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Immunization with Glucose-6-Phosphate Isomerase Induces T Cell-Dependent Peripheral Polyarthritis in Genetically Unaltered Mice¹

David Schubert,^{2*} Bert Maier,^{2*} Lars Morawietz,[†] Veit Krenn,[†] and Thomas Kamradt^{3*}

Rheumatoid arthritis is a chronic inflammatory disease primarily affecting the joints. The search for arthritogenic autoantigens that trigger autoimmune responses in rheumatoid arthritis has largely focused on cartilage- or joint-specific Ags. In this study, we show that immunization with the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (G6PI) induces severe peripheral symmetric polyarthritis in normal mice. In genetically unaltered mice, T cells are indispensable for both the induction and the effector phase of G6PI-induced arthritis. Arthritis is cured by depletion of CD4⁺ cells. In contrast, Abs and FcγR⁺ effector cells are necessary but not sufficient for G6PI-induced arthritis in genetically unaltered mice. Thus, the complex pathogenesis of G6PI-induced arthritis in normal mice differs strongly from the spontaneously occurring arthritis in the transgenic K/B × N model where Abs against G6PI alone suffice to induce the disease. G6PI-induced arthritis demonstrates for the first time the induction of organ-specific disease by systemic autoimmunity in genetically unaltered mice. Both the induction and effector phase of arthritis induced by a systemic autoimmune response can be dissected and preventive and therapeutic strategies evaluated in this model. *The Journal of Immunology*, 2004, 172: 4503–4509.

The autoantigen(s) that are recognized in chronic inflammatory arthritides such as rheumatoid arthritis (RA)⁴ are unknown. Traditionally, the search for arthritogenic autoantigens has focused on cartilage- or joint-specific Ags. The most commonly used murine model of RA is collagen type II-induced arthritis (CIA) (1–3) although there is a lack of solid evidence for collagen as a pathogenetically relevant autoantigen, diagnostic marker, or therapeutic target in RA (4). In a more recently developed model, TCR transgenic K/B × N mice spontaneously develop autoimmune arthritis (5). Arthritis development depends on the recognition of the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (G6PI) first by the transgenic T cells and then by Abs (6). Once the Abs are produced, T cells are no longer necessary for arthritis pathogenesis and transfer of serum or combinations of monoclonal anti-G6PI Abs derived from arthritic K/B × N mice can induce arthritis in recipient mice (6, 7). However, a transgenic model is somewhat removed from the clinical situation and it is currently unclear whether G6PI or other systemically expressed autoantigens are relevant to human autoimmune diseases (8–12).

In this study we report that immunization with heterologous G6PI in adjuvant induces symmetric polyarthritis of the small distal joints in genetically susceptible normal mice. CD4⁺ T cells are necessary both for the induction and the effector phase of the disease. Abs alone do not transfer disease; however, effector cells bearing the activating receptors FcγRI or III are crucial for disease induction and the arthritis severity is down-modulated by signaling through the inhibitory FcγRIIB. Thus, G6PI-induced arthritis in normal mice provides a new model to study arthritis pathogenesis and bridges the gap between transgenic mouse models and the clinical situation.

Materials and Methods

Mice

AKR, C57BL/6, B10.A, BALB/c, and DBA/1 were bred and maintained under specific pathogen-free conditions in our animal facilities. B10.Q and SWR mice were obtained from The Jackson Laboratory (Bar Harbor, ME). DBA/1 mice deficient for the FcγR^{-/-} common γ-chain or the FcγRIIB^{-/-} (13) were from S. Kleinau (Uppsala University, Uppsala, Sweden). All animal experiments were performed according to institutional and state guidelines.

G6PI cloning and preparation

Human G6PI cDNA was amplified from the vector pGEX-5X-3 (from H. Kanno, Tokyo Women's Medical University, Tokyo, Japan; Ref. 14). Mouse G6PI cDNA was obtained from mouse heart cDNA using 5' 5'-ACTGACATATGGCTGCGCTCACCC-3' and 3' 5'-ACTGAAGCTTATCTAGTTTGGTGTCCC-3' primers. G6PI cDNA fragments were introduced into a modified pQE100 expression vector (from M. Rosowski, Deutsches Rheumaforschungszentrum, Berlin, Germany) for expression of His-tagged proteins in *Escherichia coli* strain BI21. Expression in bacteria cultures was induced for 3 h at OD₆₀₀ 0.7 with 2 mM isopropyl-β-D-thiogalactopyranoside. Bacteria were lysed using a French press, and the supernatant was subjected to purification over a Ni-NTA column (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Purity was checked by a standard SDS gel.

Induction and scoring of arthritis

Mice were immunized s.c. with 250–450 μg recombinant human G6PI (rhu G6PI) in CFA (Sigma-Aldrich, Taufkirchen, Germany). The indicated amount of Ag was mixed with CFA in a 1:1 ratio (v/v) and emulsified by

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⁴ Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen type II-induced arthritis; G6PI, glucose-6-phosphate isomerase; rhu G6PI, recombinant human G6PI; rmu G6PI, recombinant murine G6PI; SI, stimulation index; wt, wild type.

sonification. One hundred microliters of the emulsion were given s.c. at each side of the base of the tail. The clinical index was evaluated over time for each paw independently. A score of 0 indicates no clinical signs of arthritis, 1 indicates slight swelling and redness of the paw. A massive swelling and redness gives the maximal score of 2. A trained observer who was blinded to the immunization status of the mice performed the scoring.

Histopathology

Mouse legs were fixed in 10% formalin and then decalcified with EDTA at 56°C. Tissue samples were paraffin embedded, and microsections of 1–2 µm were made. H&E stainings were performed according to standard procedures. Five criteria were taken into account for histopathological assessment of joint inflammation: 1) synovitis, 2) peri-arthritis, 3) tenosynovitis, 4) periostitis, and 5) cartilage/bone destruction. Each criterion was graded according to the synovitis score for human tissue (Ref. 15 and www.charite.de/ch/patho/Webpage/pages/forschung/arbeitsgruppen/ag-krenn/index.htm).

Ig purification and transfer

Sera from arthritic mice were pooled 14–18 days after immunization with rhu G6PI, and poured over a protein G column to purify IgG. Indicated amounts of IgG were adjusted to 250 µl with PBS and were injected i.p. on days 0 and 2.

Proliferation assays

Mice were sacrificed at the indicated time points and inguinal lymph nodes were taken. Single-cell suspensions were made in RPMI 1640 with glutamine (PAA Laboratories, Coelbe, Germany) supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 mg/ml), and 50 µM 2-ME. Cells were plated into 96-well round-bottom microtiter plates with a density of 2×10^5 cells/well. They were restimulated in triplicates with either 10 µg/ml rhu G6PI or PBS at 37°C in 5% CO₂ for 72 h. After 54 h, 1 µCi/well of [³H]thymidine (Amersham Pharmacia Biotech, Freiburg, Germany) was added. [³H]thymidine incorporation was measured with a β-scintillation counter. Results were displayed as the stimulation index (SI), which is the quotient of the mean counts of cells cultured with rhu G6PI and the mean counts of cells cultured in medium alone.

Analysis of cytokine production by flow cytometry

Analysis of intracellular cytokines and surface markers was performed as described (16). FITC-conjugated anti-TNF-α mAb (MP6-XT22, IgG1) and anti-mouse-IFN-γ mAb (XM61.2, IgG1); PE-conjugated anti-mouse-IL-4 mAb (11B11, IgG1), anti-mouse-IL-6 mAb (MP5-20F3, IgG1) and anti-mouse-IL-17 mAb (TC11-18H10.1, IgG1); APC-conjugated anti-mouse-IL-2 mAb (JES6-5H4, IgG2b) and anti-mouse-IL-10 mAb (JES5-16E3, IgG2b) and FITC- and PE-conjugated rat-IgG1 isotype control mAb (R3-34) and APC-conjugated rat-IgG2b isotype control mAb (A95-1) were purchased from BD Biosciences (Heidelberg, Germany). Anti-CD16/CD32 mAb (2.4G2) and anti-CD4 mAbs (YTS191.1 and GK1.5) were purified from hybridoma supernatants with protein G-Sepharose. Anti-CD4 mAb (GK1.5) was biotinylated according to standard protocols. Samples were analyzed by four-color cytometry on a FACSCalibur (BD Biosciences).

ELISA

Five micrograms per milliliter either rhu G6PI or recombinant murine G6PI (rmu G6PI) in PBS was coated on ELISA microtiter plates (Corning, Wiesbaden, Germany) overnight at 4°C. Ab titers were defined using 4-fold serial dilutions of the sera (1:100 to 1:409,600) as described (9). The titer was defined as the last dilution that gave an OD, which was at least 3-fold higher than that of the background. For the determination of the isotypes of the G6PI-specific Abs, goat anti-mouse IgG1, IgG2a, IgG2b, IgG3, and IgM-specific Abs were used as secondary Ab (Sigma-Aldrich). Binding of the secondary Abs was detected using a peroxidase-conjugated donkey anti-goat-IgG (1:3000) (Santa Cruz Biotechnology, Santa Cruz, CA). Titer was defined as written above.

Treatment with Abs and soluble receptors

CD4⁺ T cells were depleted by i.p. injection of 300 µg of anti-CD4 mAb (YTS191.1) (17) in 200 µl of PBS at the indicated time points. Efficiency of depletion was >90% (data not shown). Control mice were treated with 300 µg of rat IgG (Dianova, Hamburg, Germany) in PBS. To investigate the role of TNF-α, DBA/1 mice were immunized s.c. with 400 µg of rhu G6PI in CFA and were treated with the soluble dimeric human TNFR p75-IgG-Fc fusion protein (Etanercept; Immunex, Seattle, WA). Mice were treated with either 100 µg of TNFR p75 adjusted to 200 µl with PBS

from days 0 to 9 and then every third day until day 27 or with 30 µg of TNFR p75 adjusted to 200 µl of PBS daily until day 25 by i.p. injection. Mice were examined daily for clinical signs of arthritis over 30 days.

Results

G6PI immunization induces severe symmetric polyarthritis in genetically susceptible normal mice

We immunized normal mice with rhu G6PI and scored arthritis development. In DBA/1 mice (H-2^q haplotype) the incidence of arthritis was >90% in multiple independent experiments. Other strains, including the H-2^q strains B10.Q and SWR were resistant (Table I). G6PI immunization induced severe swelling of both the hind and front paws (Fig. 1A–D). Clinical signs of arthritis first appeared 9 days after immunization, rapidly progressed, reached a maximum around day 15, and then resolved slowly (Fig. 1E). Immunization of DBA/1 mice with rmu G6PI induced arthritis with approximately the same incidence and clinical severity as immunization with rhu G6PI (our unpublished observations). Histopathological analyses revealed that G6PI-injected DBA/1 mice had severe symmetric arthritis of the distal joints. At the front limbs, the wrists, metacarpal joints, and the proximal and distal interphalangeal joints were affected. At the lower limbs, the tarsal and ankle joints and knee joints were affected. Neither the spine nor the elbow, shoulder, or hip joints were affected in any of the mice analyzed. The histopathological findings were maximal at day 15. Similar to RA, severe synovitis, tenosynovitis, sometimes even with rupture of tendons, and destruction of bone and cartilage were detectable in almost all affected joints. In addition, noncellular markers of inflammation such as fibrin extravasation occurred (Fig. 1F–J). At later points, there was little if any inflammatory activity, instead regenerative processes and fibrosis started to occur (Fig. 1K). There were no pathological findings in the CNS, heart, lung, liver, spleen, pancreas, kidney or skeletal muscle in any of the G6PI-immunized mice. We also immunized DBA/1 mice with denatured G6PI. These mice developed arthritis with the same kinetics and severity as mice immunized with native, enzymatically active G6PI (data not shown). In contrast, immunization with recombinant murine creatine kinase in CFA or fructose-1,6-bisphosphatase in CFA, which were produced in the same expression system as G6PI, did not induce clinical or histopathological signs of arthritis (our unpublished observations).

Cellular immune responses against G6PI

DBA/1 mice mounted maximal proliferative responses to rhu G6PI with SI of ≈7 on days 9 and 12 after immunization. In other mouse strains the SI rarely exceeded 2 and were <4 at all time points examined (Fig. 2A). Thus, only the DBA/1 mice, which are susceptible to G6PI-induced arthritis, developed a sustained proliferative immune response against G6PI. A small percentage of the CD4⁺ cells from the draining lymph nodes or spleen of G6PI-immunized DBA/1 mice produced the proinflammatory cytokines

Table I. Incidence of GPI-induced arthritis in inbred strains of mice

Strain	H-2 Haplotype	Arthritis Incidence
C57BL/6	b	0/8
BALB/c	d	0/8
DBA/2	d	0/11
AKR	k	0/8
B10.A	k	0/8
DBA/1	q	168/185
B10.Q	q	0/8
SWR	q	0/10

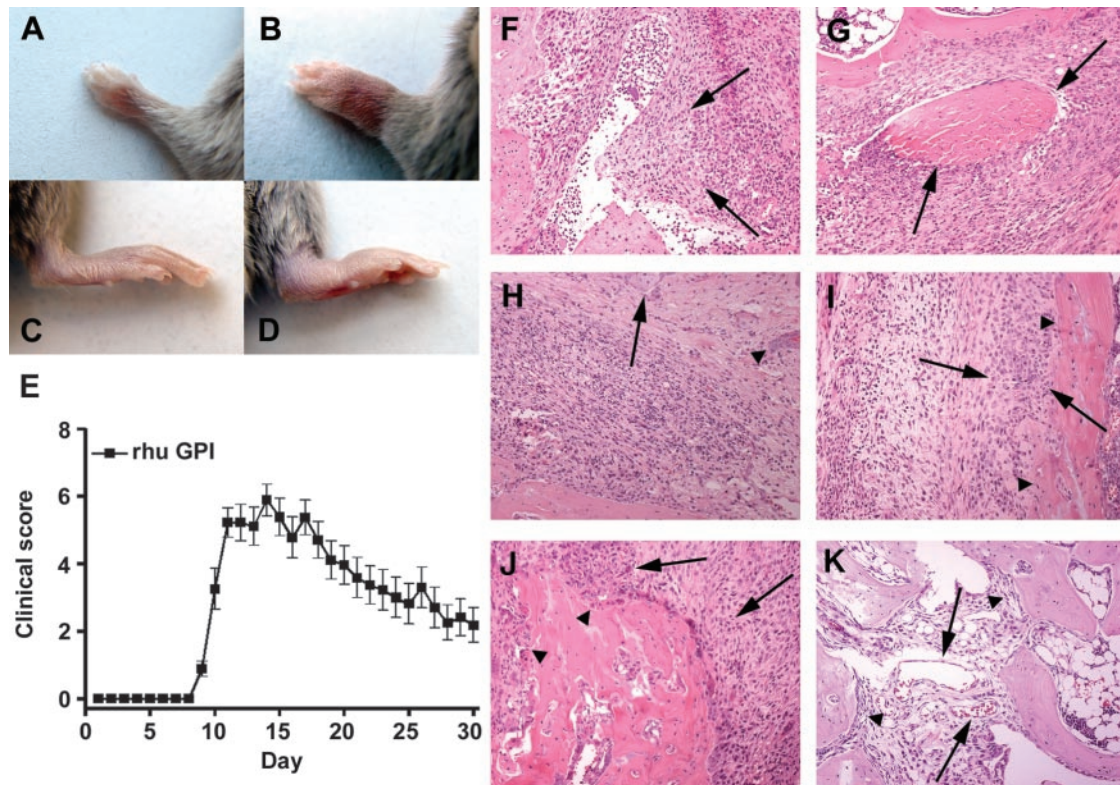


FIGURE 1. Clinical course and histopathology of G6PI-induced arthritis. *A–E*, DBA/1 mice were immunized with rhu G6PI in CFA and arthritis was evaluated macroscopically over 30 days. Front and hind limbs from a G6PI-immunized (*B* and *D*) or control (*A* and *C*) DBA/1 mouse. *E*, Clinical score (mean \pm SEM) of 27 mice immunized with rhu G6PI. *F–K*, Histological analysis of G6PI-induced arthritis. *F*, Severe synovitis. Dense inflammatory infiltrates consisting of lymphocytes, plasma cells, and granulocytes within the synovial membrane (arrows) accompanied by strong activation of resident cells (fibroblasts, macrophages). *G*, Tenosynovitis. A dense inflammatory infiltrate around a tendon (arrows). *H*, Periarthritis with strong inflammatory reaction in s.c. tissue adjacent to the talocalcaneal joint. Arrow, sebaceous gland; arrowhead, hair follicle. *I*, Periostitis with strong stroma activation and moderate inflammatory infiltrate inside the periost (between arrows) adjacent to the shank (arrowheads). *J*, Bone destruction (arrowheads) mediated by pannus (arrows), consisting of strongly activated fibrous tissue. *K*, Regenerative process with fibroblastic synovial tissue containing small blood vessels (arrows) and osteoblasts repairing bone destruction (arrowheads) (H&E staining: original magnification, *F* and *H*, $\times 100$; *G*, *I*, *J*, and *K*, $\times 200$).

TNF- α ($\sim 0.5\%$), IL-17 ($\sim 0.15\%$), or IL-6 ($\sim 0.1\%$) upon in vitro stimulation with G6PI (Fig. 2, *B* and *C*). IL-6 expressing CD4⁺ cells were more frequent in the spleen ($\sim 0.5\%$) than in the lymph nodes. Very few, if any, CD4⁺ cells expressed IFN- γ , IL-2, IL-4, or IL-10 (Fig. 2*C*).

Depletion of CD4⁺ cells cures G6PI-induced arthritis

To determine the pathogenic relevance of the anti-G6PI T cell responses we injected DBA/1 mice i.p. with either 0.3 mg of the depleting rat anti-mouse anti-CD4 mAb YTS191.1 (17) or polyclonal rat IgG. Treatment with anti-CD4 mAb on days -3 , 0, and 5 prevented arthritis development (Fig. 3*A*). Ab treatment at days 6 and 9, i.e., at time points when immune responses and histological alterations were already evident, also prevented clinical arthritis (Fig. 3*A*). When mice were treated at days 8 and 11 there was only very mild arthritis (score 1) in only two of five mice (Fig. 3*A*). Injection of anti-CD4 mAbs at days 11 and 14 after G6PI-immunization, when clinically overt arthritis was already present, induced rapid and stable resolution of arthritis (Fig. 3*A*). Thus, CD4⁺ T cells are required for both the induction and the effector phase of G6PI-induced arthritis.

Arthritis-susceptible and arthritis-resistant mouse strains produce high titers of anti-G6PI Abs

Abs against both human G6PI and murine G6PI were detectable in the serum of DBA/1 mice already 6 days after the immunization

(Fig. 4*A*). The anti-G6PI titers reached their maximum at day 9, and remained almost constant up to day 30 when the clinical disease had already resolved. Low titers of IgM and IgG1 anti-rhu G6PI Abs were already detectable at day 6 after immunization. Anti-rhu G6PI Abs of the IgG2a, IgG2b, IgG3, or IgM isotypes were detectable from day 9 after immunization. Throughout the observation period IgG1 Abs that are the pathogenic Abs in the K/B \times N model (7) and IgG2 Abs that are pathogenic in CIA (2) were detectable in high titers (Fig. 4*B*). Arthritis-resistant mouse strains also produced high titers of Abs against human (Fig. 4*C*) and murine G6PI (Fig. 4*D*).

Abs are not sufficient for the pathogenesis of G6PI-induced arthritis

The Ab response against G6PI was significantly decreased after early depletion of CD4⁺ cells. In contrast, anti-G6PI Ab titers in mice that had received anti-CD4 treatment at days 6 and 9 were not different from controls (Fig. 3*B*). These mice did not have any clinical or histological signs of arthritis despite high Ab titers. To assess the pathogenicity of anti-G6PI Abs further we pooled sera from arthritic mice 14–18 days after G6PI-immunization, purified IgG, and transferred different amounts of IgG at 2 consecutive days into recipient DBA/1 mice. Transfer of 2×1 mg IgG (four mice), 2×2 mg (four mice), or even 2×5 mg (three mice) did not induce arthritis. Histological analyses revealed no signs of arthritis in the recipients. Thus, in contrast to the TCR transgenic

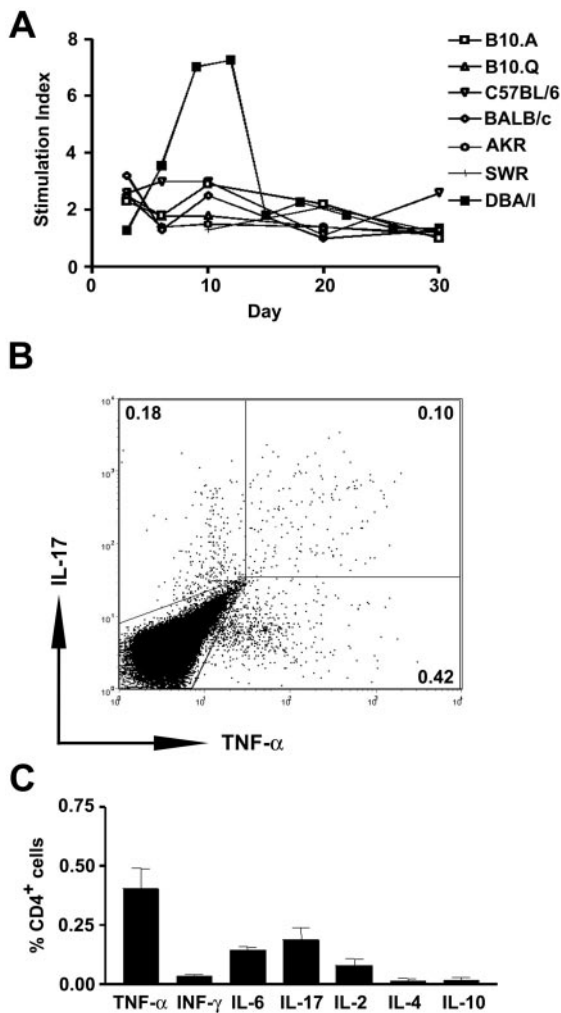


FIGURE 2. T cell responses against G6PI. *A*, Proliferation. At the indicated time points after G6PI immunization, single-cell suspensions prepared from the draining lymph nodes of three (DBA/1) or two (all other strains) mice. Cells were cultured with or without the addition of rhu G6PI for 72 h with [³H]thymidine added for the last 18 h. The values represent stimulation indices as detailed in *Materials and Methods*. *B* and *C*, Cytokine production. Single-cell suspensions were prepared from the draining lymph nodes and spleens 11 days after G6PI immunization, cultured in the presence of G6PI and stained with mAb against CD4. *B*, After fixation and permeabilization, cells were stained for intracellular IL-17 and TNF- α . *C*, The percentages of IL-2, IL-4, IL-6, IL-10, IL-17, TNF- α , and IFN- γ expressing CD4⁺ cells (mean \pm SEM) are summarized for the draining lymph nodes. Data are representative of three different mice.

K/B \times N mice, the DBA/1 mice produce anti-G6PI Abs, which alone are not sufficient to induce arthritis.

Abs are necessary for G6PI-induced arthritis

Next, we immunized DBA/1 mice lacking either the Fc γ R common γ -chain or the Fc γ RII (13) with G6PI. Only 8 of 24 DBA/1 mice deficient for the common γ -chain developed mild arthritis that was much less severe than in the control DBA/1 mice (Fig. 5A). In contrast, all of the DBA/1 mice lacking the inhibitory Fc γ RIIB developed arthritis and both the clinical score and the duration of the disease were more pronounced than in the DBA/1 control mice (Fig. 5A). The histological findings, too, were much more severe in the Fc γ RIIB-deficient mice than in wild-type (wt) animals (Fig. 5B). Ab titers against both human and murine G6PI were similar in Fc γ R γ -chain-deficient mice, the Fc γ RIIB-defi-

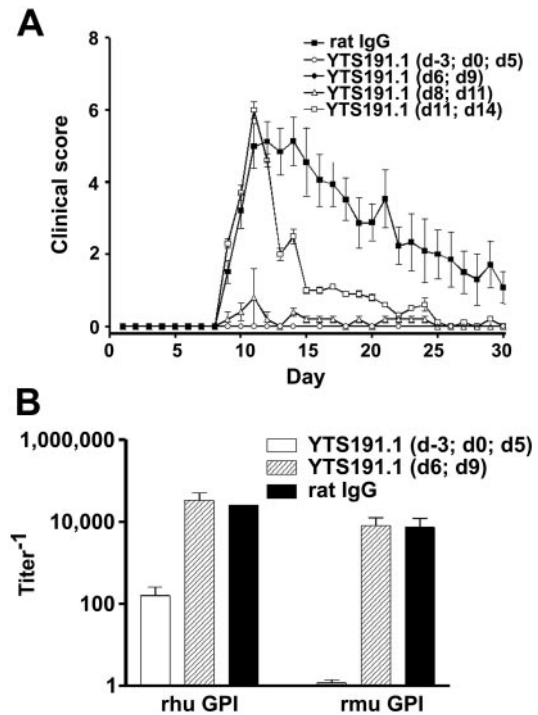


FIGURE 3. Depletion of CD4⁺ cells. *A*, DBA/1 mice were immunized with G6PI at day 0 and received i.p. injections of 300 μ g of polyclonal rat IgG (■, showing the mean of 10 mice) or the CD4-depleting mAb YTS191.1 at days -3, 0, 5 (○, showing the mean of 10 mice); days 6 and 9 (●, showing the mean of 10 mice); days 8 and 11 (△, showing the mean of 5 mice); or days 11 and 14 (□, showing the mean of 10 mice). Arthritis development was scored over 30 days. Ab titers in mice that were G6PI-immunized and treated with anti-CD4 or control IgG. *B*, DBA/1 mice were immunized with G6PI at day 0 and received i.p. injections of 300 μ g of polyclonal rat IgG (■) or the CD4-depleting mAb YTS191.1 at either days -3, 0, 5 (□) or 6 and 9 (▨). Ab titers against G6PI were determined by ELISA on day 23. The highest serum dilution where anti-G6PI Abs were still detectable is shown on the y-axis. Data represent mean \pm SEM from at least five different mice per time point.

cient mice, and DBA/1 control mice (data not shown). Thus, Fc γ R⁺ effector cells and therefore IgG Abs are crucial for the induction of G6PI-induced arthritis.

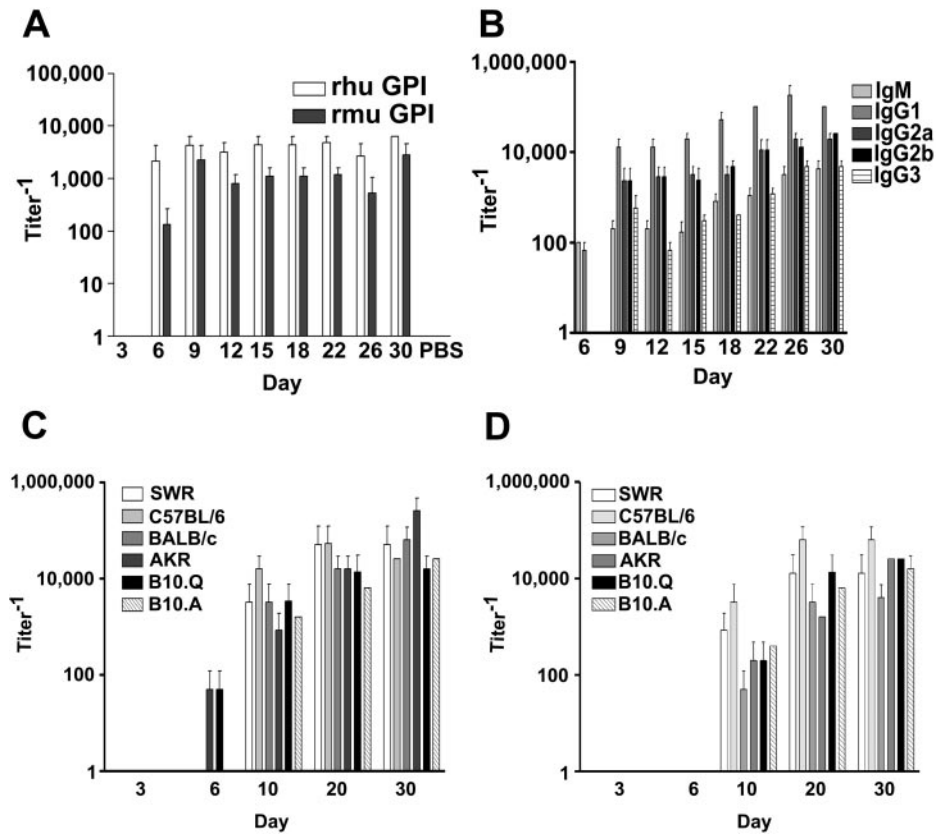
TNF- α is necessary for G6PI-induced arthritis

G6PI-specific Th cells produce TNF- α upon antigenic stimulation in vivo. TNF- α is an important effector-cytokine in the pathogenesis of RA and several murine arthritis models (18, 19). We asked whether TNF- α was critical to the pathogenesis of G6PI-induced arthritis. Injection of 100 μ g of the soluble dimeric TNFR p75-IgG-Fc fusion protein (Etanercept; Immunex) i.p. daily from days 0 to 9 and every third day thereafter until day 27 completely blocked arthritis development (Fig. 6). The effect was dose-dependent as treatment with 30 μ g of the TNFR p75 was only partially effective in reducing the incidence and severity of G6PI-induced arthritis (data not shown).

Discussion

The etiology and pathogenesis of chronic inflammatory arthritides such as RA are ill defined. Two transgenic mouse models have challenged the concept that organ-specific diseases are initiated by autoimmune responses against organ-specific Ags. In the K/B \times N model, arthritis is triggered by an autoimmune response against G6PI (5, 6). In the other model, autoimmune peripheral neuritis is

FIGURE 4. Abs against G6PI. At the indicated time points after G6PI immunization, three mice were sacrificed, and serum was taken, serially diluted, and assayed by ELISA for the presence of Abs against rhu and rmu G6PI. The highest serum dilution where anti-G6PI Abs were still detectable is shown on the y-axis. Data represent mean \pm SEM from at least three different mice per time point for anti-human (open bars) or anti-murine (filled bars) total IgG (A) or several different isotypes of Abs against rhu G6PI (B). Mice of the indicated strains were immunized and ELISAs were performed to detect Abs against rhu G6PI (C) or rmu G6PI (D).



triggered by autoreactive CD4⁺ T cells that recognize I-A^b/E α ₅₂₋₆₈ (20). In this study, we describe a model where arthritis develops in genetically unmanipulated mice upon immunization with a ubiquitously expressed self-antigen. This brings up several questions: How does systemic autoimmunity induce arthritis? Why is G6PI the target of a pathogenic autoimmune response? How relevant is this model to understanding the pathogenesis of human autoimmune diseases such as RA?

How does systemic autoimmunity induce arthritis?

Systemic autoreactivity against G6PI induces symmetric polyarthritis upon G6PI immunization in DBA/1 mice and spontaneously in the transgenic K/B \times N model. The clinical features are similar but the pathogenesis is strikingly different in the two models. One major difference between the two models is the differential requirement for CD4⁺ T cells. In genetically unaltered mice, CD4⁺ T cells are critical for the pathogenesis of G6PI-induced arthritis throughout the effector phase. CD4 depletion cures G6PI-induced arthritis in DBA/1 mice. Thus, G6PI-induced arthritis in normal mice is clearly a T cell-dependent disease. In contrast, T cells are dispensable both in the K/B \times N model (6) and in CIA (21) once the arthritogenic Abs have been produced. The incidence and severity of CIA is unaltered in DBA/1 mice that lack CD4⁺ T cells (22).

The second major difference between G6PI-induced arthritis on the one side and CIA or the K/B \times N model on the other is the different role of Abs in the pathogenesis of arthritis. Similar to earlier findings in CIA (2, 23, 24), adoptive transfer of serum, purified IgG, or mAbs from arthritic K/B \times N mice induces disease in recipient mice (6, 7, 25). In contrast to these models, transfer of serum or purified IgG, even in large amounts, from DBA/1 with G6PI-induced arthritis does not induce clinical or histological signs of arthritis in recipient mice. Moreover, arthritis-resistant

mouse strains produce high titers of anti-G6PI IgG just as the susceptible DBA/1 mice. Finally, G6PI-induced arthritis resolves in DBA/1 mice despite the presence of high titers of anti-G6PI Abs and depletion of CD4⁺ cells cures arthritis but does not affect Ab titers. Thus, in contrast to both CIA and the K/B \times N model, Abs alone are not sufficient for the pathogenesis of G6PI-induced arthritis in normal mice. Nonetheless, Abs are necessary as Fc γ R common γ -chain-deficient DBA/1 mice are protected from G6PI-induced arthritis whereas Fc γ RII-deficient mice suffer from an exaggerated arthritis.

Why, then, does transfer of IgG from arthritic DBA/1 mice not induce arthritis in the recipients? It is possible that only a fraction of the anti-G6PI Abs produced in the DBA/1 mice are arthritogenic. Important criteria include the isotype, specificity, and avidity of the Abs. The arthritogenicity of Abs depends on their isotype in the K/B \times N and CIA models. Whereas IgG2 Abs are required to induce arthritis in the CIA model (24, 26), arthritogenicity is exclusively linked with IgG1 Abs in the K/B \times N model (7). In both models the combination of Abs with different specificities is necessary to transfer arthritis (7, 24). Thus, although we transferred large amounts of purified Ig from the arthritic DBA/1 mice, it is still possible that a combination of Abs of a specific isotype, recognizing particular—and perhaps different—epitopes, might be able to transfer G6PI-induced arthritis in our model. This possibility is currently addressed in studies using a panel of monoclonal anti-G6PI Abs from DBA/1 mice.

The expression of Fc γ R is tightly regulated and cytokines produced by Th cells, including IFN- γ and TNF- α , are known to modulate Fc γ R expression (27, 28). Upon antigenic stimulation the G6PI-specific Th cells produce TNF- α , IFN- γ , IL-6, and IL-17. These cytokines are key mediators both in RA and in different murine models thereof (18, 29). Therefore, Th cells not only help B cells to produce Abs but also have additional critical functions,

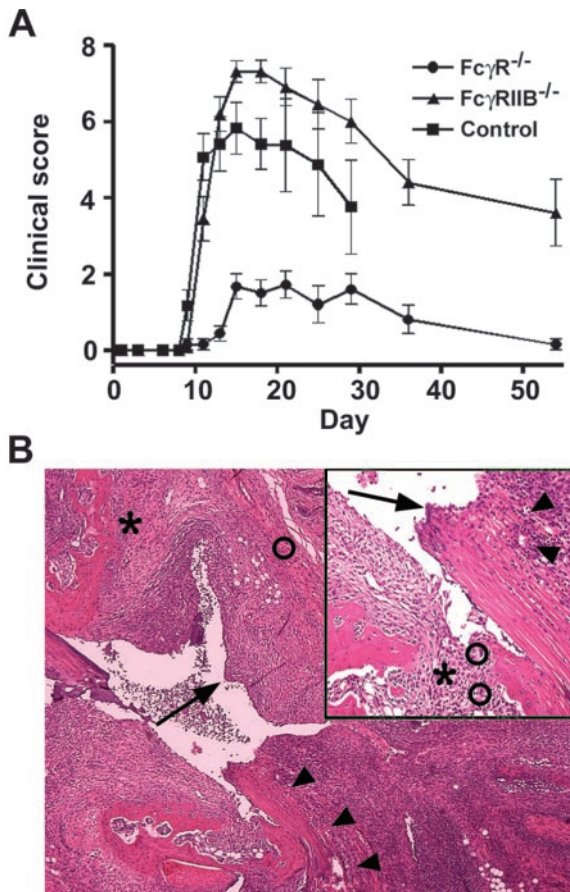


FIGURE 5. $Fc\gamma R$ are critical for G6PI-induced arthritis. **A**, DBA/1 wt (■) or DBA/1 $Fc\gamma R$ common γ -chain-deficient (●) or DBA/1 $Fc\gamma RIIB$ -deficient mice (▲) were immunized with rhu G6PI and arthritis was evaluated macroscopically over 54 days. Data represent mean clinical scores \pm SEM of those mice that developed arthritis. Arthritis incidence was 10 of 11 in DBA/1 wt, 8 of 24 in $Fc\gamma R$ common γ -chain-deficient, and 16 of 16 in $Fc\gamma RIIB$ -deficient mice. Histological analysis was performed on day 21. **B**, Overview shows an H&E staining ($\times 100$ magnification) of a highly inflamed joint of an $Fc\gamma RIIB^{-/-}$ mouse with a high-grade arthritis including synovialitis (arrow), periostitis (*), tenosynovitis (arrowhead), and periarthritis (O). The *inset* depicts a section of the overview ($\times 200$ magnification) showing multinucleated osteoclasts (O), disrupted tendon with necrotic tissue (arrow), and a very dense inflammatory infiltrate (arrowhead); and pannus formation (*) consisting of fibroblasts, lymphocytes, mast cells, neutrophil granulocytes, and macrophages resulting in a complete destruction of bone tissue.

perhaps in modulating effector cell functions such as the expression of $Fc\gamma R$. This complexity distinguishes G6PI-induced arthritis from models such as CIA (2), K/B \times N serum transfer (6, 25), Ag-induced arthritis (30), or immune complex induced arthritis (31) where Abs but not T cells mediate the effector phase. The pathogenesis of G6PI-induced arthritis is also distinct from adjuvant induced arthritis, which can be transferred to naive recipients with Th cells without need for Abs (3).

Why is G6PI the target of a pathogenic autoimmune response?

G6PI is identical with neuroleukin, autocrine motility factor, and maturation factor (32). It has been reported to be secreted by lectin-stimulated T cells and to induce Ig synthesis in cultured human PBMC (33), stimulate cell migration (32), and stimulate the differentiation of hemopoietic cells (32). Each of these functions might enhance the immune response to G6PI. However, immuni-

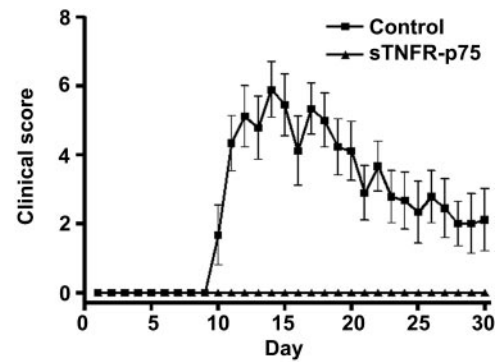


FIGURE 6. TNF- α blockade prevents G6PI-induced arthritis. DBA/1 mice were immunized with G6PI at day 0 and received i.p. injections of 100 μ g of the soluble TNFR p75 in 200 μ l PBS i.p. (▲, 0 of 10 mice developed arthritis). The clinical course of treated mice and control mice (■, showing the mean \pm SEM of 9 of 11 mice) was followed over 30 days. The experiment was performed twice with similar results.

zation with denatured G6PI also induces arthritis, indicating that G6PI's biological functions are irrelevant to its antigenicity. All cells express G6PI and small amounts of G6PI are present in the serum. It is likely that some APC will present G6PI to T cells. Clearly, however, this is not a sufficient condition to induce systemic autoimmunity: immunization with fructose-1,6-bisphosphatase, a key enzyme in gluconeogenesis, does not induce arthritis or any other clinical symptoms of autoimmunity in mice (our unpublished observations). Cationic Ags such as G6PI can easily bind to the negatively charged structures of the joint (34–36). This could lead to a locally increased concentration of G6PI, exceeding a critical threshold necessary to set off a sustained immune response. Support for this possibility comes from recent findings in the K/B \times N model. There, the B cell response against the ubiquitously expressed G6PI was initiated in and focused to the lymph nodes draining the distal joints (37). Other joint-specific factors likely contribute to the pathogenesis of G6PI-induced arthritis. One such factor may be the tissue-specific regulation of the expression of cytokines and other effector molecules. For example, in DBA/1 mice transgenic for human *tnf- α* , human TNF- α is expressed in the synovial lining cell layer but not in resting macrophages, T cells, or B cells of the spleen or lymph nodes (38).

Is G6PI-induced arthritis relevant for patients?

An initial publication reported the detection of Abs against G6PI in >60% of RA patients (8). To date, these findings have not been reproduced by others despite extensive efforts (9–12) and it is most likely that the initial findings were due to contaminations present in the commercially available G6PI preparation (9). Although it is unlikely that G6PI is a relevant autoantigen for any form of chronic inflammatory arthritis in patients, other ubiquitously expressed self-antigens may well be. Thus far, there has been a gap between the two transgenic models where either arthritis (5, 6) or peripheral neuritis (20) are induced by systemic autoimmunity and a number of clinical situations where Abs against ubiquitously expressed Ags are highly specific for certain organ-specific autoimmune diseases. Prominent examples include the association of primary biliary cirrhosis with antimicrobial Abs that recognize the E2 subunit of mitochondrial pyruvate dehydrogenase (39) and the association of antineutrophil cytoplasmic Abs targeting proteinase 3 with Wegener granulomatosis (40). These Abs are highly specific and sensitive diagnostic markers for the respective disease; however, their pathogenic significance remains unknown partly due to the lack of suitable animal models.

In summary, G6PI-induced arthritis demonstrates for the first time the induction of organ-specific disease induced by systemic autoimmunity in genetically unaltered mice. The complex interactions between cells of the adaptive and innate immune system, which are likely to be also mandatory for the pathogenesis of multifaceted human diseases such as RA, can be studied and modulated in this model system.

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