by individual QTLs, as is suggested to occur in the NZB/W mouse model for lupus nephritis (28). A similar dimorphism was observed for squalene-induced arthritis in the LEW.1F strain (13).

Besides experimental arthritis, the Oia3-region has been linked to experimental autoimmune encephalomyelitis, collagen-induced arthritis, diabetes and hypertension (19–24), suggesting that there is a gene(s) within Oia3 that affects general aspects of immune function and inflammation in the rat. In fact, Oia3 may also be of importance for immune regulation in other species, since the homologous region in the mouse (chromosome 11) and human (17q11–17q25) has been linked to inflammatory diseases, including experimental diabetes (29), experimental systemic lupus erythematosus (30), RA (31,32) and psoriasis (33), and to different forms of cancer (34–37) (see Fig. 1, where some candidate disease genes are listed). These linkages suggest that there are one or several genes within Oia3 that affect general aspects of immune function and inflammation in the rat, mouse and human.

As a first step to dissect the disease promoting Oia3 region we developed three subcongenic strains that were concomitantly phenotyped in SIA. This experiment revealed that the telomeric part of Oia3 (Oia3b, 23.7–33.0 cM, as determined from two genetic linkage maps by Å.M. Jansson, H. Öhrström, H. Luthman and J.C. Lorentzen, unpublished data) transferred arthritis susceptibility whereas the remaining centromeric part of Oia3 (Oia3a, 16.4–26.9 cM) was less efficient. The Oia3b region conferred arthritis to both males and females but the telomeric part of this region (Oia3c, 4.0–19.0 cM) transferred the risk only to females. Thus, Oia3c harbors a gene(s) which predisposes female rats to arthritis development, whereas the larger Oia3b harbors this gene(s) together with a susceptibility gene(s) expressed in males alone or in both sexes. Altogether, the experiment provides evidence for two, possibly three QTLs within the Oia3 region. The actual number of QTLs, and potential interaction between them, can be determined by a high-resolution intercross between Oia3-congenics and

### Table 1. Susceptibility to squalene-induced arthritis of Oia3-congenic rats and parental strains

<table>
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<tr>
<th>Strain</th>
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<th>Max scorec</th>
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<td>2/11 (18)</td>
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<td>8 (2; 12)</td>
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<tr>
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*aIllustrated as absolute numbers of rats with percentage in parentheses.

*bOf arthritic animals only.

*cPresented as median (min; max) values of arthritic rats only.

Figure 2. Development of squalene-induced arthritis, illustrated as mean scores for DA, LEW.1AV1 and Oia3-congenic rats versus days post-injection with squalene. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. Standard errors of the mean are indicated as bars. A more severe arthritis is observed in the Oia3-congenic strain compared with the parental LEW.1AV1 strain.
induced with squalene. Plasma AGP is presented as a percentage of normal standard serum levels. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. At day 20 p.i. (acute phase), the fibrinogen levels of Oia3-congenic animals are significantly higher than individual LEW.1AV1 plasma samples (P < 0.001). (A) Body-weight development in animals induced with squalene, illustrated as normalized weight means versus days post-induction with squalene. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. Standard errors of the mean are indicated as bars. At day 20 p.i. (acute phase), the weights of Oia3-congenic rats are significantly lower than LEW.1AV1 rats (P < 0.001). (B) Median plasma fibrinogen levels in animals induced with squalene. Plasma fibrinogen is presented as a percentage of normal standard serum levels. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. At day 20 p.i. (acute phase), the fibrinogen levels of Oia3-congenic animals are significantly higher than that of LEW.1AV1 (P < 0.01). (C) AGP levels in pooled plasma from animals induced with squalene. Plasma AGP is presented as µg/ml plasma. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. At day 20 p.i. (acute phase), the AGP levels of individual plasma samples from Oia3-congenic animals are significantly higher than individual LEW.1AV1 plasma samples (P < 0.001).

Figure 3. (A) Body-weight development in animals induced with squalene, illustrated as normalized weight means versus days post-induction with squalene. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. Standard errors of the mean are indicated as bars. At day 20 p.i. (acute phase), the weights of Oia3-congenic rats are significantly lower than LEW.1AV1 rats (P < 0.001). (B) Median plasma fibrinogen levels in animals induced with squalene. Plasma fibrinogen is presented as a percentage of normal standard serum levels. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. At day 20 p.i. (acute phase), the fibrinogen levels of Oia3-congenic animals are significantly higher than that of LEW.1AV1 (P < 0.01). (C) AGP levels in pooled plasma from animals induced with squalene. Plasma AGP is presented as µg/ml plasma. Closed squares represent DA animals, closed circles represent Oia3-congenic animals and open squares represent LEW.1AV1 animals. At day 20 p.i. (acute phase), the AGP levels of individual plasma samples from Oia3-congenic animals are significantly higher than individual LEW.1AV1 plasma samples (P < 0.001).

LEW.1AV1. We are also dissecting the Oia3c subregion through production of new recombinants.

The eventual identification of disease gene(s) within Oia3 will also depend on the chosen procedure for arthritis induction to attain large allele effects. We have previously advocated the use of simple disease triggers to reduce genetic complexity (9,13). Squalene is suitable in this respect since it is a structurally defined molecule and available as a pure oil. This oil is also attractive because it functions as an adjuvant in humans. It constitutes 5% of the MF59 adjuvant formulation that was recently approved for use in human vaccination formulations in European countries (15,38). The safety of this practice is questioned; it has been suggested that Gulf War syndrome is a consequence of vaccinations containing squalene. This demonstration of the arthritogenic nature of squalene makes it a potential etiological factor to Gulf War syndrome, in one of its forms characterized by generalized joint pain, fatigue, headache, memory or concentration difficulties, sleep disturbances and rashes (39). It has also been reported that a majority of patients have developed non-physiological anti-squalene antibodies (14).

The pathogenicity of squalene is under debate, but it is obvious in this new Oia3-specific disease model. We have established an experimental situation that facilitates the identification of a gene(s) regulating the detrimental effects of adjuvant exposure. We therefore believe that the Oia3-congenic rats, similar to MHC-congenic animals, may enable the identification and functional characterization of genes influencing immunity, autoimmunity and inflammation.

MATERIALS AND METHODS

Rats

Inbred DA and LEW.1AV1 [LEW-Rt1(DA)] rats were originally derived from Zentralinstitut für Versuchstierzucht, Hannover, Germany. The genetics and characteristics of these rat strains are described by Greenhouse et al. (40). The Oia3 region was transferred from the DA rat onto the genome of the MHC identical LEW.1AV1 rat as follows: an F1 (DA × LEW.1AV1) intercross rat was backcrossed to LEW.1AV1 and a progeny rat (F1N) heterozygous for the Oia3 boundary markers D10 Rat20 and D10 Mgh1 was selected for further backcrossing onto LEW.1AV1. This selective backcross breeding procedure was repeated for nine consecutive generations. Thereafter, one male and one female littermate (F1N) rat were intercrossed, and one male and one female offspring (F2N) homozygous for DA genes in the Oia3 region were selected and used as founder animals for the Oia3-congenic [LEW-Rt1(DA)-Oia3 (DA)] strain. Subsequent genotyping revealed that this strain was homozygous for DA alleles in the Oia3 region defined by the following markers: D10Rat92, D10Rat20, D10Arb21, D10Rat93, D10Rat99, D10Wox17, D10Wox20, D10Rat18, D10Rat17, D10n11Mit58, D10Rat15, D10Rat9, D10Rat11, D10Rat28, D10Rat227, D10Rat8, D10Rat6, D10Rat7, D10Rat5, D10Arb22 and D10Mgh1. After nine backcross generations, 99.8% of the genome was expected to be LEW-Rt1(DA)-Oia3 (DA) homozygous.

Three recombinant founders were selected for one generation of backcross onto LEW.1AV1, followed by intercrossing of heterozygous males and females for each Oia3-subregion expressing DA alleles as follows: Oia3a, D10Rat24 to D10Wox17, but not D10Rat39 or D10Mit58; Oia3b, D10Wox17 to D10Mgh1, but not D10Rat55 or D10Rat2; and finally Oia3c, D10Got154 to D10Rat135, but not D10Rat9, D10Got151 or D10Rat2. After these additional two backcross generations, 99.95% of the genome was expected to be LEW-homozygous. Rats were bred, kept and used under specific
pathogen-free conditions at the Biomedical Center in Uppsala, Sweden. The rats were sex- and age-matched for each experiment. They were kept in a climate-controlled environment with 12 h light/dark cycles, housed in polystyrene cages containing wood shavings and had free access to standard rodent food and water. Experimental procedures involving animals were performed according to guidelines provided by the central board for animal experiments at the Swedish Department of Agriculture, and were approved by the Ethical Board for animal experiments in Stockholm-North.

**Induction and evaluation of SIA**

Arthritis was induced under anesthesia by an intradermal injection at the base of the tail with 300 µl squalene (C_{30}H_{50}, density = 0.86 g/ml, derived from shark liver), >99.8% pure according to the supplier (Sigma Chemicals) or IFA (Difco). The squalene was analyzed for nitrogen content by Saybolt using an assay for detection of protein residues in oils (ASTM D4629). No nitrogen could be detected with the detection limit of 0.0001% w/w, which equals a protein content of <0.0006% w/w, assuming 16% nitrogen in protein. Thus, in squalene-induced arthritis each rat received <1 µg of shark liver protein, a dose that is extremely unlikely to be responsible for arthritis induction. Arthritis development was monitored every second to fourth day using a macroscopic scoring system ranging from 0 to 4 for each of the four limbs (1, enlargement of one type of joint; 2, enlargement of two types of joints; 3, more than two types of joint involved; and 4, severe arthritis in the whole paw), yielding a score of 0–16 per animal. The clinical data are presented as: incidence, the cumulative disease frequency (i.e. affecteds); ankylosis, rats in a late stage of disease with deformed paws without active inflammation; day of onset, the time from injection to the first recorded sign of joint inflammation; and finally, arthritis severity, the maximal score attained by each individual affected rat. From day 40 p.i., and every tenth day, the appearance of arthritis in each individual rat was also graphically depicted on paw maps.

**Immunohistochemical staining for cell surface markers**

Cryostat sections (8 µm) of decalcified rat paws (41) were mounted on gelatin-coated microscope slides (Novakemi), dried and stored at −80°C. The sections were stained as described previously (13). A panel of mouse mAbs against the cell surface markers αβ-TCR (R73), CD11b/c (ox-42, purchased from Serotec, Novakemi) and isotype matched control (IgG1, Dakopatts) were used at a concentration of 5–10 µg/ml. R73 Abs were purified from supernatant of hybridomas kindly provided by Dr Tomas Hünig, Würzburg, Germany.
Genetic analysis

The tips of the tails were collected and genomic DNA was purified according to a standard protocol (42). Genotyping was performed by PCR amplification of tandemly repeated sequences (microsatellites) that were polymorphic between the two parental strains, essentially as described previously (22), except that [γ-32P]ATP was used to label one of the primers in each pair. The following genomic markers were used: D4Mh27, D4Rat57, D4Rat60, D4Wox14, D4Rat66 and D4Mgh21 (markers for Oia2); D4Mgh17, D4Mit24 and D4Wox24 (markers for OiaW); D10Rat29, D10Mgh20, D10Mgh9 and D10Rat40 (markers for Oia4); D10Rat92, D10Arb21, D10Rat93, D10Rat20, D10Rat99, D10Mit20, IGFBP4, D10Rat55, D10Wox17, D10Wox20, D10Rat18, D10Rat17, D10M11Mit58, D10Rat15, D10Got147, D10Rat9, D10Rat11, D10Got151, D10Got154, D10Got156, D10Rat228, D10Rat227, D10Rat8, D10Rat7, D10Rat6, D10Rat5, D10Mgh1, D10Rat135 and D10Arb22 (markers for Oia3).

Quantification of fibrinogen and AGP in plasma

Plasma was collected from DA, LEW.1A1V1 and Oia3-congenic rats at day –16 (normal individuals), 10, 20, 30, 40 and 65 post-squalene-injection. Fibrinogen levels were determined for all individual animals at all time-points using nephelometry, as described previously (43). AGP was determined for all individual animals at all time-points using nephelometry, as described previously (43). AGP was determined for all individual animals at all time-points using nephelometry, as described previously (43).

Statistical methods

Non-parametrical two-tailed ranking tests were used in all statistical analyses (Mann–Whitney and Kruskall–Wallis), except for the nephelometrical analysis, where the Student’s t-test was used. P values <0.05 were considered significant.

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