Endophilin A2 deficiency protects rodents from autoimmune arthritis by modulating T cell activation

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The introduction of the CTLA-4 recombinant fusion protein has demonstrated therapeutic effects by selectively modulating T-cell activation in rheumatoid arthritis. Here we show, using a forward genetic approach, that a mutation in the SH3gl1 gene encoding the endocytic protein Endophilin A2 is associated with the development of arthritis in rodents. Defective expression of SH3gl1 affects T cell effector functions and alters the activation threshold of autoreactive T cells, thereby leading to complete protection from chronic autoimmune inflammatory disease in both mice and rats. We further show that SH3GL1 regulates human T cell signaling and T cell receptor internalization, and its expression is upregulated in rheumatoid arthritis patients. Collectively our data identify SH3GL1 as a key regulator of T cell activation, and as a potential target for treatment of autoimmune diseases.

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A berrant activation of autoreactive T cells has been suggested to initiate and drive autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). It is believed that RA is initiated years before the clinical onset through an activation of major histocompatibility complex (MHC) class II-restricted autoreactive T cells that give specific help to B cells, leading to production of autoantibodies. In RA, the chronic inflammatory attack of the joints, initiated by the adaptive immune system, is followed by an increased production of inflammatory cytokines such as TNF and IL-6 and during the last decades effective pharmaceutical treatments neutralizing this late inflammatory phase have been developed. Although early treatment has been shown to be more effective, as it improves the possibility to interfere with the underlying cause of autoimmune diseases, patients receive treatment well after disease is established. Thus, a better understanding of the role of the adaptive immune system that drives the early stages of the disease i.e. before the disease is clinically overt, is needed to improve treatment of autoimmune diseases. Genetic predisposition of RA is known to be strongly associated with MHC class II genes implicating a major contribution of T cells. Additionally, a recent genome-wide meta-analysis revealed over 100 non-MHC risk loci where many were suggested to be related to T cell immune functions. T cells can be found in abundance in the synovium of RA patients and the successful use of T cell targeting therapy, such as the CTLA-4-Ig fusion antibody abatacept, confirm the importance of T cells in the chronic stage of the disease. These observations are corroborated in animal models for RA, where depletion of T cells by use of antibodies or gene deletion protects against disease induction. A specific role of the T cell receptor (TCR) in regulating development of arthritis has been further illustrated in the SKG mouse, where a mutation in TCR signaling molecule Zap70 leads to altered TCR signaling. This in turn skews T cell development in the thymus increasing the number of autoreactive T cells leading to subsequent development of arthritis.

Herein, we have identified Endophilin A2 (EA2), encoded by the SH3gl1 gene, as a regulator of TCR internalization, signaling and downstream T cell effector functions. So far, EA2 has predominantly been studied in synaptic transmission in the central nervous system and in cancer. We demonstrate that deficient expression of SH3gl1, caused by either a spontaneous mutation in the DA rat, or in genetically modified mice, leads to protection against autoimmunity. We hereby report that EA2 has a fundamental role in autoimmunity and limits the induction of autoreactive T cells. The discovery of the EA2’s impact on T cell activation opens up to explore new pathways and treatment possibilities for not only RA but for all T cell dependent inflammatory diseases.

Results

A spontaneous mutation in the SH3gl1 gene protects against autoimmune arthritis. The DA rat is commonly used in autoimmune research due to its high susceptibility to a number of chronic inflammatory disorders such as arthritis and experimental autoimmune encephalomyelitis. We noticed an increased variability in susceptibility to arthritis in our inbred DA rat colony. Arthritis-resistant rats were selected and bred to establish a new line (denoted DA mutated (DAMut)), which was found to be completely protected against pristane-induced arthritis (PIA; Fig. 1a, b). We suspected the underlying cause to be of a genetic origin and not environmental. To test this hypothesis, the DAMut colony was re-established by cesarean sectioning into a SPF (FELASA II)-controlled facility. We injected the rats with pristane and followed arthritis development. The resulting clinical scores were similar to the conventional facility ruling out obvious environmental factors (Supplementary Fig. 1a, b). To genetically position the mutation, we crossed DAMut rats to the genetically different, arthritis susceptible, E3. The Pia43 rat strain and F2 offspring from this cross were immunized to induce PIA. Subsequent linkage analysis disclosed a significant association with arthritis incidence for a polymorphic marker on chromosome 9. Typing with additional markers lead to the identification of a quantitative trait locus (Pia43), at the telomeric end of chromosome 9 (Fig. 1c, d). The Pia43 locus had not been identified in previous E3xDA crosses, confirming that this locus was unique to the DAMut line. The fragment was introgressed into a congenic strain DAMut.E3-Pia43 and minimized to 2 Mb by new recombinations (Fig. 1c, f).

To identify the specific genetic alteration, we sequenced the DAMut rat genome and aligned it to the BN rat genome reference (Rno5). All variants in the DAMut.E3-Pia43 congenic region were manually compared to two previously sequenced DA genomes. No single nucleotide polymorphism (SNP) or short insertion or deletion (indel) was detected that could distinguish DAMut from the other DA strains in the congenic region, e.g., all 337 SNPs detected between DAMut and the reference sequence were also found in the other DA genomes. However, a structural variant was revealed, which was unique to the DAMut rat, and appeared to be the result of an insertion of a long terminal repeat (LTR) element of the ERV class I (ERV1) in intron 1 of the SH3gl1 gene (Fig. 2a).

Retrotransposons such as LTR elements have been shown to regulate gene expression in both mice and man. To investigate if this was also the case for the ERV1 insertion in the SH3gl1 gene in DAMut rats, we determined the levels of lysine 4 methylation in histone 3 (H3K4me3) and the acetylation of histone 4 (H4Ac) in DAMut rats to the genetically different, arthritis susceptible, E3. In contrast, levels in regions situated downstream of the insertion were down-regulated (Fig. 2B, C), indicating that SH3gl1 gene transcription was not active in DAMut rats. To determine if this had any impact on gene expression we analysed peripheral blood mononuclear cells (PBMCs) from DA and DAMut rats and quantified gene expression with qPCR. Strikingly, the DAMut rats had almost no expression of the SH3gl1 gene compared to DA rats (Fig. 2d). This vastly reduced gene expression also translated to a reduced level of the SH3gl1 encoded protein EA2 as determined by western blotting (Fig. 2e). To confirm that the arthritis resistance observed in the DAMut rats was due to a deficiency in EA2 expression we introgressed a deletion of the SH3gl1 gene into arthritis-susceptible B6.N.Q mice through backcrossing and evaluated them with collagen-induced arthritis (CIA). Similar to the DAMut rats, the SH3gl1 deficient mice were also protected from arthritis, in contrast to their SH3gl1 sufficient wild-type littermates (Fig. 2f). To investigate whether the effect was restricted to control only arthritis development or also other autoimmune diseases, we tested experimental autoimmune encephalomyelitis (EAE), a T-cell-dependent model of MS, which confirmed the EA2 mediated protective effect seen in arthritis (Fig. 2g). Additionally, we tested the T-cell independent collagen antibody induced arthritis model and observed no difference in disease severity or induction (Fig. 2h, i). We conclude that EA2 is a major regulator of T cell dependent autoimmune disease.

EA2 deficiency alters the induction threshold of autoreactive T cells. Since EA2 deficiency had such a great impact on T cell
dependent autoimmune disorders we investigated if the expression of \textit{SH3gl1} increased in T cells after arthritis induction. Indeed 8 days after in vivo activation the expression of \textit{SH3gl1} had more than doubled (Fig. 3a) suggesting an important function of \textit{SH3gl1} during T cell priming and activation. To further elucidate if the protection in \textit{SH3gl1} deficient rats is mediated by T cells we used the CD4$^+$ αβ$^+$ T cell dependent pristane-induced adoptive transfer model\textsuperscript{21}. Pristane-primed lymph node cells from DA or DAMut rats were expanded for T cells ex vivo before transfer into naïve DA recipients. Only cells from DA rat donors induced severe arthritis (Fig. 3b), indicating that the DAMut rats were not able to generate arthritogenic T cells. To confirm that the arthritis resistance was intrinsic to T cells, we transferred thymocytes from \textit{SH3gl1} deficient or wild-type littermate mice into TCRβ knockout mice before the induction of glucose-6-phosphate isomerase (GPI) induced arthritis. Mice receiving

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**Fig. 1** Arthritis regulating loci in the DAMut rat is restricted to a 2-Mb region on chromosome 9. a Mean arthritis score after pristane-induced arthritis in 5 DA and 7 DAMut rats. The arthritis data has been reproduced three times with the same results. b Incidence of arthritis after pristane-induced arthritis in DA and DAMut rats. c LOD score plot for incidence of PIA in S1 (E3.DA-Pia457 x DAMut) x DAMut rats of all genotyped chromosomes. d LOD score plot for incidence of PIA in (E3.DA-Pia457 x DAMut) DAMut rats on chromosome 9, broken line at 2.63 indicate significant linkage. e Schematic figure of the DAMut. E3-Pia43 congenic fragment on chromosome 9 with location of the SH3gl1 gene. Microsatellite markers in the Actbg2 and Uxs1 genes indicate inner border of congenic fragment and markers Mllt1 and Dazl indicate outer borders. f Mean arthritis score after pristane-induced arthritis in 8 DAMut and 4 DAMut E3-Pia43 heterozygote littermates. Arthritis has been reproduced in several different congenic fragments with the same results. Non-parametrical Mann-Whitney U test was used for statistical evaluation of data. Data are presented as mean with error bars indicating ± SEM with each dot representing an individual value.
$SH3gl1$ deficient thymocytes developed nearly no arthritis (Fig. 3c) and had fewer antigen-specific cells compared to the wild-type recipients as determined by an in vitro recall assay using the GPI peptide while no significant difference could be seen in mitogen activated cells (Fig. 3d). To determine if this reduction in antigen-specific cells was due to a defect in the thymic T cell development, we assessed thymic T cell populations by flow cytometry. No difference in T cell populations could be observed between the $SH3gl1$ deficient mice compared to their wild-type littermates indicating that the reduced numbers of
Fig. 3 T cells regulate Endophilin A2 mediated protection against arthritis. a Expression of SH3gl1 in sorted T cells from lymph nodes from DA rats at Day 0 and Day 8 after pristane immunization. b Mean arthritis score after adoptive transfer of pristane-primed DA and DA^Mut^ cells to 9 respectively 4 naïve DA recipients. The adoptive transfer experiment has been reproduced a total of three times with the same results. c Mean arthritis score after GPI-induced arthritis in 17 and 19 TCRβ knockout recipients that had received either SH3gl1+/− or SH3gl1+/+ thymocytes respectively, 7 days prior to disease induction. Data is pooled from three individual experiments. d Number of antigen specific IL-2, IL-17A and IFNγ producing T cells after recall stimulation with GPI peptide and IL-2 producing cells after ConA stimulation day 44 after GPI-induced arthritis. Data from 9 TCRβ knockout mice reconstituted with wild-type T cells and 8 TCRβ knockout mice reconstituted SH3gl1^−/− T cells. Recall experiment has been repeated twice in SH3gl1+/− mice. e Frequency of double negative (DN), double positive (DP), single positive CD4 (SP CD4), and single positive CD8 (SP CD8) thymocytes in 8 SH3gl1+/− and 6 SH3gl1+/+ mice. Data has been reproduced twice. f Frequency of CD25, CD44, PD-1, CD73 FR4, Ki-67, and FoxP3 positive cells in CD4^+ TCRβ^+ cells from inguinal lymph nodes ten days after GPI-peptide immunization from 6 SH3gl1+/− and 6 SH3gl1+/+ mice. g Mean arthritis score in naïve DA recipients after adoptive transfer of pristane-primed DA^Mut^ cells only, DA cells only or DA cells in addition with DA^Mut^ cells, 7 rats per group. h Arthritis sum score of the DA recipients above after adoptive transfer of pristane-primed DA cells only or addition with DA^Mut^ cells, 7 rats per group. Non-parametrical Mann–Whitney U test was used for statistical evaluation of data. Data are presented as mean with error bars indicating ±SEM with each dot representing an individual value.

EA2 deficient rodents develop normally and mount a normal response to cancer and infection. EA2 is a ubiquitous protein with high expression in the central nervous system. Thus, EA2 deficiency could potentially affect many cell types and impact development. However, we did not observe any major physiological disturbances caused by EA2 deficiency in DA^Mut^ rats by general measurements such as blood cell counts, body weight, breeding capacity and life span (up to 1.5 years) in which the DA^Mut^ rats were identical to their normal DA littermates (Supplementary Fig. 3a–e). Similarly to the DA^Mut^ rats, the SH3gl1 deficient mice develop normally as compared to their wild-type littermates. Given that the EA2 deficient rodents exhibited a profound protection against autoimmune diseases, we assumed that they could have a severely defective immune system. Our animal facility is specific pathogen free but had an outbreak of S. aureus caused infections which provoked septic arthritis in immunodeficient mice. However, no SH3gl1 deficient animals developed a clinical S. aureus infection during this outbreak. To further challenge the SH3gl1 deficient mice towards bacterial infections, an arthritogenic S. aureus LS-1 strain was inoculated intravenously. This septic arthritis model using S. aureus LS-1 strain has been shown to be dependent on T cells via activation of T cells by the superantigen, toxic shock syndrome toxin-1 (TSST-1). In this model of septic arthritis, we did not observe any difference in bacterial clearance or development of arthritis in SH3gl1 deficient mice compared to wild-type littermates. However, no SH3gl1 deficient animals developed a clinical S. aureus infection during this outbreak. To further challenge the immune system in EA2 deficient mice, we injected SH3gl1 deficient and wild-type littermate mice with B16F10 melanoma cells and followed tumor growth. No excessive tumor growth was observed in EA2 deficient mice compared to wild-type littermates. To further challenge the immune system in EA2 deficient mice, we injected SH3gl1 deficient and wild-type littermate mice with B16F10 melanoma cells and followed tumor growth. No excessive tumor growth was observed in EA2 deficient mice compared to wild-type littermates.
EA2 deficiency leads to reduced T cell activation via the TCR.
Considering the endocytic function of EA2 and the profound effect that loss of EA2 expression had on T cells specifically, we reasoned that EA2 might be important for the internalization of the TCR. Thus, we checked whether EA2 co-localized with the TCR upon activation using a proximity-ligase assay. While no co-localization could be found in un-stimulated cells, we could see that EA2 and the TCR co-localized after only three minutes of anti-CD3/CD28 stimulation (Fig. 4a and Supplementary Fig. 4). We next studied the kinetics of the TCR internalization and indeed, SH3g1 deficient T cells internalized their TCRs at a slower rate compared to SH3g1 sufficient T cells (Fig. 4b) with a reduced internalization seen already at 15 min after stimulation. The observed decreased rate of TCR internalization was not due to an increased recycling of TCR as differences in TCR internalization between the SH3g1 deficient and sufficient T cells sustain even after Brefeldin A treatment (Fig. 4c). To investigate if this had any impact on the responsiveness of the T cells we investigated downstream TCR signaling molecules in SH3g1 deficient and wild-type T cells. A reduced TCR signaling cascade was observed in SH3g1 deficient T cells compared to wild-type T cells, with reduced levels of phosphorylated Zap70 and activation of ERK1/2 (Fig. 4d–f). This reduction in responsiveness in the SH3g1 deficient T cells translated to reduced T cell proliferation following in vitro stimulation via CD3/CD28 (Fig. 4g, h). The observed reduced proliferation was not the result of reduced internalization of the IL-2 receptor as previously described but must stem from a reduced activation via the TCR (Supplementary Fig. 5).

SH3GL1 regulates TCR responses in human T cells and its expression is upregulated in RA patients. Since EA2 is highly conserved and had such a profound effect on T-cell-mediated arthritogenicity in both mice and rats, we next investigated to what extent SH3GL1 is of importance in human T cells. To demonstrate the relevance of EA2 in human T cells we made SH3GL1 CRISPR knock-out Jurkat T cells (Supplementary Fig. 6) and stimulated them in vitro with anti-CD3/CD28. Both TCR signaling and TCR internalization were reduced in SH3GL1 deficient Jurkat cells compared to wild-type control and normal Jurkat T cells (Fig. 5a, b). We addressed the possibility that SH3GL1 could be overexpressed in T cells from RA patients and determined the expression of both SH3GL1 and the TCR molecule CD3e from whole blood of RA patients and healthy controls and found that the SH3GL1 expression correlated with expression of the CD3E molecule (Fig. 5c). Similar to the findings in animal models, the levels of SH3GL1 were higher in RA patients when normalized for CD3E (Fig. 5d) corroborating the data from the NCBI GEO database where SH3g1 gene expression was found to be upregulated in sorted CD4+ T cells from RA patients compared to healthy control (Fig. 5e). Thus, SH3GL1 has a conserved mechanistic function in T cells across species and is relevant in a human disease setting.

Discussion
Aberrant activation of autoreactive T cells is the key for the self-perpetuating vicious circle of activation of both the innate and adaptive immune response that leads to chronicity and lack of resolution in autoimmune diseases. Using a rat animal model of RA, we discovered SH3GL1 as a major regulator of T cell effector function and autoimmune diseases. We show that deficiency of SH3GL1 leads to a complete protection against autoimmunity diseases in both mutated DA rats and in SH3gl1 knockout mice and that this results from loss of T cell effector functions.
Furthermore, we show that SH3g1l expression was increased in T cells during autoimmune arthritis in both rodents and RA patients.

TCR internalization and surface recycling following peptide-MHC recognition on the antigen presenting cell plays a pivotal role in establishing a stable immunological synapse and subsequent T cell activation. To limit potential induction of autoreactive responses, T cell activation is tightly regulated via the TCR and co-stimulatory molecules. Both the surface expression of the TCR as well as the adapters in the TCR signaling cascade are of importance in dictating the effector functions of the T cells. For example, CD4+ T cells deficient in the WASH protein show reduced trafficking of the TCR and proliferation of T cells. Similar to SH3g1l deficient animals, CD4+ conditional WASH knockout mice are also protected from EAE. Several earlier studies demonstrate that SH3G1l is necessary for internalization and trafficking of a number of receptors. Our data demonstrate that SH3G1l also regulates the internalization of the TCR. Because of the importance of TCR internalization and formation of the immunological synapse for efficient TCR signaling, a lack of SH3G1l leads to reduced T cell signaling and would subsequently result in reduced numbers of autoreactive T cells, in turn limiting the arthritis development. The importance of adapters in the TCR signaling cascade was recently demonstrated by targeting the adapter protein NCK. In SH3g1l deficient animals, inhibition of NCK reduce proliferation of the T cells and leads to induction of immunosuppression and protection against EAE. Although the regulation of the TCR is different between SH3G1l and NCK the resulting outcome seems to be similar. Development of effective tissue restricted immunotherapies in the field of autoimmune diseases has proven difficult, with a variety of antigen-specific vaccination strategies developed in the 1990s with limited success. Consequently, instead of attempting to address antigen specificity, the field moved more into the depletion of molecules (e.g. TNF blockade) or whole cell populations (e.g. B cells using anti-CD20 antibody) involved in the pathogenesis. Although these therapies have been beneficial they are not curative and there is still a substantial number of patients who are non-responders or develop severe side-effects such as increased susceptibility to infections. Our results with SH3G1l in rodents demonstrate an interesting pathway by which activation of autoreactive T cells is modulated via the TCR while retaining the ability to respond to pathogens. Thus, SH3G1l targeting could be a promising therapeutic treatment strategy for RA as well as in other T cell-mediated diseases.

Methods

Animals. The E3/ZtmRhod and DA/ZtmRhod rats originated from the Zentralinstitut für Versuchstierzucht, Hannover, Germany and were bred for more than 20 brother/sister mating generations in the animal facility of Medical Inflammation Research Lab. The DAMut rats were isolated from the DA/ZtmRhod stock and bred for more than 10 brother/sister mating generations. To obtain the (E3.DA-Pia457x DAMut) DAMut rats used in the linkage analysis, 2 male DAMut rats were bred with 4 female E3.DA-Pia457x rats. Subsequently, 10 female F1 hybrids were bred with 6 DAMut male rats to produce 51 males of the F2 offspring. The C57BL/6.129-Sk3gl1tm1Pdc/J, titled SH3G1l+/− in paper, mice were a kind gift from Professor Pietro de Camilli and backcrossed for five generations to the arthritis susceptible C57BL/6 mouse, expressing MHC class II A(q)39. C57BL/6.129-Sh3gl1tm1Pdc/J, titled SH3G1l−/− in paper, mice, were bred with C57BL/6.129-Tcrb+Mopu2 (Stock No: 002118), titled TCRb−/− in paper, mice, were bought from Jackson Laboratory and backcrossed to C57BL/10 expressing the MHC class II A(q) for more than ten generations. All animals included in the experiments were kept in a specific pathogen-free environment following the Federation of European Animal Laboratory Science Association guidelines (FELASA II), in a climate-controlled...
environment with a 12-h light/dark cycle and fed standard rodent chow and water ad libitum. All animal experiments followed the ARRIVE guidelines, they were performed in accordance with the ethical standards and with written informed consent, and approved by the local ethical committees (Malmo/Lund, Goeteborg and Stockholm, Sweden, ethical permits M109/07, M107/07, N67/10, N69/10, N169/10, N940/12, N134/13, 353-2012, N35/16, and N288/15).

Patients and healthy controls. Whole blood from RA patients and healthy controls was collected in PAXgene tubes at the Rheumatology Clinic at Karolinska University hospital. RA patients all met the 1987 American college of rheumatology criteria for diagnosing RA. Informed consent was obtained from all the participants and the Stockholm ethical review board approved the study.

Experimentally induced arthritis. Pristane-induced arthritis (PIA) was induced by a single injection (100 µl) of pristane (2, 6, 10, 14-tetramethylpentadecane; ACROS Organics) at the base of the tail. Collagen-induced arthritis (CIA) was induced by injection of 100 µg of pepstatin-digested rat collagen type II (as described in17) emulsified in 100 µl complete Freund’s adjuvant (Difco) with a boost injection at day 35 with collagen type II in incomplete Freund’s adjuvant (Difco). Glucosamine-induced arthritis was induced by inoculation of the S. aureus TST-1-producing; LS-1 strain intravenously in one of the tail veins with 2.07 x 10^8 S. aureus LS-1/ml in a total of 0.1 ml phosphate-buffered saline (PBS). Arthritis development in PIA, CIA, and PIA experiments were monitored using a macroscopic scoring system. Each limb with the highest possible count of 15, thus with a total possible score of 60 per animal described in more detail for rats in18 and for mice in19. The S. aureus-induced arthritis development was monitored using a macroscopic scoring system where each limb was scored according to a scoring scheme (0, neither swelling nor erythema; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; and 3, marked swelling and erythema). The total score was calculated by adding up all the scores within each animal tested.

Experimental autoimmune encephalomyelitis. Spinal cord from naive DA rats was taken and homogenized. EAE was induced by a single injection of spinal cord homogenate emulsified in incomplete Freund’s adjuvant (Difco). EAE was macroscopically scored according to the following scoring scheme: 0 = Normal, 1 = Tail weakness, 2 = Tail paralysis, normal gait, 2.5 = Tail paralysis, little affected gait, 3 = Tail paralysis, low back and mild waddle, 3.5 = Tail paralysis and low back, severe waddle, 4 = Tail paralysis, severe waddle, less sure footing, 4.5 = Tail paralysis, severe waddle, falling and lost balance, 5 = Tail paralysis and paralysis of one limb, 6 = Tail paralysis and paralysis of a pair of limbs, back is affected, 7 = Tetraparesis, and 8 = Paraplegia or deceased.

Adoptive T cell transfer of arthritogenic cells. Eight days after pristane injection, rats were euthanized by CO2 inhalation and inguinal lymph nodes were taken and mechanically homogenized through 40 µm filters. Cells were washed in PBS and T cells were depleted and enriched in vivo in D-MEM supplemented with HEPES (GIBCO), streptomycin/D-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies), β-mercaptoethanol (GIBCO), 5% fetal calf serum (GIBCO) and Concanaval A (3 µg/ml; Sigma-Aldrich). Cells were incubated at 37 °C and 5% CO2 for 48 h. Cells were washed in PBS and transferred to naïve DA rats.

Genotyping and linkage analysis. Toe biopsies were sampled and DNA was prepared by alkaline lysis. The DNA was amplified using fluorescence-marked microsatellites and standard polymerase chain reaction (PCR) reagents (dNTPs, hGPI325-339 emulsion phosphate isomerase (GPI)-induced arthritis was induced by a single injection of synthetically produced peptide corresponding to the human GPI protein aa325-339 (hGPI325-339) emulsified in complete Freund’s adjuvant (Difco)20. S. aureus-induced arthritis was induced by inoculation of the S. aureus TST-1-producing; LS-1 strain intravenously in one of the tail veins with 2.07 x 10^8 S. aureus LS-1/ml in a total of 0.1 ml phosphate-buffered saline (PBS). Arthritis development in PIA, CIA, and PIA experiments were monitored using a macroscopic scoring system. Each limb with the highest possible count of 15, thus with a total possible score of 60 per animal described in more detail for rats in21 and for mice in22. The S. aureus-induced arthritis development was monitored using a macroscopic scoring system where each limb was scored according to a scoring scheme (0, neither swelling nor erythema; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; and 3, marked swelling and erythema). The total score was calculated by adding up all the scores within each animal tested.

Chromatin-immunoprecipitation and quantitative PCR. Based on previous ChIP-Seq results from BN and SHR rats23, we identified regions with high levels of H3K4me3 and H3K27Ac modifications in the Sh3gl1 promoter. The Sh3gl1-ΔCMT (100 mg) from 3 DA and 3 DAmut rats were used for nucleolus isolation and subsequent ChIP analysis was performed according to24. The regions of interest were analyzed by ChIP followed by qPCR on ABI 7900HT detection system (Applied Biosystems). PCR primers were designed to amplify designated genomic regions using PRIMER EXPRESS software. qPCR analyses were carried out in 384-well plates with a final volume of 20 µl each for 40 cycles. We used Power SYBR Green PCR Master Mix (Applied Biosystems) with diluted ChIPed DNA or un-enriched input DNA as template. Enrichment ratios were calculated according to the 2^-ΔΔCT method with endogenous control genes similar to gene expression data sets. For H3K4me3 and H3ac modification see supplementary Table 1 for sequences of primers used for quantification.

RNA extraction and quantitative- polymerase chain reaction. Mononuclear leukocytes from blood (separated from Ficoll-density gradient) or sorted T cells (Pan T Cell MicroBeads, Miltenyi Biotec) were obtained from naive DA and DAmut rats and RNA was isolated (TriZol and Pure-link mRNA Kit, Invitrogen). Whole blood from RA patients and healthy controls were collected in PAXgene Blood RNA Tubes and total RNA was extracted with the PAXgene Blood RNA Kit (PreAnalytiX, Feldbachstrasse, Switzerland) according to the manufacturer’s protocol. cDNA conversion was performed with an Script cDNA synthesis (Bio-Rad, Hercules, CA, USA). Gene expression experiments were performed using TaqMan gene expression assays(Hs04325362_g1(Sh3gl1), Hs01495281_s1 (PPIA), Hs01062241_m1(CD3e), Rn01527769_g1(Sh3gl1), Rn01527840_m1(Hprt1), Applied Biosystems) and samples were run on a CFX96 RT-PCR(Ro-Bad) according to gene manufacturers description. Relative fold change was calculated according to the 2^-ΔΔCT method after normalization to reference gene, Hprt1 for rat gene and human gene expression experiments.

Western blotting. Whole cell lysates from DA and DAmut rats were obtained from brain samples by lysis in RIPA buffer (ThermoScientific). Samples were run in a NuPAGE 4–12% Bis-Tris gel (Novex, Invitrogen) and transferred to a PVDF membrane (EMD, Millipore). Blots were blocked with 5% bovine serum albumin (Sigma-Aldrich) in Tris-buffered saline with 0.1% Tween 20 (Cell Signaling Technology) and subsequently incubated with mouse anti-α-endoplasmic Rabinofilin 2 (clone SS1-1, Oigenic Technologies, Inc) and rabbit anti-histone 2B (Cat. No C49810, LifeSpan Biosciences, Inc). Blot was incubated with peroxidase-conjugated goat anti-mouse IgG(IgG(cat. no. 115-035-062, Jackson ImmunoResearch laboratories Inc.) or peroxidase-conjugated donkey anti-rabbit IgG (IgG cat.no.771-036-152, Jackson ImmunoResearch laboratories Inc.) respectively and developed using enhanced chemiluminescince. Pictures included in the entire blot can be found in the Source Data file.

Determining number of antigen-specific cells with enzyme-linked immuno- spot (ELISPOT) assay. Single cell suspension from spleens of mice previously treated with prime-boosted antigen to be used for ELISPOT assays were first pre-coated with antibodies to IL-2 (clone JES6-1A12, IL-17 (clone TC11-18H10) and a IFNγ(A18N) and stimulated for 48 h with 10 µM hGPI325-339 in D-MEM supplemented with HEPES (GIBCO), streptomycin/D-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies), β-mercaptoethanol (GIBCO), 10% fetal calf serum (GIBCO). Plates were washed and incubated with biotinylated antibodies to IL-2 (clone 5H4), IL-17 (clone TC11-8H4) and IFNγ (R46A2). Plates were washed and Extravidin-Phosphatase (Sigma) was added to wells and incubated for 45 min and washed. Plates were subsequently developed using BGI/PNT(Bigma). Plates were scanned, and number of active and inactive cells were counted with Immunospot software (Cellular Technology Ltd.).

Cell development. Single cell suspension from thymus of C57BL/6J129- Sh3gl1ΔCMT/I and their wild-type littermates were stained with anti-mouse monoclonal antibodies (B220-Flc, CD5-PE, CD25-PECy7, TC8r-PerCP5.5, CD4- Q605, CD8-BV650, CD69-APC, CD44-AT700) for 20 min at 4 °C. After washing with PBS, cells were acquired on a LSRII (BD Biosciences, Franklin Lakes, NJ, USA) using the BD FACSDiva™ software (BD Biosciences) with gates set to include all viable cells and later analyzed by FlowJo (Tree Star, Inc.) software.

Cell proliferation assays. T cells from DA and DAmut lymph nodes were negatively sorted using biotinylated mouse anti-rat CD11b/ (Clone C042X, Biologend) and mouse anti-rat CD45R (Clone C0X33, Biologend) and anti-biotin Microbeads (Miltenyi Biotec). Sorted T cells were then labeled with CellTrace-Violet (Mole- cular Probes) (Cool blood) and plates coated with plate coated using peroxidation tests (n = 10000), in which p < 0.05 was considered significant. The genetic map was generated in the R/qtl environment based on the recombinations in the backcross.
TCR internalization assay. Lymph node cells from DA and DA\textsuperscript{Nat} rats were stained with mouse anti-CD3 (Clone 1F4) conjugated with FITC (Biologen) at 4 °C. Labeled cells were added to plates coated with anti-rat \textit{g}TCR (Clone RT3) and anti-rat CD28 (Clone JJ319) in DEM-supplemented with HEPES (GIBCO), streptomycin/\textit{p}-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies), b-mercaptoethanol (GIBCO), 10% fetal calf serum (GIBCO), and incubated for 15, 30, and 60 min at 37 °C or left on ice for zero time point. Cells were washed and split into two fractions, one was quenched by stripping of surface anti-CD3-Alexa488 antibodies with PBS (GIBCO) pH 2 for 1 min and the other fraction left untreated. For the TCR internalization assay with the CRISPr Jurkat T cells were stained with anti-human CD3 (Clone UTCH1) conjugated with Alexa488 (BD Biosciences) at 4 °C. Labelled cells were added to plates coated with anti-human CD3 (Clone H13a, BD Biosciences) and anti-human CD28 (Clone CD28.2, BD Biosciences) in RPMI1640 (GIBCO), supplemented with streptomycin/D-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies) and 10% fetal calf serum (GIBCO) and incubated for 15, 30, and 60 min at 37 °C or left on ice for zero time point. Cells were washed and split into two fractions, one was quenched of surface anti-CD3-Alexa488 signal with unlabelled Alexa488 (MolecularProbes, Invitrogen) and the other fraction left untreated. For the Brefeldin A inhibitor experiment lymph node cells from SH3gl1<sup>−/−</sup> and SH3gl1<sup>+/−</sup> mice were stained with Armenian hamster anti-mouse TCRβ (Clone H57-597, Biologen) conjugated with Alexa488 at 4 °C. Labeled cells were added to plates coated with anti-mouse CD3e (Clone 145-2C11, BD Biosciences) and anti-mouse CD28 (Clone 37.51, BD Biosciences) in DEM supplemented with HEPES (GIBCO), streptomycin/D-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies), b-mercaptoethanol (GIBCO), 10% fetal calf serum (GIBCO) and together with 1 μg/ml of Brefeldin A (Sigma) and incubated for 30 min at 37 °C or left on ice for zero time point. Cells were washed and split into two fractions, quenched or no quench, and subsequently stained with rat-anti-mouse CD45R-PE-Cy7 (Clone RA3-6B2, BD Biosciences), rat anti-mouse CD4- BV405 (Clone RM4.5, BD Biosciences), rat anti-mouse CD3-PacificBlue (Clone 17A.2, Biologen) and anti-Alexa-488 (MolecularProbes, Invitrogen) to half of the samples (quenched). Cells were acquired on a LSRII (BD Biosciences, Franklin Lakes, NJ, USA) using the BD FACSDiva® software (BD Biosciences) with gates set to exclude doublets and include all viable cells determined as LIVE/DEAD® ~Near-In (Invitrogen) negative cells and later analyzed by Flowjo (Tree Star, Inc.) software. Percentage of internalized CD3/CD8α/8β was calculated as (Qx-Q0)/(Q0+Qx)100, where Qx is the mean fluorescence of cells quenched with anti-Alexa488 at each time point, Q0 is the mean fluorescence of cells quenched at time zero, and T is the mean fluorescence of cells that were not quenched.

TCR signaling. T cells from lymph nodes and spleens of C57BL/6J, Sh3gl1<sup>−/−</sup>, Sh3gl1<sup>−/−</sup> and wild-type littermates were sorted (Untouched CD4 + T cells MicroBeads, Miltenyi Biotec) and resuspended in DEM-supplemented with HEPES (GIBCO), streptomycin/D-penicillin (104 IU/ml penicillin, 10 mg/ml streptomycin; Invitrogen Life Technologies), b-mercaptoethanol (GIBCO), 10% fetal calf serum (GIBCO). Cells were rested for one hour at 37 °C and later added to plates coated with 10 μg/ml rat anti-mouse CD3 (Clone 145-2C11, BD Biosciences) and 5 μg/ml rat anti-mouse CD28 (Clone 37.51, BD Biosciences) and stimulated for 3 and 7 min. Cells were lysed using M-PER (Thermo Scientific) and supplemented with 1 μg/ml rat anti-Phospho-epitope specific antibody (Cell Signaling Technology), anti- phosphor-phosphorylated p44/p42 MAPK (Erk1/2), Thr202/Tyr204 (Cell Signaling Technology) and rabbit anti-anti-histone 2B (Cat. no. ab1790 Abcam) as loading control. Blot was incubated with peroxidase-conjugated donkey anti-rabbit IgG (Cat no.70-036-152, Jackson Immunoresearch Laboratories Inc.) and developed using enhanced chemiluminescence. Pictures including the entire blot can be found in the Source Data file.

Whole genome sequencing and bioinformatics analysis. Genomic DNA from liver from DA and DA\textsuperscript{Nat} rats was isolated using the nuclear lysis buffer, followed by ethanol precipitation and diluted with water. DNA Integrity (Trexus) DNA and paired-end sequencing (2x 100 bp). Sequences were aligned to the RN reference sequence Rn05 and compared to previous sequenced DA genome (Gibson 2016). Visual inspection of DExM expression alignments indicated that Genomics Viewers (IGV), Broad Institute, revealed a region of partial or improperly paired alignments in intron 1 of the Sh3gl1 gene and indicated an insertion. The reads aligned in the region and their paired sequences were assembled using Tri

References

1. Bos, W. H., van de Stadt, L. A., Sohrabian, A., Rönnelid, J. & van Schaardenburg, D. Development of anti-citrullinated protein antibody and rheumatoid factor isotypes prior to the onset of rheumatoid arthritis. \textit{Arthritis Res. Ther.} 16, 405 (2014).

2. Biotech, T., Teukis, J. & McInnes, I. Cytokines as therapeutic targets in rheumatoid arthritis and other inflammatory diseases. \textit{Pharmacol. Rev.} 67, 308–320 (2015).

3. Okada, Y. et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. \textit{Nature} 506, 376–381 (2014).

4. Van Bael, J. A. & Paget, S. A. Predominantly T-cell infiltrate in rheumatoid synovial membranes. \textit{N. Engl. J. Med.} 293, 517–520 (1975).

5. Ranges, G. E., Sirram, S. & Cooper, S. M. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. \textit{J. Exp. Med.} 162, 1105–1110 (1985).

6. Corhoy, A., Johansson, A., Vestberg, M. & Holmdahl, R. Collagen-induced arthritis development requires alpha beta T cells but not gamma delta T cells.

Statistical analysis. Visualization and calculations of arthritis and EAE data and immunosassays was done using GraphPad Prism v5 software program. The non-parametrical Mann–Whitney U test was used for statistical evaluation of arthritis scoring data and immunosassay. P values < 0.05 were considered significant.

Tumor model. B16F10 melanoma cells were obtained from ATCC and cultured in 5% CO\textsubscript{2} at 37 °C using 10% FBS in DMEM media supplemented with 4.5 g/ml of glucose, 1 mM pyruvate, 1.5 g of NaHCO\textsubscript{3}/500 ml of DMEM (GIBCO, MA, Thermofisher). Cells were washed two times with 37 °C PBS, trypsinized with 1X EDTA trypsin, then neutralized with 1:1 ratio of 10% FBS DMEM growth medium to trypsin and 200 μl/mouse of serum free cell growth medium containing 1×10\textsuperscript{5} B16F10 (passage 10) p10 melanoma cells were injected subcutaneously. Tumor volume was assessed using an electronic caliper and caliper function with formula (Length x Width\textsuperscript{2}) / 2. Maximum allowed tumor size is 1.5cm\textsuperscript{2} as calculated by (Length x Width\textsuperscript{2}) / 2.

Generation of Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 – SH3GL1 knock-out Jurkat cells. SH3GL1 CRISPR knockout cells were generated and bought from GenScript Ltd. Two clones T3-5 and T3-21 were predicted to be SH3GL1 knock-outs and T3-23 to be wild type. The three clones were confirmed to be either SH3GL1 knock-out or wild-type Jurkat cells by quantitative–polymerase chain reaction and western blotting (Supplementary Fig. 4a, b).

Proximity- ligation assay. Co-localization of EA2 and TCR was analysed by proximity ligation assay using the Duolink® PLA Starter Kit Mouse/Rabbit from Sigma (Cat. No DUC9210) according to the manufacturer’s description. EA2 was detected using primary rabbit anti-human EA2 (Sigma HPA021485) at 1μg/ml. TCR was detected using primary mouse anti-human CD3 (BD Biosciences Clone H13a) at 1μg/ml. Primary antibodies were detected using Duolink® In Situ PLA Probe Anti-Rabbit PLUS, Affinity purified Donkey anti-Rabbit IgG (H + L) and Duolink® In Situ PLA™ Probe Anti-Mouse MINUS, Affinity purified Donkey anti-Mouse IgG (H + L) provided by the kit according to manufacturer’s recommendations. Nuclei were stained using DAPI at 0.5μg/ml for 20 min in PBS. Cells were imaged on a Zeiss LSM 800 confocal laser scanning microscope (Zeiss). TexasRed was detected at 594nm/664nm and DAPI at 358nm/461nm. For image analysis, events were counted using the Analyze Particles function on ImageJ. The number of DAPI positive events was related to the number of TexasRed positive events per cell.
studies with T cell-deficient (TCR mutant) mice. *Int. Immunol.* **11**, 1065–1073 (1999).

7. Sakaguchi, N. et al. Altered thymic T-cell selection due to a mutation of the Zap-70 gene causes autoimmune arthritis in mice. *Nature* **426**, 454–460 (2003).

8. Milošević, I. et al. Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fusion. *Neuron* **72**, 587–601 (2011).

9. Baldassarre, T. et al. Endophilin A2 promotes TNBC cell invasion and tumor metastasis. *Mol. Cancer Res.* **13**, 1044–1055 (2015).

10. So, C. W. et al. EEN encodes for a member of a new family of proteins containing an Src homology 3 domain and is the third gene located on chromosome 19p13 that fuses to MLL in human leukemia. *Proc. Natl. Acad. Sci. USA* **94**, 2563–2568 (1997).

11. Backdahl, L., Ekman, D., Jagodic, M., Olsson, T., & Holmdahl, R. Identification of candidate risk gene variations by whole-genome sequence analysis of four rat strains commonly used in inflammation research. *BMC Genomics* **15**, 391 (2014).

12. Rintisch, C., Förster, M. & Holmdahl, R. Detection of arthritis-susceptibility loci, including Ncf1, and variable effects of the major histocompatibility complex region depending on genetic background in rats. *Arthritis Res. Ther.* **60**, 419–427 (2008).

13. Bergsteindottir, K., Yang, H. T., Pettersson, U. & Holmdahl, R. Evidence for common autoimmune disease genes controlling onset, severity, and chronicity based on experimental models for multiple sclerosis and rheumatoid arthritis. *Immunology* **164**, 1564–1568 (2000).

14. Vingbo-Lundberg, C. et al. Genetic control of arthritis onset, severity and chronicity in a model for rheumatoid arthritis in rats. *Nat. Genet.* **20**, 401–404 (1998).

15. Backdahl, L., Ekman, D., Jagodic, M., Olsson, T. & Holmdahl, R. Identification of candidate risk gene variations by whole-genome sequence analysis of four rat strains commonly used in inflammation research. *BMC Genomics* **15**, 391 (2014).

16. Yau, A. C. Y. & al. et al. Conserved 33-kb haplotype in the MHC class III region regulates chronic arthritis. *Proc. Natl. Acad. Sci. USA* **113**, E3716–E3724 (2016).

17. Makskova, I. A. et al. Retroviral elements and their hosts: insertional mutagenesis in the mouse germ line. *PLoS Genet.* **2**, e2 (2006).

18. Santos-Rosa, H. et al. Active genes are tri-methylated at K4 of histone H3. *Cell** **136**, 215–223 (2009).

19. Holmberg, J. et al. Pristane, a non-antigenic adjuvant, induces MHC class II-deficient arthritis in rats. *Arthritis Res. Ther.* **164**, 1144–1155 (2013).

20. Tuncel, J. et al. Animal models of rheumatoid arthritis (I): pristane-induced arthritis in the rat. *PloS ONE** **11**, e0155936 (2016).

21. Backdahl, L., Ekman, D., Holmdahl, R. & Tarkowski, A. Clonal expansion of D. WASH knockout T cells demonstrate defective receptor trafﬁcking in vascular smooth muscle cells. *J. Immunol.* **183**, 1073–1083 (2014).

22. Rintisch, C. et al. Novel variation of histone modiﬁcations in the SH3GL1 gene body – potential. *PLOS One** **9**, e107397 (2014).

23. Smolen, J. S. & Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat. Rev. Rheumatol.* **11**, 276–289 (2015).

24. Winthrop, K. L. Infections and biologic therapy in rheumatoid arthritis: our changing understanding of risk and prevention. *Rheum. Dis. Clin. North Am.* **38**, 727–745 (2012).

25. Pizzolla, A., Wang, K. & Holmdahl, R. A glucose-6-phosphate isomerase peptide induces T and B cell-dependent chronic arthritis in C57BL/10 mice: arthritis without reactive oxygen species and complement. *Am. J. Pathol.* **183**, 973–1073 (2013).

26. Gluud, K. J. et al. Additional information is available from the European Community’s IMI project BTCURE.

27. Bäcklund, J. et al. C57BL/6 mice need MHC class II Aq to develop collagen-induced arthritis dependent on autoreactive T cells. *Ann. Rheum. Dis.* **72**, 1225–1232 (2013).

28. Lu, S., Carlsen, S., Hansson, A. S. & Holmdahl, R. Immunization of rats with homologous type XI collagen leads to chronic and relapsing arthritis with different genetics and joint pathology than arthritis induced with homologous type II collagen. *J. Autoimmun.* **18**, 199–211 (2002).

29. Tuncel, J. et al. Animal models of rheumatoid arthritis (I): pristane-induced arthritis in the rat. *PLoS ONE** **11**, e0155936 (2016).

30. Holmdahl, R. et al. Genetic analysis of mouse models for rheumatoid arthritis. *Human Genome Methods** **215**, 213–238 (1998).

31. Bäcklund, J. et al. C57BL/6 mice need MHC class II Aq to develop collagen-induced arthritis. *PLoS ONE** **11**, e0155936 (2016).

32. Holmdahl, R. et al. Genetic analysis of mouse models for rheumatoid arthritis. *Human Genome Methods** **215**, 213–238 (1998).

33. Bäcklund, J. et al. C57BL/6 mice need MHC class II Aq to develop collagen-induced arthritis. *PLoS ONE** **11**, e0155936 (2016).

34. Sieper, J. et al. Oral type II collagen treatment in early rheumatoid arthritis. A double-blind, placebo-controlled, randomized trial. *Arthritis Rheum.* **39**, 41–51 (1996).

35. Elliott, M. J., Feldmann, M. & Maini, R. N. TNF alpha blockade in rheumatoid arthritis: rationale, clinical outcomes and mechanisms of action. *Int. J. Immunopharmacol.* **17**, 141–145 (1995).

36. Cohen, M. D. & Keystone, E. Rituximab for rheumatoid arthritis. *Rheumatol. Ther.* **2**, 99–111 (2015).

37. Smolen, J. S. & Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat. Rev. Rheumatol.* **11**, 276–289 (2015).

38. Winthrop, K. L. Infections and biologic therapy in rheumatoid arthritis: our changing understanding of risk and prevention. *Rheum. Dis. Clin. North Am.* **38**, 727–745 (2012).

39. Pizzolla, A., Wang, K. & Holmdahl, R. A glucose-6-phosphate isomerase peptide induces T and B cell-dependent chronic arthritis in C57BL/10 mice: arthritis without reactive oxygen species and complement. *Am. J. Pathol.* **183**, 942–953 (2014).

40. Grabbherr, M. G. et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652 (2011).

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42. Author contributions

U.N. together with R.H. designed the overall study, performed, planned, analysed the data, and wrote the manuscript. C.R. performed and analysed the linkage analysis and ChiP sequencing and reviewed the manuscript. F.F., L.M., J.T., K.K., M.B., J.B., M.Y., K. S., M.B., M.Y.B., G.F.L., J.J., and L.B. performed experiments and reviewed the manuscript. D.E. performed the bioinformatic analyses and reviewed the manuscript. I.G. and N.H. provided advice and reviewed the manuscript. R.H. supervised the project.

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