GENETIC SUSCEPTIBILITY TO MURINE COLLAGEN II AUTOIMMUNE ARTHRITIS

Proposed Relationship to the IgG2 Autoantibody Subclass Response, Complement C5, Major Histocompatibility Complex (MHC) and Non-MHC Loci

By WILLIAM C. WATSON and ALEXANDER S. TOWNES

From the Veterans Administration Medical Center and Department of Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38104

Collagen II autoimmune arthritis (CII AIA),[1] an experimental model of rheumatoid arthritis, is induced in rats or mice by immunization with heterologous CII (1, 2). There is considerable evidence to support the concept that the initiation of disease is related to the production of a crossreactive IgG autoantibody to CII, which deposits in articular cartilage and activates the complement cascade. After immunization with heterologous CII, arthritic rats produce an IgG antibody which is more crossreactive with rat CII than the IgG produced by nonarthritic rats (3). Affinity-purified rat anti–rat CII IgG will passively transfer arthritis to normal rats (4). Complement depletion inhibits the development of arthritis after active immunization with CII (3) or passive transfer with CII antibody (5). In addition, we recently have shown that in contrast to C5 normal B10.D2/new line mice, C5-deficient B10.D2/old line mice are resistant to arthritis by passive transfer with CII antibody, and that, when bound to cartilage in vitro, either rat or mouse CII antibody will activate C5 to C5a (6).

In the murine model of CII AIA, Wooley et al. (7, 8) suggested that susceptibility to CII AIA is linked to the I-Aq haplotype of the H-2 locus of the MHC, which in turn is linked to high antibody responsiveness to CII. However, these investigators noted that some strains produce substantial antibody responses, but fail to develop arthritis. Based upon studies reported herein, we propose that the specific responses of the complement-fixing IgG2 subclasses, as well as the availability of complement C5, represent important determinants of susceptibility to CII AIA. In addition, these studies implicate genes outside the MHC in

A preliminary account of part of this work was presented to the ASBC/AAI FASEB June, 1984 meeting in St. Louis, Missouri [Watson, W. C., and A. S. Townes. 1984. Genetic restriction of the IgG2a autoantibody subclass response to collagen II (CII): an hypothesis for resistance to CII autoimmune arthritis in C57B1 mice. *Fed. Proc.* 43:1992 (Abstr. 3343)]. This work was supported by program directed research funds from the Veterans Administration. Address correspondence to: W. C. Watson, Rheumatology Research Laboratory, VA Medical Center (151), 1030 Jefferson Ave., Memphis, TN 38104.

[1] *Abbreviations used in this paper:* CII AIA, collagen II autoimmune arthritis; CFA, complete Freund's adjuvant; ELISA, enzyme-linked immunosorbent assay; IFA, incomplete Freund's adjuvant; MHC, major histocompatibility complex.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/85/12/1878/14 $1.00
Volume 162 December 1985 1878–1891

determining susceptibility and provide additional support for an important role for autoantibody to CII in the initiation of arthritis in this animal model.

Materials and Methods

Mice. 7–8-wk-old mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Breeding between C57BL/6 (B6) and DBA/1 (D1) mice and backcrosses to D1 [(B6D1)F_1 × D1] was done at the Memphis VAMC. Complement, specifically C5, is thought to be required for the initiation of CII AIA (6), and female mice are known to be relatively deficient in C5, as well as C6 and C7 (9). To control for this sex-related difference in complement and its possible effect on susceptibility to CII AIA, all studies used male mice, with the exception of the B6D1 × D1 backcross study. Only five backcrosses were available and two of these were female. Since the study was performed primarily to examine the mode of inheritance of IgG2a autoantibody responsiveness, rather than the susceptibility to arthritis of B6D1 × D1 backcrosses, these two females were included.

Collagen. Chick CII was extracted from sternal cartilage and purified according to published methods (10). Mouse CII was a generous gift from Dr. Michael A. Cremer, who prepared it according to the same procedures used in preparing chick CII. The purity of both preparations was confirmed by electrophoresis (10).

Immunization. Mice were injected subcutaneously in the tail with chick CII emulsified 1:1 with complete Freund's adjuvant (CFA). The CFA was prepared by grinding mycobacteria in incomplete Freund's adjuvant (IFA) (Difco Laboratories, Detroit, MI). The mycobacteria were heat-killed *M. tuberculosis* strains C, DT, and PN (Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey, England). Each mouse received 0.1 ml chick CII/CFA containing 100 μg CII and 10, 100, 250, or 500 μg mycobacteria as indicated in the text.

Arthritis. Mice were observed for 4–5 mo for the presence or absence of arthritis. An animal was judged to be arthritic if one or more joint regions (digits, wrist, ankle) were red and swollen.

Purification of Chick CII Antibody. The following procedure was used to obtain purified D1 and B6 antibody for studies of crossreactivity and affinity and as a reference for estimating IgG autoantibody subclass serum levels in individual mice as explained below. Chick CII–immune mice were bled from the retroorbital sinus at 7–10-d intervals beginning ~30 d after immunization and ending on about day 70. Serum aliquots were stored at −70°C. Pooled serum was incubated with chick CII–Sepharose overnight at 4°C. The immunosorbent was prepared according to standard procedures using CNBr-activated Sepharose 4B (Sigma Chemical Co., St. Louis, MO). Unbound protein was removed by washing the immunosorbent wtih 0.1 M Tris, 0.15 M sodium chloride, pH 7.4, on a sintered glass funnel until the filtrate absorbance at 280 nm achieved constant background levels (<0.05 optical density units). Chick CII antibody then was eluted with 0.2 M glycine, pH 2.8, neutralized with 1 M Tris, pH 8, concentrated in an Amicon cell (Amicon Corp., Danvers, MA), dialyzed into PBS, and stored at −70°C.

IgG Subclass Enzyme-linked Immunosorbent Assay (ELISA). A "sandwich" ELISA (11) was used in the determination of levels (μg/ml) of IgG1, 2a, 2b, and 3 in the purified chick CII antibody preparations. These values were used in the estimation of antibody affinity and IgG autoantibody subclass serum levels, as explained in subsequent sections. 96-Well microtiter plates (Nunc; Vanguard International, Neptune, NJ) were coated with goat anti-mouse IgG $F(ab')_2$–specific antibody (Cappel Laboratories, Cochranville, PA) diluted 1:75 in 0.1 M sodium carbonate, pH 9.6. Coating was done overnight at 4°C. The plates were washed in 0.15 M sodium chloride, 0.05% Tween-20 with a Dynawasher I (Dynatech Laboratories, Inc., Alexandria, VA) and incubated overnight at 4°C with affinity-purified chick CII antibody diluted in 0.1 M Tris, 0.15 M sodium chloride, 0.5% Tween-20, pH 7.4 (ELISA buffer). After washing, the plates were incubated for 2 h at 4°C with rabbit anti–mouse IgG1, 2a, 2b, or 3 sera (Miles Laboratories, Inc., Elkhart, IN) diluted 1:1000 in ELISA buffer. The crossreactivity of these subclass antisera was

found to be <10% under the conditions of this subclass ELISA on the basis of their relative reactivities with purified mouse myeloma IgG subclass proteins (Litton Bionetics Inc., Kensington, MD). After washing, the plates were incubated for another 2 h at 4°C with peroxidase-conjugated goat anti-rabbit IgG, Fc-specific sera (Cappell Laboratories) diluted 1:7000 in ELISA buffer, washed again, and then incubated with peroxidase substrate for 60 min at room temperature. The substrate consisted of 2.21 mM *o*-phenylenediamine, 26.59 mM citric acid, 51.40 mM sodium phosphate, 0.012% hydrogen peroxide, and 0.1% Tween-20. A volume of 0.15 ml/well was used in all incubations. Absorbances at 450 nm were measured with a Microelisa Auto Reader MR580 (Dynatech Laboratories, Inc.).

The following procedure was used to convert absorbances to μg/ml values for each IgG subclass. Various dilutions of Miles Laboratories mouse reference serum were assayed on the goat anti-mouse IgG $F(ab')_2$–coated plates. This reference serum is supplied with μg/ml values for each IgG subclass. The absorbance values (y) and the corresponding log μg/ml values (x) were entered into a TI-55 calculator (Texas Instruments, Inc., Dallas, TX), preprogrammed to compute slope (m) and y-intercept (b) values in the straight line equation: $y = (m)(\log x) + b$. The calculator used a linear regression program to solve for m and b; correlation coefficients (r values) for the relationship between ELISA absorbance and μg/ml values of IgG1, 2a, 2b, or 3 were not <0.990. The observed y absorbance values for each IgG subclass in the affinity-purified chick CII antibody preparations then were entered into the calculator to compute the corresponding μg/ml values by solving for log x in this equation.

IgG Autoantibody Subclass ELISA. Arthritic mice were bled within 48 h after the onset of disease and nonarthritic mice were bled at the peak of the serum response, i.e., day 40–45. These two times were similar, since the mean onset day (±SE) for arthritic D1 mice (Table I) was 43 ± 2, $n = 25$. An exception to the above protocol is noted for the study described in Table IV. Serum samples were stored at −70°C before assaying for IgG autoantibody as follows. The serum samples were diluted in ELISA buffer and incubated overnight at 4°C on microtiter plates coated with 2 μg/ml mouse CII in 0.145 M potassium phosphate buffer, pH 7.6. Absorbance values for each IgG subclass were developed with the rabbit anti-mouse subclass sera, peroxidase-conjugated goat anti-rabbit IgG Fc-specific serum, and peroxidase substrate as described above. Known concentrations of anti-chick CII IgG1, 2a, 2b, and 3 in affinity-purified chick CII antibody preparations were assayed simultaneously. These concentrations were established by IgG subclass ELISA on goat anti-mouse $F(ab')_2$–coated microtiter plates as described above. The resultant pairs of values (absorbance and μg/ml IgG antibody subclass) were subjected to linear regression analysis to compute m and b values for each subclass; correlation coefficients (r values) for the relationship between ELISA absorbance and μg/ml IgG1, 2a, 2b, or 3 were not <0.980. The observed y absorbance values for each IgG autoantibody subclass in individual serum samples then were entered into the calculator to compute the corresponding μg/ml values.

Competitive Inhibition ELISA. Anti-chick CII IgG2a was purified from an aliquot of D1 anti-chick CII IgG with protein A–Sepharose CL-4B (Sigma Chemical Co.), according to the general procedure described by Ey et al. (12). The following modifications were made. After eluting IgM and IgG1 with pH 6 buffer, the buffer pH was changed to 3.5, to elute IgG2a plus IgG2b plus IgG3. The pH of this mixture was readjusted to 5.5, the mixture loaded back onto the column, and IgG2a was eluted by washing the column with pH 5.5 buffer. The IgG2a eluate was immediately neutralized with 1 M Tris, pH 8, concentrated in an Amicon cell, and dialyzed into PBS. Analysis of the eluate by IgG subclass ELISA revealed 97% IgG2a. Horseradish peroxidase (type IV; Sigma Chemical Co.) then was conjugated to the IgG2a antibody with glutaraldehyde, according to the method described by Avrameas et al. (13). Various concentrations of pooled D1 or B6 chick CII antibody were incubated with the coupled antibody in mouse CII–coated microtiter wells for 18 h at 4°C using a volume of 0.1 ml/well; concentrations were established by IgG subclass ELISA using plates coated with goat anti-mouse IgG $F(ab')_2$, as explained in the previous section. The plates then were washed and peroxidase substrate

was added. Absorbances were measured with the Microelisa Auto Reader. Relative antibody affinity was defined as the concentration of uncoupled D1 or B6 antibody required to reduce the ELISA absorbance by 50%.

Results

Analysis of the IgG Autoantibody Subclass Response in the CII AIA-susceptible D1 Strain. The relationship between susceptibility to CII AIA and the magnitude of the total IgG autoantibody response in the D1 strain is shown in Table I. With variably increasing amounts of mycobacteria used in the adjuvant, the serum level of IgG autoantibody increased from an average of 14 μg/ml, without mycobacteria, to a maximum of 789 μg/ml with 250 μg mycobacteria per mouse. Arthritis failed to develop in mice immunized without mycobacteria in which serum levels varied between 8 and 29 μg/ml. In mice immunized with mycobacteria, arthritis was found to be associated with serum levels of 81–1,943 μg/ml. Analysis of the IgG subclass response revealed that most of this IgG autoantibody was IgG2a, averaging 73% of the total IgG autoantibody (range, 45–97%; n = 25). It is interesting to note that a high percent of IgG2a autoantibody responsiveness appears to be an inherent trait of the D1 strain, since IgG2a autoantibody also was the predominant IgG subclass in the nonarthritic D1 mice immunized with or without mycobacteria, averaging 55% (range, 40–71%; n = 9). In terms of the magnitude of the IgG2a autoantibody response, susceptibility appeared to be associated with a response of ~50 μg/ml or more, since the lowest serum IgG2a autoantibody level associated with disease was 62 μg/ml and the highest level associated with the absence of disease was 44 μg/ml (Fig. 1).

Comparison of IgG Autoantibody Subclass Responses in Susceptible D1 and Resistant B6 Strains. As shown in Fig. 1, IgG1, 2b, and 3 autoantibody responses of nonarthritic B6 mice generally overlapped those of arthritic D1 mice. In marked contrast, there was a clear separation between IgG2a autoantibody responses:

TABLE I

A Comparison of Arthritic and Nonarthritic IgG Autoantibody Serum Levels in D1 Mice

Group (n)	Arthritis	Anti-mouse CII IgG				
		Total	Mean percent (range)			
			1	2a	2b	3
		μg/ml				
$D1_1$ (7)	–	14 (8–29)	28 (17–43)	55 (40–71)	14 (7–24)	3 (2–6)
$D1_2$ (8)	+	364 (81–836)	16 (7–33)	69 (57–84)	12 (5–25)	3 (1–9)
$D1_2$ (1)	–	69	7	55	29	9
$D1_3$ (10)	+	420 (171–858)	14 (3–27)	67 (45–87)	9 (1–32)	10 (2–36)
$D1_4$ (7)	+	789 (260–1943)	12 (1–31)	83 (63–97)	3 (1–5)	2 (1–4)
$D1_4$ (1)	–	81	4	54	33	9

Mice were immunized with 100 μg chick CII and either 0 ($D1_1$), 10 ($D1_2$), 100 ($D1_3$), or 250 ($D1_4$) μg mycobacteria in IFA. Total and percent IgG autoantibody subclass values were derived from individual μg/ml autoantibody subclass values determined by IgG autoantibody subclass ELISA on mouse CII-coated microtiter plates as explained in Materials and Methods.

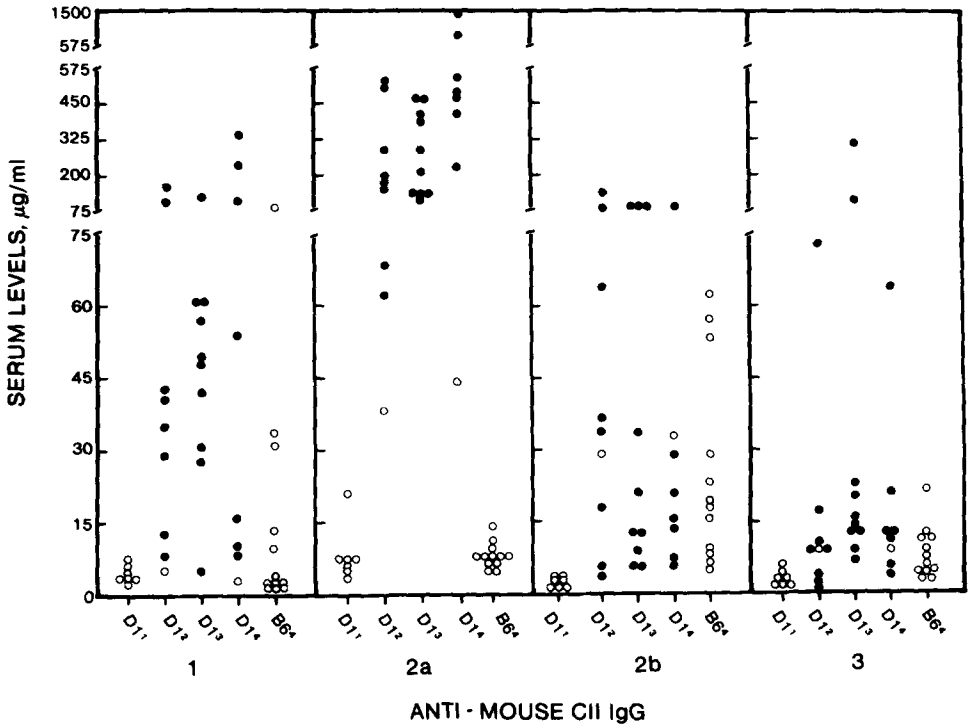

FIGURE 1. Comparison of IgG autoantibody subclass responses of the CII AIA-susceptible D1 strain (Table I) with those of the resistant B6 strain. Immunization schedule for D1 mice is described in the legend to Table I. B6 mice were immunized with 100 μg chick CII and 250 μg mycobacteria in IFA (B6-4). Serum IgG autoantibody subclass levels were determined by IgG autoantibody subclass ELISA on mouse CII-coated microtiter plates as explained in Materials and Methods. (●) Arthritic mice, (○) nonarthritic mice.

TABLE II

Comparison of the Crossreactivity of Anti-Chick CII IgG Affinity Purified from the Pooled Serum of Arthritic D1 and Nonarthritic B6 Mice

Group	Percent crossreactivity of anti-chick CII IgG			
	1	2a	2b	3
D1	57 (50-64)	50 (48-51)	56 (55-57)	53 (50-55)
B6	53 (46-63)	54 (48-60)	55 (47-64)	49 (44-55)

Antibody was purified with chick CII-Sepharose and assayed on either mouse CII- or chick CII-coated ELISA plates as described in Materials and Methods. Data represent anti-mouse CII ELISA value divided by the anti-chick CII ELISA value multiplied by 100; values represent the mean (range of triplicate determinations).

the maximal B6 IgG2a autoantibody response was 14 μg/ml (group mean, 8 μg/ml) compared with the lowest IgG2a autoantibody response associated with arthritis in D1 mice, i.e., 62 μg/ml, measured in an arthritic D1 mouse immunized with 10 μg mycobacteria.

Crossreactivity with Mouse CII of Antibodies from Susceptible D1 and Resistant B6 Strains. Table II examines whether the relative deficiency of the IgG2a autoantibody subclass response of the B6 strain shown in Fig. 1 might represent a

subclass-specific decrease in crossreactivity with mouse CII. Details of the method are described in the legend to Table II. As shown, there were no major differences in the relative crossreactivities of B6 anti-chick CII IgG1, 2a, 2b, and 3. There also was no major difference in crossreactivity when comparisons were made with D1 anti-chick CII IgG1, 2a, 2b, and 3. The overall mean percent crossreactivity of D1 or B6 anti-chick CII IgG (% IgG1 + % IgG2a + % IgG2b + % IgG3/4) was 54% (range, 50–57%) and 53% (range, 49–55%), respectively.

Affinity of Autoantibody from Susceptible D1 and Resistant B6 Strains. The relative affinities of D1 and B6 antibody for mouse CII were compared using a competitive inhibition ELISA as described in Materials and Methods. The comparison revealed that 27 μg/ml of either D1 or B6 chick CII antibody inhibited by 50% the binding of peroxidase-labeled D1 anti-chick CII IgG2a to mouse CII-coated microtiter plates. The ranges for 50% inhibition in duplicate determinations were 26–27 μg/ml for D1 antibody and 24–29 μg/ml for B6 antibody.

IgG Autoantibody Subclass Response in (B6D1)F_1 Hybrids. The suggested association between susceptibility and the IgG2a autoantibody response was further examined in (B6D1)F_1 hybrids. As shown in Table III, 12 of 12 B6D1 mice were high-percent IgG2a autoantibody responders (range, 50–95%). In the four with arthritis, total IgG autoantibody levels ranged from 65 to 358 μg/ml. As a result of high-percent IgG2a autoantibody responsiveness, most of this autoantibody was IgG2a and serum levels of IgG2a autoantibody were within the range of arthritic D1 levels. Table III also shows that, in 4 of 12 nonarthritic B6 mice, total IgG autoantibody levels were similar to arthritic B6D1 levels (B6 range, 70–182 μg/ml). However, serum IgG2a autoantibody levels remained substantially below arthritic B6D1 levels (B6 range, 6–14 μg/ml) as a result of low-percent IgG2a autoantibody responsiveness (B6 range, 6–16%).

Comparison of IgG Autoantibody Subclass Responses in Arthritic and Nonarthritic B6 and B10 Mice. A study was designed to challenge the resistance of the B6 strain by increasing the quantity of mycobacteria-containing CFA from 250 μg/mouse to 500 μg/mouse, boosting, and treating the mice with low-dose (10 mg/kg) Cytoxan 2 d before the primary and booster immunizations, in an attempt to reduce T suppressor cell activity (14). As shown in Table IV, these treatments did not produce any major changes in either the magnitude of the total IgG autoantibody response or the relative IgG subclass distribution of the total IgG autoantibody response in most of the B6 mice.

However, arthritis did develop in 2 of 20 of these mice, one that received the higher dose of CFA and the other, the higher dose of CFA plus low-dose Cytoxan. Total IgG autoantibody levels were markedly elevated (Table IV) in these arthritic mice, but most of the IgG autoantibody was IgG2b. Table IV also shows the results of a study with the B10 subline in which 1 of 12 mice developed arthritis with the lower dose of CFA (250 μg/mouse). Again, total IgG autoantibody levels were increased, but IgG2b and not IgG2a was the predominant IgG autoantibody subclass.

Comparison of IgG2a Autoantibody Responses in Strains of Mice Differing in H-2 and Ig-1 Haplotypes and Susceptibility to CII AIA. Table V presents the IgG2a

TABLE III

Comparison of Arthritic and Nonarthritic IgG Autoantibody Responses in (B6D1)F_1 Hybrids and B6 Mice

Strain	Anti-mouse CII IgG			Arthritis
	Total	IgG2a	IgG2a	
	μg/ml	%	*μg/ml*	
B6D1	358	60	214	+
B6D1	210	95	200	+
B6D1	168	53	89	+
B6D1	65	83	54	+
B6D1	39	72	28	−
B6D1	35	74	26	−
B6D1	29	66	19	−
B6D1	21	71	15	−
B6D1	18	50	9	−
B6D1	18	50	9	−
B6D1	15	60	9	−
B6D1	13	69	9	−
B6	182	6	11	−
B6	86	16	14	−
B6	73	10	7	−
B6	70	9	6	−

Mice were immunized with 100 μg chick CII and 250 μg mycobacteria in IFA. Serum IgG autoantibody subclass levels were determined by IgG autoantibody subclass ELISA on mouse CII-coated microtiter plates as described in Materials and Methods. Total and percent IgG2a were determined from individual μg/ml values of IgG1, 2a, 2b, and 3 (only those B6 mice with total responses within the range of B6D1 responses are shown here; see Table IV for the analysis of all 12 B6 mice).

autoantibody response as a simple mathematical product of the μg/ml value of total IgG autoantibody and the percent IgG2a autoantibody. H-2q/Ig-1c D1 mice were high-total and high-percent IgG2a responders, resulting in IgG2a autoantibody serum levels of 62 μg/ml or more in the arthritic animals. In the one of eight D1 mice that failed to develop arthritis, the serum IgG2a autoantibody level was 44 μg/ml.

The H-2d/Ig-1c D2 strain also was a high-percent IgG2a responder, but a low-total IgG responder. As a result, IgG2a autoantibody serum levels reached a maximum of only 22 μg/ml and arthritis was not observed. To evaluate the possibility of a link between high-percent IgG2a responsiveness and the H-2d haplotype, we analyzed the response of the B10.D2/n strain, which has the H-2d haplotype of the D2 strain on the B10 (Ig-1b) background. Unlike the D2 strain, this H-2d congenic strain was a low-percent IgG2a responder, similar to the B10 strain. Both of these latter strains also were low-total IgG responders, resulting in serum IgG2a autoantibody levels that failed to reach arthritic D1 levels. The 8% value for incidence of disease in the B10 strain shown in Table V refers to the 1 of 12 mice that developed disease and markedly elevated levels of IgG2b autoantibody (Table IV).

B6D1 F_1 hyrids (H-2 b/q, Ig-1 b/c) were low-total IgG responders similar to

TABLE IV

Comparison of Arthritic and Nonarthritic IgG Autoantibody Responses in B6 and B10 Mice

Group (*n*)	Arthritis	Anti-mouse CII IgG				
		μg/ml Total	Mean percent (range)			
			1	2a	2b	3
B6* (12)	−	57 (17–182)	20 (3–60)	20 (6–44)	45 (16–73)	16 (6–39)
$B6_2$ (9)	−	31 (19–65)	8 (5–11)	14 (11–21)	59 (49–68)	18 (15–30)
$B6_2$ (1)	+	573	2	6	78	14
$B6_2$/CYT (9)	−	89 (29–166)	6 (2–10)	17 (7–25)	62 (48–85)	16 (6–30)
$B6_2$/CYT (1)	+	555	4	4	88	4
$B10_1$ (11)	−	19 (7–43)	11 (5–23)	24 (16–30)	50 (29–65)	17 (13–32)
$B10_1$ (1)	+	122	2	13	78	7

Mice were immunized with 100 μg chick CII and either 250 ($B10_1$) or 500 ($B6_2$) μg mycobacteria in IFA: B6 mice also were boosted with 100 μg chick CII in IFA 80 d after the primary immunization. Cytoxan (CYT) (10 mg/kg) was administered by the intraperitoneal route 2 d before the primary and booster immunizations; B6 mice were bled 15 d after the booster, 4 and 7 d before the onset of arthritis in two mice; B10 mice were immunized once and bled on day 44, 5 d after the onset of arthritis in one mouse. Total and percent IgG autoantibody subclass values were derived from individual μg/ml autoantibody subclass values determined by IgG autoantibody subclass ELISA on mouse CII-coated microtiter plates, as explained in Materials and Methods.

* Nonarthritic B6 mice immunized once with 100 μg chick CII and 250 μg mycobacteria in IFA and bled on day 44 (Fig. 1).

TABLE V

Genetic Variation in the Total IgG Autoantibody Response, the Percent IgG2a Autoantibody Response, and Susceptibility to CII AIA

Strain (*n*)	H-2	Ig-1	Anti-mouse CII IgG [mean (range)]			Arthritis
			Total	IgG2a	IgG2a	
			μg/ml	%	*μg/ml*	%
D1 (8)	q	c	700 (81–1943)	79 (54–97)	560 (44–1448)	88
D2 (6)	d	c	20 (8–47)	63 (47–82)	12 (5–22)	0
B10 (12)	b	b	27 (7–122)	24 (13–38)	5 (2–16)	8
B10.D2/n (6)	d	b	6 (4–10)	38 (20–50)	2 (2–3)	0
B6 (12)	b	b	57 (17–182)	20 (6–44)	8 (5–14)	0
B6D1 (12)	b/q	b/c	82 (13–358)	67 (50–95)	57 (9–214)	33
B6D1 × D1 (5)	—	—	840 (242–1231)	79 (70–85)	666 (187–970)	100
SWR (8)	q	c	770 (121–2267)	79 (67–90)	626 (108–1945)	0

Mice were immunized with 100 μg chick CII and 250 μg mycobacteria in IFA as described previously for D1 (Table I), B10 (Table IV), B6 (Fig. 1), and B6D1 (Table III) strains; serum IgG autoantibody subclass levels were determined by IgG autoantibody subclass ELISA on mouse CII-coated microtiter plates as described in Materials and Methods. Total and percent IgG2a were determined from individual μg/ml values of IgG1, 2a, 2b, and 3.

the B6 parent strain, but high-percent IgG2a responders similar to the D1 parent strain. In those B6D1 mice at the upper range of the total response, IgG2a autoantibody levels were in the range of arthritic D1 levels and disease was

observed. In the five backcrosses to D1, total IgG responses were low in one and high in the other four, while the percent IgG2a response was high in all five. In the mouse with low total IgG autoantibody, however, the IgG2a autoantibody level was in the range of arthritic D1 levels; disease was observed in this animal as it was in the other four. Based upon the responses of both the B6D1 and D1 backcrosses, high-percent IgG2a responsiveness appears to be inherited as a dominant trait.

Finally, the IgG2a autoantibody response of the resistant and C5-deficient (15) H-2q/Ig-1c SWR strain was compared with that of the D1 strain (Table V). Arthritis was absent in all eight SWR mice, despite the induction of an autoimmune response that was equivalent to that of arthritic D1 mice in terms of total IgG autoantibody and percent IgG2a autoantibody.

Discussion

The results of this study demonstrate an important role for the IgG autoantibody subclass response in CII AIA. In the susceptible D1 strain, the IgG autoantibody response consisted predominantly of the IgG2a subclass and, in the resistant B6 strain, the IgG2a autoantibody subclass response was relatively deficient, despite similar IgG1, 2b, and 3 autoantibody responses. Estimates of B6 antibody crossreactivity and affinity for mouse CII were similar to those of D1 antibody, indicating that these parameters were not relevant to resistance to arthritis in this strain. In recent studies, B6 mice were found to readily develop arthritis after passive transfer of affinity-purified rat anti-CII antibody (unpublished data), which is thought to consist primarily of the IgG2a subclass (5, 18). Studies by Farkas et al. (16) and Ey et al. (17) suggest that rat IgG2a and mouse IgG2a are similar, since IgG2a from either species is the most potent activator of the classical complement cascade, with IgG2b being less potent but more effective than IgG1. Thus, this passive transfer study tends to rule out the possibility that resistance in the B6 strain was due to defects in humoral effector systems, e.g., complement component deficiencies and/or inflammatory cell defects, and also provides support for the link between susceptibility and the IgG2a subclass. In (B6D1)F_1 hybrids, arthritis developed only in those animals with IgG2a autoantibody responses that approximated or exceeded those in arthritic D1 animals, further supporting the importance of the IgG2a autoantibody response in determining susceptibility to CII AIA.

As additional evidence for the potential pathogenicity of the IgG2a subclass, we recently found (6) that purified D1 IgG2a antibody will activate C5 to C5a when bound to cartilage in vitro and that C5-deficient mice are resistant to arthritis by passive transfer of CII antibody. Thus, the failure of B6 mice to develop disease, despite the production of total IgG autoantibody levels within the range of arthritic D1 mice, might be explained by the relative decrease in this potent complement-activating subclass.

Aside from the greater complement-activating potency of IgG2a, it is also of interest that bound immune complexes consisting of IgG2a appear to be the most potent activators of Fc receptor–bearing macrophages (19). This finding might be relevant to a further pathogenic role for the IgG2a autoantibody

subclass in promoting the activation of mononuclear cell/macrophage–like cells found in areas of cartilage erosion in CII AIA.

It should be noted that IgG2b and 1 are not devoid of either complement- (17) or macrophage (19)-activating activity, but rather are less active than IgG2a. Thus, it would be reasonable to expect that CII AIA could be induced by either subclass, if a sufficient quantity is produced and binds to the articular cartilage. The development of arthritis in three otherwise resistant B6 and B10 mice (Table IV) appears to represent evidence for such a possibility in the case of the IgG2b subclass. The recent report by Wooley et al. provides additional support for this possibility (20).

The finding that susceptibility to CII AIA is linked to the predominant response of a single IgG subclass, e.g., IgG2a or 2b, has relevance to immunoregulatory mechanisms as well as to potential strategies for modulating diseases involving a particular autoantibody subclass. The regulation of IgG2a expression is linked with specific T cell mechanisms (21). Modulation of these potential subclass regulatory mechanisms in the D1 strain, for example, might provide a more selective immunosuppressive effect by not interfering with IgG1 or 3 (or IgE, A, etc.) antibody subclass responses to other antigens, e.g., bacterial, viral, tumor, etc.

Analysis of the immunogenetics of the IgG2a autoantibody subclass response in Table V suggests that CII AIA represents a model of autoimmunity in which susceptibility is dependent upon the interaction of both MHC and non-MHC genes. According to this model, MHC genes, specifically I-A genes, regulate the magnitude of the total IgG autoantibody response, and non-MHC genes, perhaps Igh-C genes, regulate the relative fraction of IgG autoantibody as IgG2a. Such a model was initially suggested by the dissociation of the magnitude of the total IgG autoantibody response and the relative percent of IgG2a autoantibody observed in the D1-D2 and B6-B6D1 comparisons (Table V). The general concept that H-2 genes are involved in regulating the magnitude of humoral responses is well established (22). In the case of CII AIA, Wooley et al. (7) and Huse et al. (8) used H-2 recombinant strains of mice to suggest that susceptibility and the high (total) antibody response to CII in the D1 strain is linked to the I-Aq haplotype of the H-2 or MHC.

The D2-B10.D2/n comparison in Table V provides the basis for suggesting that non-MHC genes might be involved in regulating the relative percent of total IgG autoantibody as IgG2a. The H-2d/Ig-1c D2 strain was a high-percent IgG2a autoantibody responder, while the H-2d/Ig-1b B10.D2/n congenic strain was a low-percent IgG2a autoantibody responder. Thus, high-percent IgG2a autoantibody responsiveness was not linked to the H-2 haplotype of the D2 strain. This trait also does not appear to be linked to the H-2 haplotype of the D1 strain, since IgG2b and not IgG2a was described as the predominant IgG antibody subclass in the H-2 congenic B10.Q strain (20). Both of these comparisons provide strong evidence of a link between the percent IgG2a autoantibody subclass response and a non-MHC locus (or loci).

Idiotypes and allotypes are known to be linked to immunoglobulin genes. We suggest that an Igh-C gene(s) might be involved in determining the relative fraction of total IgG autoantibody as IgG2a. This suggestion is based upon the

finding that (*a*) the percent IgG2a autoantibody response differed in H-2 congenic strains (Table V, D2 vs. B10.D2/n), thereby suggesting linkage to a locus outside the H-2 or MHC, and (*b*) there is a link between high-percent IgG2a autoantibody responsiveness in Ig-1c D1, D2, and SWR strains (Table V). An interesting consequence of Igh-C gene involvement might be the use of Ig-1 allotypes in predicting susceptibility to a disease involving a particular IgG subclass such as IgG2a. For example, in the case of an experimental disease like CII AIA, the presence of the Ig-1c allotype would predict high-percent IgG2a autoantibody responsiveness and the possibility of disease. As shown in Table V, high-percent IgG2a autoantibody responsiveness appears to be a dominant trait, so that high-percent IgG2a autoantibody responsiveness would be expected even in individuals heterozygous for the Ig-1c allotype. Of course, additional studies with allotype congenic strains are needed to determine if this trait is linked to the Igh-C locus, as well as to determine if this trait is associated with other Ig-1 allotypes (Ig-1a, d, e, etc.).

In a recent study, Wooley et al. (23) noted that the D1 and SWR strains are both H-2q and are equally immunoresponsive to CII, yet the D1 strain is susceptible to CII AIA, while the SWR strain is resistant. We analyzed the IgG autoantibody subclass response of the SWR strain and, as shown in Table V, total IgG autoantibody and percent IgG2a autoantibody responses were high and essentially identical to those of the D1 strain. Based upon the demonstrated resistance of C5-deficient mice to passively transferred disease (6), we suggest that resistance in the SWR strain is related to the fact that this strain is genetically deficient in C5 (15). C5 is linked to the Hc locus, which probably is not within the MHC (24). Thus, susceptibility to CII AIA appears to be linked to two non-MHC genes, one that determines the availability of C5 and another which ensures that a high percent of total IgG autoantibody is an appropriate complement activating subclass, and to an MHC gene that determines the overall magnitude of the IgG autoantibody response.

The data in Tables III and V emphasize that none of these genetic factors alone is sufficient for disease. The 8 of 12 B6D1 mice that failed to develop disease were high-percent IgG2a autoantibody responders, as were D2 mice. However, in these cases, total IgG autoantibody levels were too low, i.e., below the lowest IgG2a autoantibody level associated with disease. In the case of nonarthritic B6 mice, total responses were within the range of arthritic B6D1 levels, but the percent IgG2a response was too low to ensure adequate serum IgG2a autoantibody levels. Finally, the resistant SWR strain is both a high-total and a high-percent IgG2a responder, but is genetically deficient in C5.

In conclusion, susceptibility to CII AIA can be regarded as a complex interaction of multiple MHC and non-MHC genes that control not only the total humoral response, but also the quality of this response, e.g., the IgG2 subclass as well as the availability of mediators of the inflammatory response such as complement C5.

Summary

Analysis of the IgG autoantibody subclass response in the collagen II autoimmune arthritis (CII AIA)-susceptible D1 strain mice revealed that the onset of

disease was associated with a predominance of IgG2a autoantibody. In a comparative study, resistance in the B6 strain was associated with a deficient IgG2a autoantibody response. B6 IgG1, 2b, and 3 autoantibody responses generally overlapped those of arthritic D1 mice, and estimates of antibody crossreactivity and affinity were similar for both strains. In crosses between D1 and B6, arthritis developed only in those F_1 mice with IgG2a autoantibody responses approximating or exceeding those in arthritic D1 mice. Additional studies with B6 and B10 strains suggested an alternate role for the IgG2b autoantibody response.

In inbred strains with known genetic backgrounds, a dissociation between the magnitude of the total IgG autoantibody response and the percent of total as IgG2a was demonstrated. The H-2q, Ig-1c D1 strain was a high-total and high-percent IgG2a responder, while the H-2d, Ig-1c D2 strain was a low-total but high-percent IgG2a responder. The H-2b, Ig-1b B6 strain was a low-total and low-percent IgG2a responder, while the H-2b/q, Ig-1b/c (B6D1)F_1 hybrid was a low-total but high-percent IgG2a responder. A further dissociation between high-percent IgG2a autoantibody responsiveness and the H-2 haplotype was demonstrated by the H-2 congenic B10.D2/n (H-2d, Ig-1b) strain, in which a low-percent IgG2a response was observed to differ from the D2 strain. High-percent IgG2a autoantibody responsiveness also appeared to be inherited as a dominant trait based upon high responses in all (B6D1)F_1 hybrids and backcrosses to D1. These findings suggest that the H-2 haplotype is involved in the total IgG autoantibody response but that the relative fraction of the total response as IgG2a is independent of the H-2 haplotype and possibly related to Igh-C genes.

C5-deficient SWR (H-2q, Ig-1c) mice were found to have a high total autoantibody response to mouse CII and IgG2a comparable to arthritic D1 mice, but these mice did not develop arthritis.

Based upon these observations, we conclude that susceptibility to CII AIA requires the interaction of multiple genes, both major histocompatibilty complex (MHC) and non-MHC, which influence the magnitude (total IgG) and the quality (IgG subclass) of the autoimmune response and the availability of appropriate mediators (C5) to initiate the inflammatory reaction.

Received for publication 15 July 1985.

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