

Research article

Th1 and Th2 cytokines regulate proteoglycan-specific autoantibody isotypes and arthritis

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Abstract

BALB/c mice immunized with human cartilage proteoglycan (PG) develop arthritis accompanied by the production of autoantibodies to mouse cartilage PG. To determine whether the autoantibody isotype contributes to the onset and severity of arthritis, PG-specific serum IgG1 (Th2, IL-4-cytokine-supporting) and IgG2a (Th1, IFN- γ -controlling) concentrations were monitored during immunization with PG in IL-4-deficient and IFN- γ -deficient mice. Paradoxically, despite elevated IFN- γ , the PG-specific IgG1 isotype was significantly higher than the PG-specific IgG2a response, and the PG-specific IgG1 isotype was independent of IL-4. In contrast, the serum concentration of PG-specific IgG2a isotype was six times higher in IL-4-deficient mice than in wild-type controls. Moreover, the high concentration of PG-specific IgG2a isotype in IL-4-deficient mice corresponded to an increased severity of arthritis. The concentration of PG-specific IgG2a isotype was lower in IFN- γ -deficient mice than in wild-type mice, and the incidence and severity of arthritis also were significantly lower. Concentrations of PG-specific IgG2a isotype autoantibody correlated with the onset and severity of arthritis, suggesting a pathological role of this isotype, probably locally in the joint.

Keywords: antibody, arthritis, autoimmunity, cytokines, T cells

Introduction

BALB/c mice immunized with human cartilage proteoglycan (PG) (aggrecan) develop arthritis [1–4]. Autoantibody production precedes the first clinical symptoms of arthritis [5]. While these anti-PG autoantibodies alone do not transfer arthritis, autoantibodies significantly hasten the onset of disease and cause PG to be released from the cartilage [6,7].

B cells proliferate and differentiate into immunoglobulin-secreting plasma cells under the influence of a combination of cytokines derived from T cells [8]. IL-4, a T helper (Th)2 cytokine, regulates the switch from IgM/D to IgG1 and IgE in activated B cells [9,10]. However, the *in vivo* dependency on IL-4 for the IgG1 response is controversial [11–13]. The Th1 cytokine IFN- γ is important *in vitro* and *in vivo* for enhancement of IgG2a secretion [14,15]. Th1

and Th2 cytokines also function to cross-regulate Ig isotypes. For example, IFN- γ antagonizes IL-4-induced IgG1 responses at the level of IgG1 transcription [16,17], whereas IL-4 has the ability to suppress IFN- γ -driven IgG2a responses [16].

We have previously shown that PG-specific antibodies increase the severity of arthritis and that PG-induced arthritis is a Th1-type disease dominated by IFN- γ [4]. We therefore were interested in finding out how IFN- γ and IL-4 regulate isotype expression of the PG-specific antibodies and if an autoantibody isotype contributes to the severity of disease.

Materials and methods

Animals

BALB/c and IFN- γ -deficient mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). BALB/c heterozygous and homozygous nude mice were obtained from the National Cancer Institute (Frederick, MD, USA). Breeding pairs of BALB/c IL-4-deficient mice were obtained from Klinische Forschergruppe für Rheumatologie (Freiburg, Germany). CD40-deficient BALB/c mice were purchased from Taconic (Germantown, NY, USA).

Preparation of cartilage PG monomer (aggrecan) and immunization

Human cartilage was obtained during joint-replacement surgery and high-density PG was prepared as described elsewhere [1,5]. Female BALB/c mice (wild-type or gene deficient) were injected intraperitoneally on days 0, 21, and 42 with 100 μ g of human cartilage PG measured as protein, in adjuvant, as described elsewhere [1,2,5].

Measurement of immunoglobulin isotypes

Enzyme-linked immunosorbent assay (ELISA) was used to measure isotype-specific antibodies in serial dilutions (1:1000 to 1:62,500) of sera. ELISA plates were coated with either 0.5 μ g human PG or 1 μ g mouse PG, as described elsewhere [5,18]. PG-specific IgG isotypes were detected with peroxidase-labeled rabbit anti-mouse IgG1 or IgG2a (Zymed Laboratories, San Francisco, CA, USA). IgG1 and IgG2a myeloma proteins were used for a standard curve.

Assessment of cytokine production by spleen cells *in vitro*

Spleens were obtained 1 week after the third injection with PG, as described elsewhere [4]. Cells were stimulated in the presence or absence of PG (20 μ g/ml) and a capture ELISA was used to measure the released cytokines IFN- γ , IL-4, and IL-10 [4].

Assessment of arthritis

The severity of arthritis in each paw was graded according to an established scoring system [3,5] as follows: 0, normal; 1, mild erythema and/or swelling, usually in one or two toes;

2, moderate erythema and/or swelling of the paw; 3, more diffuse erythema; 4, severe erythema and swelling affecting the entire paw (maximum score for the four paws = 16). Inflammation and joint swelling were recorded twice a week by the same individual in a blinded manner.

Statistical analysis

The Mann-Whitney *U* test was used to compare nonparametric data for statistical significance. *P* values less than 0.05 were considered significant.

Results

PG-specific IgG1 isotype dominates despite a higher ratio of IFN- γ to IL-4

BALB/c mice immunized with human PG generated a PG-specific IgG1 isotype response which was significantly higher than the PG-specific IgG2a isotype (Fig. 1a). However, when the production of Th1 and Th2 cytokines was measured from splenocytes of animals immunized with PG, IFN- γ was produced at a significantly higher concentration than either IL-4 or IL-10 (Fig. 1a). These results reveal a dichotomy between the isotype of the PG-specific antibody response (IgG1) and the secretion of IFN- γ . The discrepancy could be reconciled if the PG-specific IgG1 response is independent of T cells.

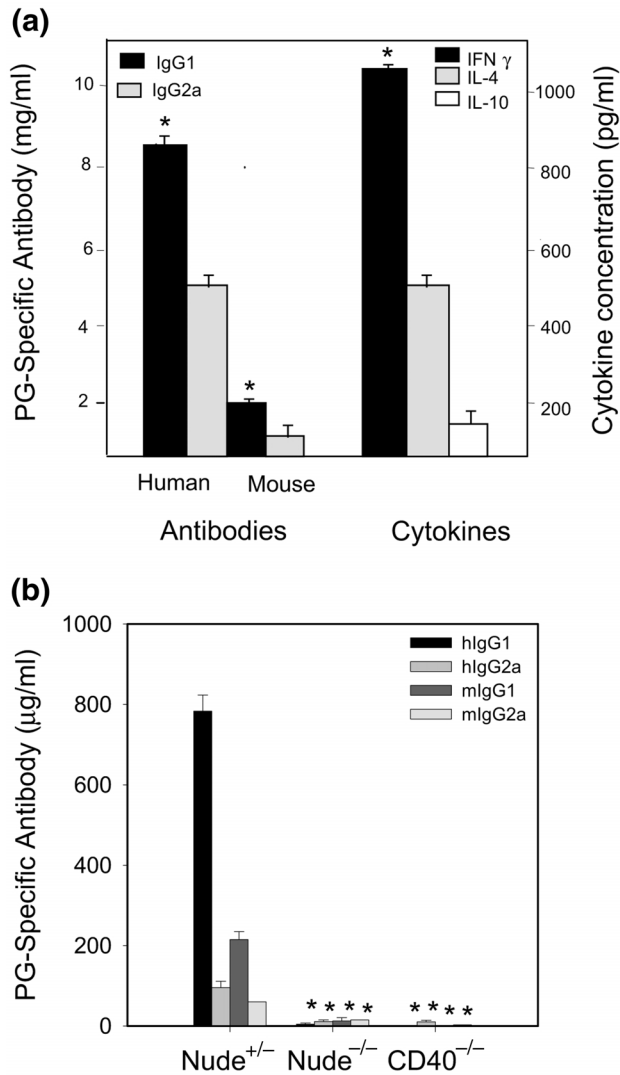
We therefore assessed the PG-specific response in heterozygous and homozygous nude mice and in CD40-deficient mice. Whereas heterozygous nude mice generated essentially the same antibody response to PG as wild-type mice (Fig. 1b), no or very little anti-PG antibody was detected in homozygous nude or CD40-deficient mice (Fig. 1b). These results show that the human and murine PG-specific antibody responses are dependent on the interaction between T cells and B cells.

IL-4 and IFN- γ regulation of the PG-specific IgG1/IgG2a isotype response

To find out if the PG-specific IgG1 response is dependent on IL-4, we immunized IL-4-deficient mice with PG and measured the PG-specific IgG1 and IgG2a isotypes. Whereas the IgG1 response to human PG was unaffected (Fig. 2a), the loss of IL-4 significantly reduced the IgG1 response to mouse PG in IL-4-deficient mice (Fig. 2b). The most striking observation, however, was the dramatic increase in PG-specific IgG2a response in IL-4 deficient mice. There was a sixfold increase in the IgG2a response in the absence of IL-4 (Fig. 2a,b). These results show that the PG-specific IgG1 response was marginally dependent on IL-4, whereas endogenous IL-4 dramatically suppressed the PG-specific IgG2a response.

To find out if IFN- γ induced a PG-specific IgG2a response *in vivo*, the PG-specific IgG2a response was examined in IFN- γ -deficient mice. The IgG2a antibody responses to both human and mouse PGs were lower in these mice

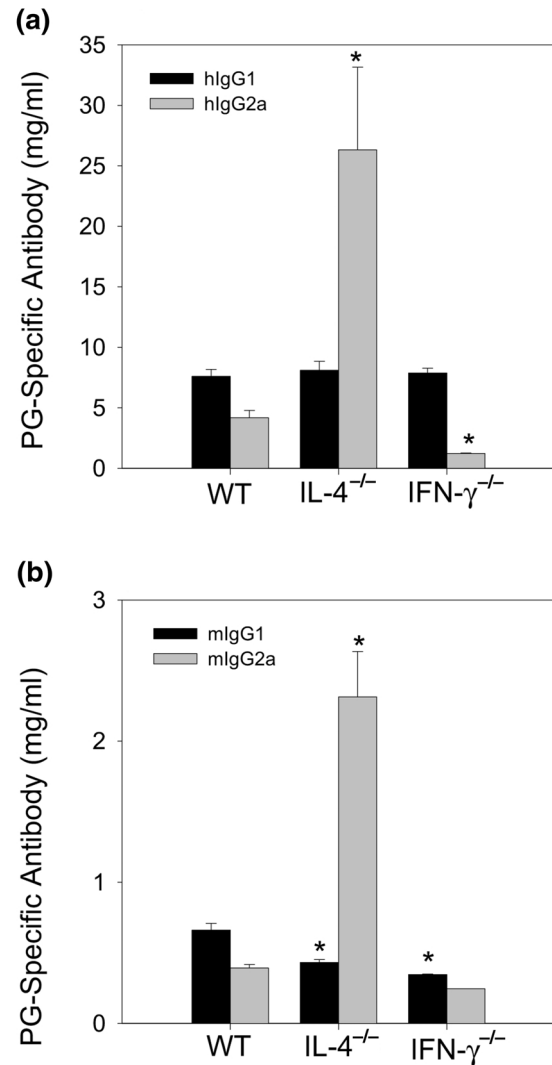
Figure 1



PG-specific antibody isotypes and cytokines in PG-induced arthritis. **(a)** Serum concentrations of PG-specific IgG1 and IgG2a isotypes and production of PG-specific cytokines by spleen cells of PG-immunized mice. PG-specific (anti-human and anti-mouse) isotypes were measured in sera ($N = 30$) and cytokines in supernatants of spleen cells ($N = 5$). **(b)** T-cell-deficient (nude) and CD40-deficient mice did not generate a PG-specific antibody response. Heterozygous and homozygous nude and CD40-deficient mice were immunized with PG. Note: Scales for antibody concentrations differ between panels A and B. Values are means; whiskers indicate SEM. h, m = respectively, antibodies to human and mouse PG; PG = proteoglycan. * $P < 0.05$ in comparison with heterozygous nude mice.

than in wild-type mice (Fig. 2a,b). These results show that the PG-specific IgG2a response was dependent on IFN- γ . If IFN- γ concentrations regulated IgG1, reduction in IFN- γ should be permissive for IgG1; in IFN- γ -deficient mice, however, there was no increase in IgG1 response to either human or mouse PG (Fig. 2a,b).

Figure 2



Production of PG-specific IgG2a isotype is increased in IL-4-deficient mice. Wild-type, IL-4-deficient, and IFN- γ -deficient mice were immunized with PG, and serum antibody isotypes to human PG **(a)** or mouse PG **(b)** were measured by ELISA. Values are means; whiskers indicate SEM. PG = proteoglycan; WT = wild-type. * $P < 0.05$ in comparison with wild-type mice.

Arthritis is associated with an increase in IgG2a

We studied the induction of arthritis in mice deficient in IL-4 and IFN- γ to find out if there is a correlation between the isotype of the antibody and disease. In wild-type and IL-4 deficient mice, paw swelling began 11.6 ± 6.6 days after the last injection with PG, whereas in the IFN- γ -deficient mice, swelling began much later at 36.8 ± 6.2 days (Table 1). The incidences of arthritis in wild-type and IL-4-deficient mice were much higher than in the PG-immunized IFN- γ -deficient mice, and the arthritis score was significantly higher in the IL-4-deficient than in the wild-type animals (Table 1). In IFN- γ -deficient mice, not only

Table 1**Arthritis is exacerbated in IL-4-deficient mice and suppressed in IFN- γ -deficient mice**

Mice	Incidence (arthritis/total) (%)	Arthritis score of diseased mice ^a	Day of onset ^b
Wild-type	9/12 (75)	5.2 \pm 2.5	11.8 \pm 6.6
IL-4 ^{-/-}	9/9 (100)	9.6 \pm 1.1*	9.5 \pm 6.0
IFN- γ ^{-/-}	5/22 (22)	2.4 \pm 2.0*	36.8 \pm 6.2**

^aMean arthritic score for wild-type and IL-4-deficient mice was maximum at day 23 and for the IFN- γ -deficient mice at day 41 after the last immunization with PG. ^bDays from last immunization with PG to onset of disease. *Score differed significantly from that for wild-type mice ($P < 0.05$). **Time to onset of disease differed significantly from that in wild-type mice ($P < 0.002$).

was the proportion of animals with arthritis lower than in the other groups, but also their reduced susceptibility was further mirrored in the significantly less severe paw swelling (Table 1). These data show that endogenous cytokine concentrations dramatically affect both the onset and the severity of inflammation.

Discussion

Because PG-induced arthritis is dominated by IFN- γ , it is considered to be a Th1-type disease [4,18]. Paradoxically, we observed higher concentrations of the IgG1 isotype than of the IgG2a isotype. To resolve this apparent paradox, we examined the anti-PG responses in cytokine-deficient animals. We found that in the absence of IL-4, the PG-specific IgG1 isotype was only minimally affected. These *in vivo* experiments are very different from those describing a dramatic *in vitro* effect of IL-4 on IgG1 induction [9,10], but are similar to other studies that showed partial elimination of IgG1 *in vivo*, either using neutralizing antibodies to IL-4 or blocking the IL-4 receptor with antibodies [11–13,19]. One possibility was that the large carbohydrate content of PG might have a promoting interaction with B cells in a mitogen-like manner, thus permitting production of IgG1. However, nude mice and CD40-deficient mice did not generate a PG-specific IgG1 response and such a response was also independent of IFN- γ . Taken together, these results are in contrast to the IL-4-dictated IgG1 switch response. Rather, they suggest that the PG-specific IgG1 response, at least in BALB/c mice, may be regulated *in vivo* by other, as-yet-unidentified, cytokine(s) [8,20–22].

In contrast to IgG1, the expression of IgG2a isotype showed a strong inverse correlation with the concentration of endogenous IL-4. In the absence of IL-4, the IgG2a response was six times greater than in wild-type mice. This finding demonstrates that IL-4 plays a critical role in regulating the PG-specific IgG2a response. The role of this cytokine in the suppression of IgG2a *in vivo* has not been well studied. The IgG2a response was only minimally enhanced after *in vivo* treatment with anti-IL-4 antibody [8], but it was increased in immunized IL-4-deficient mice [23]. Since there is no evidence that IL-4 directly inhibits

IgG2a transcription in B cells, this cytokine very likely suppresses the IFN- γ response, either directly or indirectly. Indeed, the significant reduction of the PG-specific IgG2a response in the absence of IFN- γ suggests that the response is controlled by this interferon.

We have found that the development of PG-specific antibodies correlates with the development of arthritis, and that PG-specific autoantibodies increase the severity of disease. We show here that mice deficient in IL-4 develop arthritis that is significantly more severe than in wild-type animals. The pathogenic role of IgG2a antibody was also described in a model of autoimmune hemolytic anemia [24]; however, these IgG2a autoantibodies were not pathogenic in mice that are deficient in Fc γ receptor III [24]. These two observations taken together suggest the possibility that the IgG2a PG-specific autoantibodies elicit a pathogenic effect through an Fc-receptor-mediated mechanism.

PG-induced arthritis is considered a Th1-type disease, characterized by a higher ratio of IFN- γ to IL-4 [4]. Our finding that treatment with IL-4 inhibits IFN- γ suggests that IL-4 could mediate a shift from a Th1 to a Th2 response *in vivo* [4]. In addition to the effects of IL-4 on antibody production, this cytokine inhibits Th1 responses, including IFN- γ and macrophage production of proinflammatory cytokines [25,26]. An increase in inflammatory cytokine production in IL-4-deficient mice may also contribute to exacerbation of PG-induced arthritis. In adjuvant arthritis, which may not be dependent on antibodies, disease is increased in the absence of IL-4 [27]. This finding suggests that IL-4 inhibits adjuvant arthritis by its anti-inflammatory properties and not by an alteration in isotype expression. Paradoxically, in collagen-induced arthritis (CIA), disease is suppressed in IL-4-deficient mice [28]. CIA is also different from PG-induced arthritis in regard to IFN- γ . In CIA and some other organ-specific autoimmune diseases, IFN- γ functions in a protective role, so that in IFN- γ -deficient mice disease is exacerbated [29,30]. Whether the difference between PG-induced arthritis and CIA is due to the autoantigen or to genetic differences in mouse strains is not clear at present.

Conclusion

Th1 and Th2 cytokines regulate PG-specific autoantibody IgG isotypes and susceptibility to arthritis. In the absence of IL-4, the production of PG-specific IgG2a isotype was dramatically enhanced and disease was significantly more severe. IL-4 may exert its effect through IFN- γ , since in IFN- γ -deficient mice, both the PG-specific IgG2a response and arthritis were suppressed. The correlation between PG-specific autoantibody isotype and development of arthritis suggests a pathological role for IgG2a isotype in disease.

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