

# Human antibodies induce arthritis in mice deficient in the low-affinity inhibitory IgG receptor Fc $\gamma$ RIIB

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**Rheumatoid arthritis (RA) is a complex autoimmune disease with a poorly understood pathogenesis. The disease is associated with polyclonal B cell activation and the production of autoantibodies (autoAbs), but there is a longstanding controversy as to whether such Abs contribute to, or are secondary to, the pathogenesis of RA. To address the potential pathogenicity of human RA-associated Abs, we developed a passive transfer model involving mice deficient in the low-affinity inhibitory Fc receptor, Fc $\gamma$ RIIB. We report that plasma or serum from patients with active RA can induce inflammation and histological lesions in Fc $\gamma$ RIIB<sup>-/-</sup> mice consistent with arthritis, and that this pathogenic activity is caused by the immunoglobulin G-rich fraction. Our results suggest that humoral autoimmunity can contribute directly to autoimmune arthritis, and that Fc $\gamma$ RIIB<sup>-/-</sup> mice are a promising model to evaluate the arthritogenic potential of human autoAbs.**

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Rheumatoid arthritis (RA) is a complex autoimmune disease distinguished by chronic joint inflammation that eventually leads to destruction and remodeling of joint architecture. The arthritic histopathology is characterized by the presence of leukocytic infiltrates composed of T and B lymphocytes, macrophages, neutrophils, mast cells, and dendritic cells, all of which show signs of activation (1). However, it is not known what factors trigger the activation and recruitment of these inflammatory cells to the joint.

RA is also associated with polyclonal B cell activation and the production of autoantibodies (autoAbs), but there is a longstanding controversy as to whether such Abs contribute to, or are secondary to, the pathogenesis of RA. The earliest association of RA with autoAbs was the identification of “rheumatoid factor” (RF) present in 60–80% of RA patients (2). However, not all RA patients are “seropositive” for RF and many normal individuals with other inflammatory diseases produce RF. Association of Abs to glucose-6-phosphate isomerase (GPI) with RA (3) has also been reported; however, this association has been recently challenged (4). RA has also been associated with the pro-

duction of IgG Abs against ubiquitous antigens, such as keratin, fibrinogen, filaggrin, and cyclic citrullinated proteins (CCPs; references 5 and 6). Currently it is unclear whether human IgG autoAbs are simply a marker for or are directly involved in the pathogenesis of RA.

Animal models have provided convincing evidence that pathogenic autoAbs can contribute to autoimmune arthropathies. In collagen-induced arthritis models, transient disease can be induced by passive transfer of concentrated Ig or purified anti-collagen Abs from arthritic mouse donors into naive rodent recipients (7). In the K/BxN model (8), arthritis is directly attributed to an abundance of IgG Abs specific for GPI, which localize to distal joints of mice (9), and promotes a cascade of both complement-mediated and cellular effector events via immunostimulatory Fc receptors (10). Thus, although there is excellent evidence supporting the involvement of autoAbs in mouse models of RA, major question remains concerning the extent to which these findings can be extrapolated to human RA.

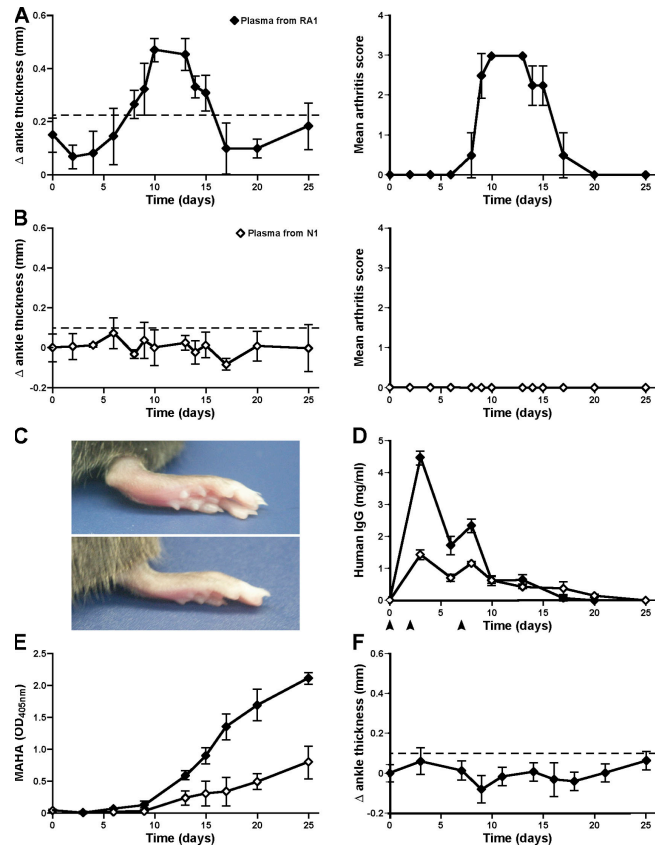
To address the potential pathogenicity of human RA-associated autoAbs, we developed a passive transfer model involving mice deficient in the low-affinity inhibitory Fc $\gamma$ RIIB

The online version of this article contains supplemental material.

receptor ( $Fc\gamma RIIB^{-/-}$ ).  $Fc\gamma RIIB$  is a monomeric inhibitory receptor that suppresses B cell, mast cell, and macrophage activation upon engagement with IgG-immune complexes and transmits inhibitory signals via its cytoplasmic immunoreceptor tyrosine-based inhibitory motif (11). Studies clearly show that  $Fc\gamma RIIB^{-/-}$  mice are hyperreactive to the transfer of pathogenic IgG (12, 13). Here, we report that plasma or serum from patients with active RA can induce inflammatory arthritis in  $Fc\gamma RIIB^{-/-}$  mice, and that this pathogenic activity is caused by the IgG-rich fraction. Our results suggest that humoral autoimmunity can contribute directly to autoimmune arthritis and that  $Fc\gamma RIIB^{-/-}$  mice are a promising model to evaluate the arthritogenic potential of human autoAbs.

## RESULTS AND DISCUSSION

Plasma from a patient with RA (RA1) and a normal donor N1 were injected i.p. into groups of 8–12-wk-old B6. $Fc\gamma RIIB^{-/-}$  mice. At this age, there is minimal evidence for lupus-like autoimmune disease reported in older B6. $Fc\gamma RIIB^{-/-}$  mice (14, 15). Mice were injected with human samples on days 0, 2, and 7 for a total of 2.5 ml plasma per mouse. Plasma from RA1 caused mild soft tissue swelling indicated by ankle thickness and an overall arthritis score in all (6/6) mice tested (Fig. 1, A and C, top). Both hind ankles were affected; however, the involvement of other joints was difficult to detect by simple visual inspection. The joint swelling was transient and resolved by day 20. In contrast, none (0/4) of the mice injected with control plasma from healthy individual N1 developed measurable ankle inflammation or swelling (Fig. 1, B and C, bottom). The transient nature of the inflammation caused by RA1 plasma is consistent with mouse serum-induced models of arthritis that generally result in more severe lesions. One explanation for the transient nature of the lesions is accelerated clearance of human Abs in recipient  $Fc\gamma RIIB^{-/-}$  mice. To test this possibility, we monitored the clearance of human IgG Abs in mice using mouse sera collected at different time points. Levels of circulating human IgG Abs in mice injected with human plasma samples from both RA1 and N1 were maximal on day 3, followed by a second peak on day 8 (Fig. 1 D). The level of human IgG in the mouse circulation gradually decreased and disappeared by day 20. A similar pattern of IgG degradation was observed in mice injected with plasma from healthy control N1; however, the level of circulating human IgGs was significantly lower at all time points compared with that from the RA patient. We then tested whether  $Fc\gamma RIIB^{-/-}$  mice developed a mouse anti-human Ab (MAHA) response, which could potentially neutralize circulating pathogenic human Abs. Plasma from both RA patient RA1 and healthy individual N1 resulted in a gradual accumulation of MAHAs after the onset of inflammation (Fig. 1 E). This temporal pattern suggests that MAHAs do not contribute to the lesions and may diminish the pathogenic activity of the human plasma. Collectively, these results provide direct evidence that plasma from a patient with RA is able to initiate acute inflammatory arthritis when injected into genetically susceptible  $Fc\gamma RIIB^{-/-}$  mice.



**Figure 1. Induction of inflammatory arthritis in  $Fc\gamma RIIB^{-/-}$  with plasma from an RA patient.** 8–12-wk-old B6. $Fc\gamma RIIB^{-/-}$  mice were injected with plasma from a patient with RA (RA1) or a healthy blood donor (N1) on days 0, 2, and 7. Arthritis was evaluated by measuring the increase in ankle thickness and arthritis score (see Materials and methods). Each solid line represents the mean  $\pm$  SEM (mm) results from both ankles. Values above the dashed line are considered statistically significant based on measuring the ankle thickness of an untreated control group of B6. $Fc\gamma RIIB^{-/-}$  mice. (A) B6. $Fc\gamma RIIB^{-/-}$  ( $n = 6$ ) mice were injected with plasma from RA1, and the animals were examined for a period of 25 d after the initial injection ( $\blacklozenge$ ). (B) B6. $Fc\gamma RIIB^{-/-}$  mice ( $n = 4$ ) injected with plasma from a healthy blood donor N1 ( $\diamond$ ). (C) Representative images of hind paws at day 14 after the initial injection with plasma from RA1 (top) and N1 (bottom). (D) Clearance of human IgG Abs in the mouse circulation during the time course of the experiment. Human blood samples were injected on days 0, 2, and 4 as indicated by arrowheads. (E) Accumulation of MAHAs was more pronounced in recipients of plasma from RA1 as compared with control individual N1. (F) B6. $Fc\gamma RIIB^{+/+}$  mice ( $n = 3$ ) injected with plasma from RA1 did not develop arthritis when injected with the same volume (2.5 ml/mouse) of arthritogenic RA1 plasma.

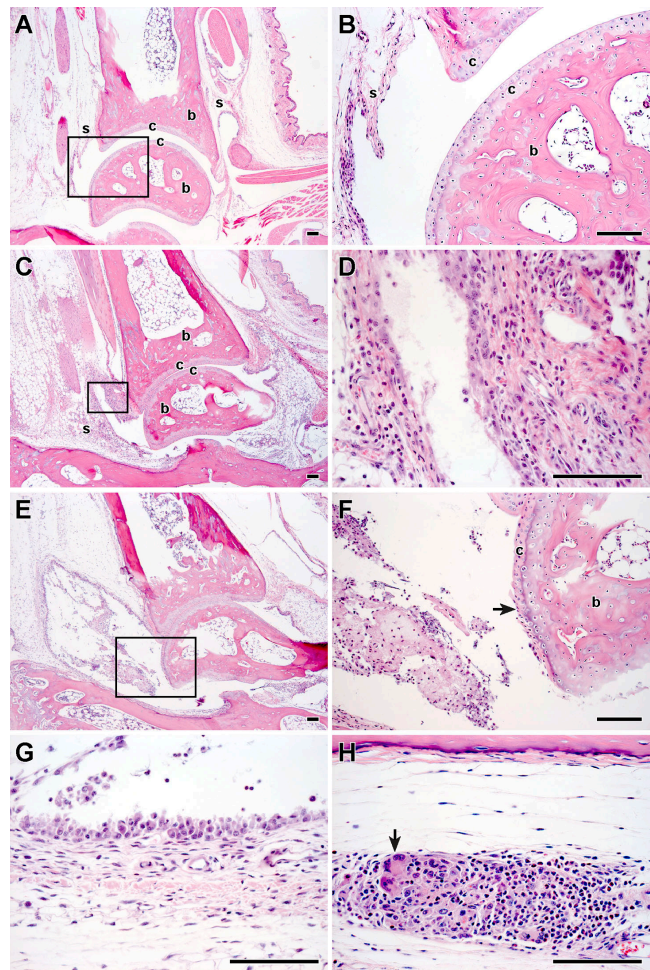
To test whether the absence of the inhibitory receptor  $Fc\gamma RIIB$  is necessary for arthritis initiation, age-matched B6 mice ( $n = 3$ ) were injected with the same dose of plasma from RA1 (2.5 ml/mouse). As illustrated in Fig. 1 F, no mice (0/3) showed joint inflammation. This result demonstrates that  $Fc\gamma RIIB$  deficiency is required for mice to become susceptible to arthritis induced by human RA plasma.

Histological analysis was performed to determine whether lesions could be detected in the joints of  $Fc\gamma RIIB^{-/-}$  mice

injected with arthritogenic human plasma (Fig. 2). We observed a normal synovial lining, acellular joint space, and smooth articular cartilage in the ankle joints of mice ( $n = 2$ ) injected with normal plasma N1 and killed on day 11 after initial injection (Fig. 2, A and B). In contrast, the joints of mice injected with plasma from RA1 and subsequently killed on days 11, 14, and 15 (total  $n = 3$ ) had arthritic lesions. The mouse killed on day 11 had marked inflammation within the joint space and subsynovial tissues (Fig. 2, C and D). Mild synovial hypertrophy and hyperplasia were present; however, the bone and articular cartilage were unaffected. The arthritic lesions were characterized by large numbers of inflammatory cells, predominantly neutrophils with some macrophages and a few lymphocytes (Fig. 2 D). The mice analyzed on days 14 and 15 ( $n = 2$ ) had edema of the periarticular soft tissue as well as mild synovial hyperplasia and hypertrophy with diffuse inflammatory cell infiltrations (representative images shown in Fig. 2, E–H). Within the joint space there was a small amount of fibrin mixed predominantly with plasma cells, a lesser number of neutrophils, and a few fibroblasts (Fig. 1, E and F). The inflammatory infiltrates present in the synovium contained predominantly lymphocytic and plasmacytic cells with a moderate number of neutrophils and a few macrophages (Fig. 2 G). In addition, there was multifocal perivascular infiltration within perivascular soft tissue that contained neutrophils, macrophages, and a few multinuclear giant cells (Fig. 2 H). These results provide direct evidence that plasma from an individual with active RA contains pathogenic components able to initiate acute inflammatory arthritis.

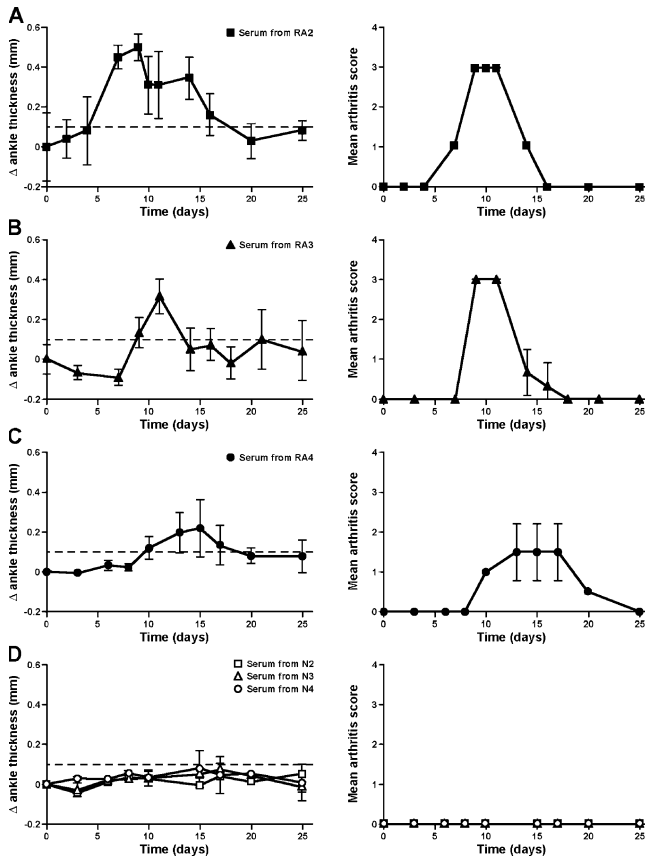
To determine whether the ability to promote inflammatory lesions is consistent among RA sera, serum samples obtained from three additional RA patients were tested (Table S1, available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>). Patients were classified as having RA on the basis of the American College of Rheumatology criteria (16). Groups of  $Fc\gamma RIIIB^{-/-}$  mice were injected with sera collected from three additional RA patients (RA2, RA3, and RA4) and three healthy blood donors (N2, N3, and N4) using the same injection protocol. Once again, we observed moderate severity and onset of arthritis in mice injected with the pathogenic sera from RA patients (Fig. 3, A–C, and Table S2, available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>), but not with sera from three additional normal blood donors (Fig. 3 D and Table S2).

Patients with RA demonstrate hypergammaglobulinemia with a wide range of RA-associated autoAbs. Therefore, we wanted to determine whether RA-related autoAbs were present in human blood samples collected from the RA patients and healthy controls. The total concentrations of IgG were variable in the samples obtained from the RA patients as well as healthy controls (Fig. 4 A). The IgG isotype ratios of sera obtained from both RA patient and healthy control groups were similar (Table S3, available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>). Of the RA-associated Abs tested, anti-GPI Ab was more prevalent in plasma from RA1, yet there was no difference in the level of this autoAb



**Figure 2. Histopathology of ankle joints.** (A) Hematoxylin and eosin-stained tissue sections from the ankle joint obtained from a representative  $B6.Fc\gamma RIIIB^{-/-}$  mouse injected with plasma collected from healthy individual N1 and killed on day 11. For orientation: s, synovium; b, bone; c, cartilage. (B) Higher magnification of selected area from A showing a normal joint space, smooth articular cartilage, and normal synovial membrane. (C) Section of ankle joint from a mouse injected with plasma from RA patient RA1 and killed on day 11 after the initial injection. Note the marked expansion of the synovial stroma by inflammatory cells. (D) Higher magnification of the synovium shows the infiltration with inflammatory cells is predominantly neutrophilic. (E–H) Representative images of the ankle joint from mice injected with plasma from RA1 and killed on days 14 and 15. (E) Inflammation and synovial hypertrophy are seen on day 14, which coincides with the peak of ankle swelling. (F) Higher magnification of a field from E showing fibrillation of the cartilage surface (arrow) and mixed inflammatory cells, predominantly neutrophils, and macrophages within the joint space. (G) Mild focal synovial hyperplasia. (H) Periarticular soft tissue with perivascular inflammation predominantly containing neutrophils, macrophages, and a few Langerhans-type multinucleated giant cells (arrow). Bar, 100  $\mu$ m.

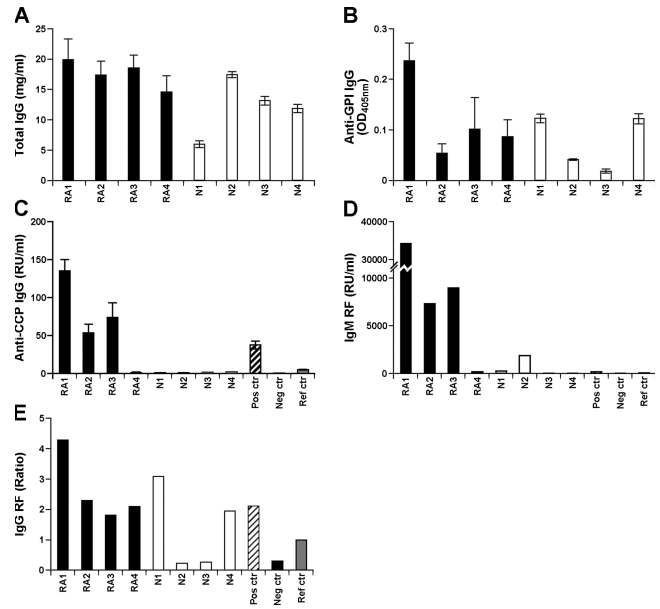
in the other three RA patients in the cohort as compared with healthy individuals (Fig. 4 B). Anti-CCP Abs were elevated in three out of four samples from RA patients (Fig. 4 C) and was not detected in sera obtained from healthy



**Figure 3. Passive transfer of arthritis with sera obtained from three additional RA patients.** *FcγRIIB*<sup>-/-</sup> mice were injected with sera from three additional arthritic patients (RA2, RA3, and RA4; A, B, and C) and three normal blood donors (N2, N3, and N4; D). Ankle thickening (mm) ± SEM and arthritis score are shown. Results for each group of mice were calculated as an average based on individual measurements of both hind paws of each individual mouse. The severity and onset of arthritis after injection with human RA samples varied among recipient groups; however, a 100% incidence was observed in each group (A, *n* = 2; B, *n* = 4; C, *n* = 2). None of the animals injected with sera from normal blood donors (A, *n* = 1; B, *n* = 3; C, *n* = 3) developed ankle swelling.

donors. All RA patients and one normal donor (N2) were positive for IgM RF (Fig. 4 D), and all four arthritic patients and two of the normal blood donors (N1 and N4) were IgG RF<sup>+</sup> (Fig. 4 E). Although there was considerable variation in autoAb levels among the RA and normal donor sera, the anti-CCP and IgM RF correlated best with the potential to induce inflammatory arthritis in mice.

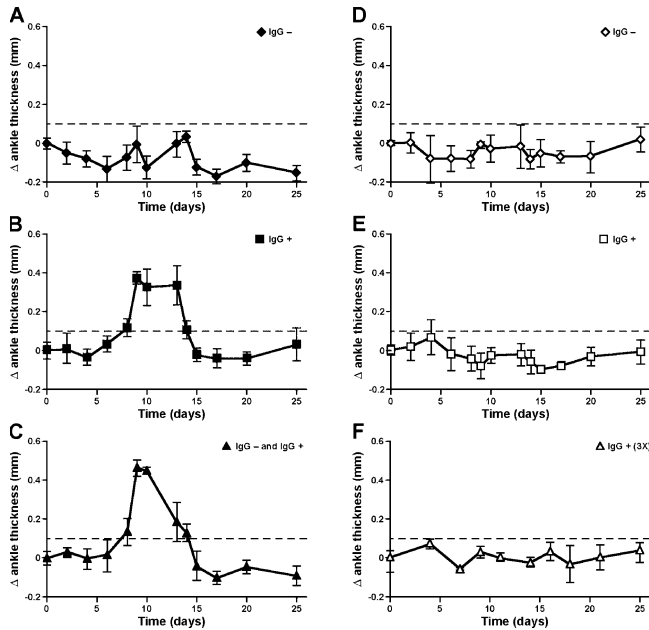
Plasma from RA patient RA1 and healthy blood donor N1 was fractionated into IgG<sup>+</sup> and IgG<sup>-</sup> components (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>), and their disease-inducing capacity was tested in *FcγRIIB*<sup>-/-</sup> mice (Fig. 5). The IgG<sup>+</sup> fraction obtained from RA patient RA1, but not the IgG<sup>-</sup> fraction, was capable of provoking arthritis in *FcγRIIB*<sup>-/-</sup> recipient mice (Fig. 5, A and B). At this concentration (total of 47.4 mg IgG/mouse), the potency of the IgG<sup>+</sup> fraction was similar to



**Figure 4. Levels of human IgG Abs.** (A) Total IgG (mg/ml). (B) Anti-GPIIb/IIIa Abs (OD, 405 nm). (C) Anti-CCP IgG Abs (relative units, RU/ml). Values > 5 RU/ml were considered positive. Mean ± SD is indicated. (D) IgM RF (values ≥ 20 RU/ml are considered positive). (E) IgG RF of each blood sample obtained from RA patients and healthy controls. Data indicate the calculated ratios, and values > 1 were considered positive.

whole plasma with the same relative IgG concentration in the starting volume (Figs. 5 B and 1 A). Furthermore, when IgG<sup>-</sup> and IgG<sup>+</sup> fractions were combined and injected (Fig. 5 C), mice developed arthritis with the same arthritic lesions as IgG<sup>+</sup> (Fig. 5 B) and the whole plasma group (Fig. 1 A). In contrast, mice injected with IgG-bound (14.7 mg IgG/mouse) or unbound IgG fractions (<0.0001 mg IgG/mouse) from nonarthritic donor N1 did not show any inflammation even when IgG concentrations three times (44.1 mg IgG/mouse) above that obtained from normal N1 plasma were used (Fig. 5, D–F). IgG<sup>-</sup> and IgG<sup>+</sup> fractions from one additional patient with established RA were purified and injected into *FcγRIIB*<sup>-/-</sup> mice (Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>). Once again the IgG<sup>+</sup> but not IgG<sup>-</sup> fraction was able to induce mild and transient arthritic lesions in mice. Collectively, these results suggest that the pathogenic component of the human plasma leading to induction of arthritis in mice resides in the IgG fraction.

To date, few studies have documented the successful passive transfer of arthritis into mice by injection of purified human IgG Abs. Transfusion of peripheral leukocytes, lymph node cells, and plasma from patients with RA into nonrheumatoid human recipients failed to provoke any evidence of rheumatic disease (17). Other experiments included injections of a patient's own IgG Abs into one knee and an identical dose of rheumatoid IgG into the other (18), resulting in RA serum-induced inflammatory reactions persisting for several days.



**Figure 5. Induction of arthritis with purified human IgG Abs from patient RA1.** Groups of  $Fc\gamma RIIB^{-/-}$  mice ( $n = 3$  each group) were injected with protein G column-separated  $IgG^{-}$  and  $IgG^{+}$  fractions of human blood samples. (A, B, and C)  $IgG^{-}$  ( $<0.0001$  mg  $IgG$ /mouse),  $IgG^{+}$  (total 47.4 mg  $IgG$ /mouse), and recombinant  $IgG^{-}$  plus  $IgG^{+}$  (47.4 mg  $IgG$ /mouse) fractions from RA1 were tested for their ability to induce arthritis in  $Fc\gamma RIIB^{-/-}$  mice. Arthritis was monitored as ankle thickening (presented here) and the overall arthritis score (not depicted). (D, E, and F)  $IgG^{-}$  ( $<0.0001$  mg  $IgG$ /mouse),  $IgG^{+}$  (14.7 mg  $IgG$ /mouse), and  $3\times$   $IgG^{+}$  (44.1 mg  $IgG$ /mouse) fractions obtained from N1 were injected in animals, and arthritis was monitored for 25 d.

In 1984, Wooley et al. (19) reported that purified anti-type II collagen IgGs from a patient with seronegative rheumatoid-like arthritis induced mild, transient arthritis in 20–25% of the injected collagen type II-susceptible mice. To our knowledge, there has been no further documentation of the reliable induction of arthritic lesions in mice after transfer of human Abs. Therefore, despite the presence of autoAbs in many patients with RA and related arthropathies, their importance in autoimmune pathogenesis remains uncertain.

We used a mouse model sensitized by a deficiency in Fc receptor  $Fc\gamma RIIB$  to exaggerate humoral immunity as a bioassay to investigate the pathogenic activity of human RA-associated Abs. Our data show that (a) plasma or serum collected from individuals with active RA was able to initiate inflammatory arthritis in  $B6.Fc\gamma RIIB^{-/-}$  mice with pathological features of human RA; (b) the inflammatory lesions in recipient animals are mild and transient in nature but highly reproducible; (c) the pathogenic component of the human plasma from RA1 leading to induction of arthritis in mice resides in the IgG fraction.

These studies suggest that human Abs from RA patients can inflict pathological lesions consistent with the underlying RA syndrome. Although too limited to establish an actual link between human-specific autoAbs and RA, they raise the

intriguing possibility that the  $Fc\gamma RIIB^{-/-}$  mouse model can be used as a bioassay to thoroughly evaluate the pathogenic involvement of humoral autoimmunity in RA. In contrast to mouse anti-GPI and anti-collagen type II Abs, the specificities of human Abs playing a role in the pathogenesis of RA are poorly defined. In particular, Abs that react with one or more ubiquitous normal protein (e.g., GPI) show poor association with human RA, whereas Abs reactive with citrullinated proteins show better correlation (20). It remains to be demonstrated whether anti-CCP, or other undefined autoAbs, are responsible for the inflammatory lesions we observed. Our studies suggest the  $Fc\gamma RIIB^{-/-}$  mouse system can be used productively for evaluating the pathological potential of currently known and novel RA-associated human Abs. Conceivably, the same model also could be used to evaluate Abs associated with other human autoimmune disorders. Finally, this model has a potential application as a disease model for examining novel anti-autoimmune therapies.

## MATERIALS AND METHODS

**Human samples.** Blood samples (serum or plasma) from healthy donors and RA patients were collected at The Jackson Laboratory, the Mount Desert Island Hospital, or the Rheumatology VA Medical Center at the University of New Mexico. All the RA patients enrolled in the study were diagnosed and selected for investigation according to the American College of Rheumatology 1987 classification criteria.

**Mice.**  $B6.Fc\gamma RIIB^{tm1Ray}$  mice were originally obtained from Taconic Farms. 8–12-wk-old male and female  $B6.Fc\gamma RIIB^{-/-}$  and  $B6.Fc\gamma RIIB^{+/+}$  control mice (The Jackson Laboratory) were used in the experiments. These mice were bred and maintained in an environmentally controlled, specific pathogen-free facility in accordance with the guidelines of the Institutional Animal Care and Use Committee.

**Serum transfer and arthritis scoring.** To induce arthritis in experimental mice using human sera or plasma, each experimental mouse was injected i.p. with 0.5 ml of human sample on day 0, followed by two more injections on days 2 (1 ml/mouse) and 7 (1 ml/mouse). Ankles were inspected for inflammation and erythema by two observers. Joint swelling was assessed by measuring the lateral diameter of the ankles as described previously (21, 22). Both ankles were measured three times and the results for each time point were presented as average ankle thickness. Delta ankle thickness (mm) was calculated based on ankle thickness before the injection with human samples. An overall arthritis score was determined by examination of ankles as follows: 0, unaffected; 1, erythema; 2, swelling of one hind limb; 3, severe swelling of both hind limbs.

**Histology.** Whole ankle joints were fixed in Bouin's solution, decalcified, and subsequently embedded in paraffin wax. 7- $\mu$ m serial longitudinal sections were prepared from tissue blocks and stained with hematoxylin and eosin. Results were interpreted by two qualified observers in a blinded manner.

**Ab detection.** Total human IgG and IgG isotype Abs were measured by ELISA using unlabeled mouse anti-human IgG Abs (IgG, IgG1, IgG2, IgG3, and IgG4) to coat and mouse anti-human IgG (Fc) Ab conjugated to alkaline phosphatase (SouthernBiotech) as the detection Ab. Data are expressed as concentration of IgG (mg/ml) based on interpolation to a titration of purified human IgG standards (Sigma-Aldrich). Anti-GPI IgG Abs were detected by ELISA using rabbit GPI (Sigma-Aldrich) for capture as described previously (22). Anti-CCP Abs were detected using an anti-CCP ELISA kit (Euroimmun US). Anti-CCP Abs were considered positive when the absorbance was higher than 5 RU/ml based on a reference sample provided by

the manufacturer. IgG RF and IgM RF were evaluated using test kits and protocols provided by the manufacturer (Euroimmun US). IgM RF values were computed as RU based on a reference sample provided by the manufacturer (values  $\geq 20$  RU are considered positive). IgG RF values are computed as ratios of the OD at 405 nm of the patient sample divided by the OD of the reference control. Values  $>1$  are considered positive. For detecting MAHA, plates were coated with human IgG (Sigma-Aldrich), and MAHAs were detected using goat anti-mouse IgG Ab conjugated to alkaline phosphatase (SouthernBiotech).

**Purification of human IgG Abs.** Human IgGs were purified from plasma using protein G columns as described by the manufacturer (GE Healthcare). Plasma, diluted 1:1 in 1X PBS, was separated using an H Trap Protein G column. The column was washed with PBS, IgG Abs were eluted in 0.1 M glycine HCl buffer, pH 2.8, and immediately brought to neutral pH with 1 M Tris-HCl, pH 9.0. The elution profile was monitored by measuring the absorbance at 280 nm. Detection of human Abs was accomplished by using a human IgG-specific ELISA. The peak of bound IgG fraction corresponded to the first five fractions after the void volume. The second peak represented the IgG-rich fraction. Fractions were then pooled into IgG-depleted (IgG<sup>-</sup>) and -enriched (IgG<sup>+</sup>) groups. The concentration of IgG in each pool (IgG<sup>-</sup> and IgG<sup>+</sup>) was then measured by ELISA. To verify the purification of IgGs, 10  $\mu$ l of untreated plasma and the IgG<sup>-</sup> and IgG<sup>+</sup> pools were separated under reducing conditions on a gradient (4–20%) SDS-polyacrylamide gel and stained with Coomassie blue staining.

**Online supplemental material.** Fig. S1 illustrates the SDS-PAGE profile of whole plasma and different IgG elution fractions, and Fig. S2 shows the induction of arthritis with purified human IgG Abs from an additional RA patient. Table S1 lists the clinical parameters of the RA patients in the study. Table S2 shows the maximum increase in ankle thickness in mice injected with human sera, and Table S3 lists the serum levels of IgG isotypes. The online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20051951/DC1>.

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