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Type II Collagen-induced Murine Arthritis

II. Genetic Control of Arthritis Induction Is Expressed on L3T4$^+$ T Cells Required for Humoral as well as Cell-mediated Immune Responses

NOBUO SEKI*

Department of Zoology, Faculty of Science, Kyoto University
Sakyo-ku, Kyoto, 606, Japan

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Abstract The present study is concerned with the cellular and molecular mechanisms responsible for the genetic control of type II collagen-induced murine chronic arthritis. Analyses were made for both humoral and cellular immune responses, since the induction of arthritis required synergy between both types of immunity. The results indicate that the genetic control of the induction of arthritis is not expressed on B cells but on L3T4$^+$ T cells which are required for generating anti-collagen humoral as well as cell-mediated immunities as assessed by DTH response *in vivo* or lymphokine productions *in vitro*.

INTRODUCTION

Incidence of collagen-induced arthritis has been demonstrated to be under genetic control in rodents with the use of inbred, congenic, and recombinant strains (Wooley et al., 1981; Griffiths et al., 1981a; Griffiths et al.,1981b; Wooley et al.,1983). The susceptibility is mapped mainly to the I region of the H-2^q haplotype (Wooley et al., 1981), especially to I-A^q subregion subregion (Huse et al., 1984; Holmdahl et al., 1986), and genes other than I-A^q also appear to be involved in the genetic control (Wooley et al., 1983; Griffiths et al., 1984). Such a genetic control has been established based on the observation that the ability to mount a type II collagen-antibody response is associated with genes inside and/or outside the major histocompatibility complex (MHC). However, recent studies from our laboratories have revealed that a chronic, perpetuating arthritis can not be induced merely by humoral immunity but by the synergy between humoral and L3T4$^+$ T cell-mediated immunities (Seki et al., 1988).Since anti-collagen antibody response is generated by B cells and helper T cells assisting B cell responses, the overall anti-collagen immune responses consist of a complex network of B cells and various functionally distinct T cells. These considerations prompted us to investigate more accurately the cellular and molecular mechanisms underlying the genetic control in the induction of chronic arthritis.

In the present study, an attempt was made to localize cellular site(s) at which genetic control is expressed. The results demonstrate that B cells themselves from high and low

*Present Address: New Drug Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 2-1-6, Kashima, Yodogawa, Osaka, 532 Japan

responder strains have the potentitial to mount comparable anti-collagen antibody responses. These observations, together with evidence of high and low levels of antibody production in these strains, suggested the localization of genetic defect in the anti-collagen antibody responses at the helper T cell level. In fact, this was verified by the results that $L3T4^+$ T cells from high and low responders are associated with potent and weak production of lymphokines such as interleukin(IL) 6, which represents an aspect of helper T cell function in antibody responses. Moreover, it was demonstrated that there exist appreciable differences between high and low responder $L3T4^+$ T cells in the expression of cell-mediated immunity as exemplified by delayed-type-hypersensitivity (DTH) response in vivo or the production of IL-2 and γ-interferon(γ-IFN) *in vitro*. These results indicate that genetic control of anti-type II collagen immune responses is expressed at the level of $L3T4^+$ T cell subset responsible for assisting anti-collagen antibody responses, as well as expressing cell-mediated immunity, both of which represent absolute requirements for inducing a chronic arthritis.

MATERIALS AND METHODS

Mice

DBA/1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and C3H/He, C57BL/6, and BALB/c mice were obtained from Japan SLC Inc. (Shizuoka, Japan). All mice were used at 6 to 8 wk of age.

Collagen

Bovine type II collagen was obtained from Cosmo-Bio,Co.Ltd., (Tokyo, Japan) and dissolved in 0.1 M acetic acid. Denatured collagen was prepared by placing collagen solution in a boiling water bath for 5 min (Seki et al.,1988). For in vitro studies, collagen solution was dialyzed against RPMI1640 medium.

Mouse thyroglobulin

Mouse thyroglobulin was prepared according to the procedure previously described (Sugihara et al., 1988). As analyzed by polyacrylamide gel electrophoresis, more than 95% of the peparation was 19S thyroglobulin and the remaining consisted of 27S iodoprotein.

Sensitization to collagen

Mice were sensitized at tail base with 200μg native or denatured collagen emulsified in complete Freund's adjuvant containing *Mycobacterium tuberculosis* strain $H_{37}R_V$ (Wako Pure Chemical Industries Ltd., Osaka, Japan), and boosted with the same preparations of antigen plus complete Freund's adjuvant 3 weeks later.

Incidence and scoring of arthritis

Incidence of arthritis was determined 5 weeks after the first immunization with collagen unless otherwise indicated. An animal was judged to be arthritic if one or more joint regions (digitus, wrist, or ankle) were red and swollen. In some experiments, severity was graded visually by using a method adapted from that previously described for rats (Rogers et al.,1980), and expressed by the arthritic index which was obtained by scoring

each limb from 0–3 severity-grade and by summing up scores of four limbs.

Immunoassay of antibody to collagen

An enzyme-linked immunosorbent assay (ELISA) was used to detect anti-collagen or anti-thyroglobulin antibodies. The method was adapted from that previously described (Sugihara et al., 1988; Stuart & Dixon,1983; Fujitsu et al.,1986). Briefly, wells of flat-bottomed microplates (Dynatech Corp., Chantilly, VA) were coated with 50 μl of native collagen (50μg/ml) or thyroglobulin (10μg/ml) in phosphate-buffered saline (PBS; 10mM phosphate, 150 mM NaCl, pH7.6), at 37°C for 1 hr. The wells were then washed three times with PBS containing 0.05% Tween 20 and 0.1% BSA, and 50 μl of sample diluted with PBS containing 0.05% Tween 20 and 0.5% BSA was added. After incubation for 1 hr at room temperature,the wells were washesd three times with PBS containing 0.05% Tween 20 (PBS-Tween), incubate with 50μl of a 1:200 dilution of goat anti-mouse IgG coupled to horseradish peroxidase (Sigma Chemical Co., St.Louis, MO) at room temperature for 30 min, washed three times with PBS-Tween, and developed at room temperature for 20 min in the dark with 0.2ml of 0.4mg/ml O-phenylene-diamine and 0.003% H_2O_2 in 0.05M citrate-0.1M phosphate buffer pH 4.5. The reaction was stopped by adding 50μl of 6.6% sulfuric acid, and the OD at 490 nm was read using Micro ELISA Mini Reader MR 590 (Dynatech Corp.). As a reference, a serum pool from DBA/1 mice exhibiting typical arthritis was given arbitarily a value of 100 U/ml.

In Vitro Cultures, Polyclonal activation of splenic B cells with lipopolysaccharide (LPS)

Spleen cells (1 x 10^6/well) were cultured with or without LPS (1 μg/well) in RPMI-1640 medium supplemented with 10% fetal calf serum and 5 x 10^{-5}M 2-mercaptoethanol in a 2-ml volume in 24-well culture plates (No.25820, Corning Glass Works, Corning, NY) at 37°C in a humidified CO_2 incubator (5% CO_2, 95% air) for 7 days. Culture supernatants were collected and anti-collagen or anti-thyroglobulin antibody contained in these culture supernatants were assessed by ELISA.

Production of IL-2 and IL-6

Lymph node cells (4 x 10^6/well) were cultured with 10–100 μg/ml collagen in RPMI-1640 medium supplemented with 0.5% normal mouse serum and 5 x 10^{-5}M 2-mercaptoethanol in a 1-ml volume in 24-well culture plates for 1 day. IL-2 or IL-6 activity was measured by using the respective lymphokine-dependent CTLL-2 (Baker et al.,1979) or PIL6 cell line (Takai et al.,1989). It was previously confirmed that CTLL-2 responds to IL-2 but not to IL-4 (Ogata et al.,1987). Briefly, 4 x 10^3 CTLL-2 or 10^4 PIL6 cells were cultured in a volume of 200 μl containing the above culture supernatant. After 1 (CTLL-2) or 5 days (PIL6) of incubation at 37°C, cells were pulsed with 0.5 μCi/well of ^{3}H-TdR and harvested 6 hr later. The incorporated radioactivity was measured with a scintillation spectrometer (Aloka, Co., Ltd., Tokyo, Japan). The results of the ^{3}H-TdR incorporation assay were expressed as the mean cpm of triplicate cultures.

Absorption of culture supernatants with collagen-or thyroglobulin-coated microplates

Culture supernatants obtained after polyclonal stimulation of splenic B cells with LPS were absorbed once or twice with collagen or thyroglobulin by placing in wells of microplates coated with collagen or thyrogrobulin at room temperature for 1 hr. The

procedure of coating was similar to the case of ELISA.

DTH response to collagen

Assay system was essentially the same as described for viral protein (Takai et al.,1985). Briefly, anti-collagen DTH response was assessed by the direct challenge of 100 μg native collagen in a 25 μl volume into hind footpads of collagen-primed mice. Twenty four hours after the direct challenge, the footpad thickness was measured by using a micrometer. Footpad swelling was expressed by the mean thickness increment ± S.E. of five mice/group.

Preparation of serum concentrate for transfer studies

Immune sera for transfer were obtained from arthritic DBA/1 mice 5 weeks after primary immunization with collagen. The sera were pooled, and an equal volume of saturated solution of ammonium sulfate was added to precipitate the immunoglobulin. The precipitate was collected by centrifugation, ressolved in a minimal volume of water, and dialyzed against 0.15M NaCl and 0.01M sodium phosphate (PBS) at pH 7.2. The volume was then adjusted to 1/3 that of the orignal serum, the concentrate was sterilized by microporous filtration through a 0.45-μm disposable filter unit. The concentrate was stored at-80°C until use.

Treatment of immune lymphoid cells with antibody plus complement

Monoclonal antibody to Lyt-2 antigen was used. The 3.155 monoclonal antibody-producing hybridoma cell line, specific for the Lyt-2 molecule was obtained from ATCC (Rockville, MD). Lymphoid cells (5×10^7) from immunized mice were incubated at room temperature for 40 min with ascitic form of anti-Lyt-2 monoclonal antibody at a dilution of 1:10. Cells were washed and incubated at 37°C for 45 min with rabbit C preabsorbed with syngeneic mouse spleen cells at a final dilution of 1:10.

RESULTS

Comparable anti-collagen responsiveness of B cells between high and low responder strains

As is shown in Table 1, whether homozygously or heterozygously, the mouse strains expressing H-2^q haplotype (e.g., DBA/1) are highly susceptible to collagen-induced arthritis compared to those bearing H-2 haplotypes other than H-2^q such as H-$2^{b,d,or\,k}$, and that these high and low susceptibilities are associated with the respective high and low levels of production of anti-collagen antibody after immunization with collagen (Wooley et al., 1981; Wooley et al., 1983; Huse et al., 1984; Holmdahl et al., 1986). In order to investigate whether the difference in the magnitude of anti-collagen antibody responses is ascribed to B cells, helper T cells, or both, we first examined the potential anti-collagen responsiveness of B cells from high and low responder mice. Thus, spleen cells from various strains of normal unimmunized mice were stimulated *in vitro* with LPS and anti-collagen antibody activity produced in culture supernatant after polyclonal activation with LPS was assessed by ELISA. The results of Table 1 show that culture supernatants of spleen cells from two low responder strains (C57BL/6 & C3H/He) exhibit comparable anti-collagen reactivity to those from high responder strains [DBA/1 and (C57BL/6 x

DBA/1)F1], although another low responder (BALB/c) spleen cells produced a moderately reduced magnitude of anti-collagen reactivity for undetermined reasons.

To examine the specificity of anti-collagen reactivity generated in the culture supernatants, such supernatants were absorbed with microplates coated with either collagen or mouse thyroglobulin as control (Fig. 1). Analysis of the resultant culture supernatants in ELISA have revealed that the absorption with collagen or thyroglobulin resulted in appreciable reduction of anti-collagen or anti-thyroglobulin reactivity, respectively. Thus, the results of Figure 1 indicate the production of antibodies specific for collagen or thyroglobulin in the supernatants from polyclonally stimulated cultures and again demonstrate comparable magnitudes of anti-collagen antibody responses by high (DBA/1) and low (C57BL/6) responder splenic B cells. This also suggests that genetic difference in the magnitude of anti-collagen antibody response is controlled at the level of helper T cells rather than B cells.

Strain difference in the induction and expression of anti-collagen L3T4$^+$ T cell-mediated immune responses

High and low responder strains of mice were sensitized with denatured collagen and their ability to express anti-collagen DTH response was assessed by the direct challenge with native collagen (100 or 10 μg/head) into their footpads. The results (Fig. 2) demonstrate that high (DBA/1) resdponder mice exhibit potent and low (C57BL/6, BALB/c, C3H/He) responder mice exhibit weak (100 μg challenge) or only marginal (10 μg challenge) footpad reactions.

Lymph node cells from the same batches of denatured collagen-sensitized mice as used in Figure 2 were assessed for their capacity to produce lymphokines. The results (Table 2) demonstrate that L3T4$^+$ T cells from DBA/1 mice exhibit potent capabilities of the production of IL-2 and IL-6, whereas either whole or L3T4$^+$ T cells from low responder mice produce appreciably (C57BL/6) or apparently (BALB/c and C3H/He) reduced amounts of these lymphokines. Similar strain difference was observed for the

Table 1. Induction of type II collagen-induced arthritis and production of anti-collagen antibody in various strains of mice.

Mouse strain	Incidence of arthritis[a)]	Anti-collagen antibody response	
		serum (U/ml)[b)]	Culture SN (OD490nm)[c)]
DBA/1	30/30	309 ± 21	0.548 ± 0.005
C57BL/6	2/30	43 ± 9	0.502 ± 0.087
BALB/c	0/30	13 ± 1	0.202 ± 0.092
C3H/He	1/30	69 ± 10	0.488 ± 0.015
(C57Bl/6 x DBA/1)F1	30/30	405 ± 74	0.529 ± 0.094

a) Various strains of mice were immunized with 200 μg native bovine type II collagen in complete Freund's adjuvant at tail base and boosted 3 weeks later. Diagnosis of arthritis was performed 5 weeks after the first immunization.

b) Titers of anti-type II collagen antibody was determined by ELISA at 5 weeks after the first immunization and expressed by the mean U/ml ± S.E., as previously described (Seki et al., 1988).

c) Spleen cells (1 x 10^6) from normal unimmunized mice were stimulated *in vitro* with LPS (1 μg/ml) for 7 days. Anti-collagen antibody activity produced in culture supernatants (SN) was determined by ELISA and expressed by the mean OD490 ± S.E. of triplicate cultures.

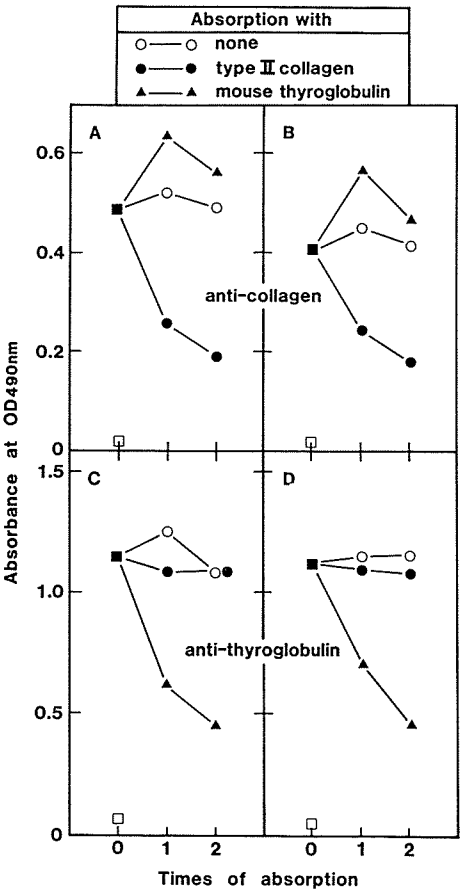

Fig. 1. Comparable anti-collagen antibody production of splenic B cells from high and low responders following polyclonal activation with LPS. Splenic B cells (1 x 10^6) from DBA/1 (panels A & C) or C57BL/6 (panels B & D) were stimulated in vitro with LPS (1 μg/ml) for 7 days. Culture Supernatants were unabsorbed or absorbed with either collagen or thyroglobulin, and anti-collagen (panels A & B) or anti-thyroglobulin (panels C & D) antibody was determined by ELISA. Symbols □ and ■ represent supernatants obtained after culturing without and with LPS, respectively. Each value was expressed the mean of triplicate assays.

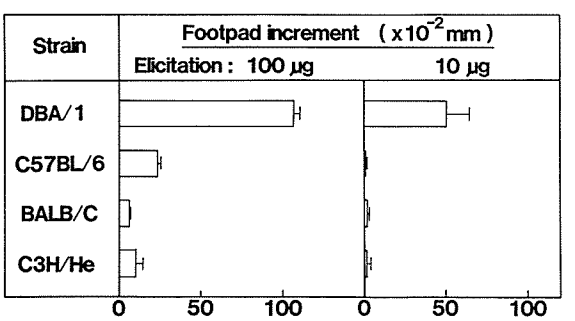

Fig. 2. Strain difference in DTH response. Various strains of mice (5 mice/group) were sensitized with 200 μg denatured collagen. Two weeks later, these mice received the direct challenge of 100 μg or 10 μg native collagen in their footpads.

production of γ-interferon (γ-IFN) and IL-5 by L3T4$^+$ T cell subset (data not shown). Thus, these results indicate that genetic difference in anti-collagen immune response is detected at the level of L3T4$^+$ T cell-mediated immunities which represent (1) DTH responses associated with the production of IL-2 and γ-IFN, and (2) helper T cell response for humoral immunity as monitored by the production of IL-6 and IL-5.

Table 2. Strain difference in capacities of lymphokine production following stimulation with collagen.

Strain	T cell subset[a]	Stimulation with denatured collagen (μg/ml)	^{3}H-TdR uptake[b] (cpm) of cells:			
			CTLL-2		PIL6	
			Exp.1	Exp.2	Exp.1	Exp.2
DBA/1	whole	10	3284	1259	15823	7961
		100	5625	4045	22027	12148
	L3T4$^+$ T	10	N.D.	5396	N.D.	8853
		100	N.D.	10063	N.D.	16147
C57BL/6	whole	10	0	0	2241	3048
		100	1446	211	2909	4968
	L3T4$^+$ T	10	N.D.	0	N.D.	3713
		100	N.D.	1748	N.D.	8615
BALB/c	whole	10	80	20	0	269
		100	94	0	1559	255
	L3T4$^+$ T	10	N.D.	0	N.D.	0
		100	N.D.	0	N.D.	121
C3H/He	whole	10	47	0	1024	0
		100	408	0	921	551
	L3T4$^+$ T	10	N.D.	0	N.D.	80
		100	N.D.	191	N.D.	1279

[a] The whole fraction of spleen cells or L3T4$^+$ T-enriched fraction were stimulated *in vitro* with denatured collagen at doses indicated. To obtain the L3T4$^+$ T-enriched fraction, spleen cells were treated with anti-Lyt-2 antibody plus C. The resultant cells were seeded onto anti-mouse immunoglobulin-coated dishes and nonadherent cells were collected. Flow microfluorometric analyses revealed that approximately 80 % cells were of L3T4$^+$.

[b] Data represent the values after the subtraction of ^{3}H-TdR uptake by culture supernatant without stimulation from ^{3}H-TdR uptake by culture supernatant with stimulation. S.E. were omitted from the data for simplicity since they were less than 10 % in each group.

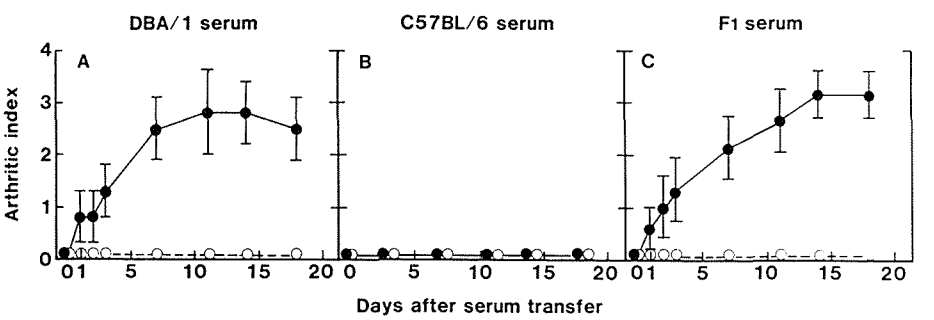

Fig. 3. Strain differense in the induction of arthritis by synergy between cell-mediated and humoral immunities. DBA/1 (●——●) or C57BL/6 (○——○) mice sensitized twice with heat-denatured collagen were transferred with concentrated serum (0.2ml) from DBA/1 (panel A), C57BL/6 (panel B), or (C57BL/6 x DBA/1)F1 (panel C) mice which had been immunized with native collagen twice. Serum transfers were performed on days 0 and 1. Arthritic index was expressed by the mean ± S.E. of 5 mice/group.

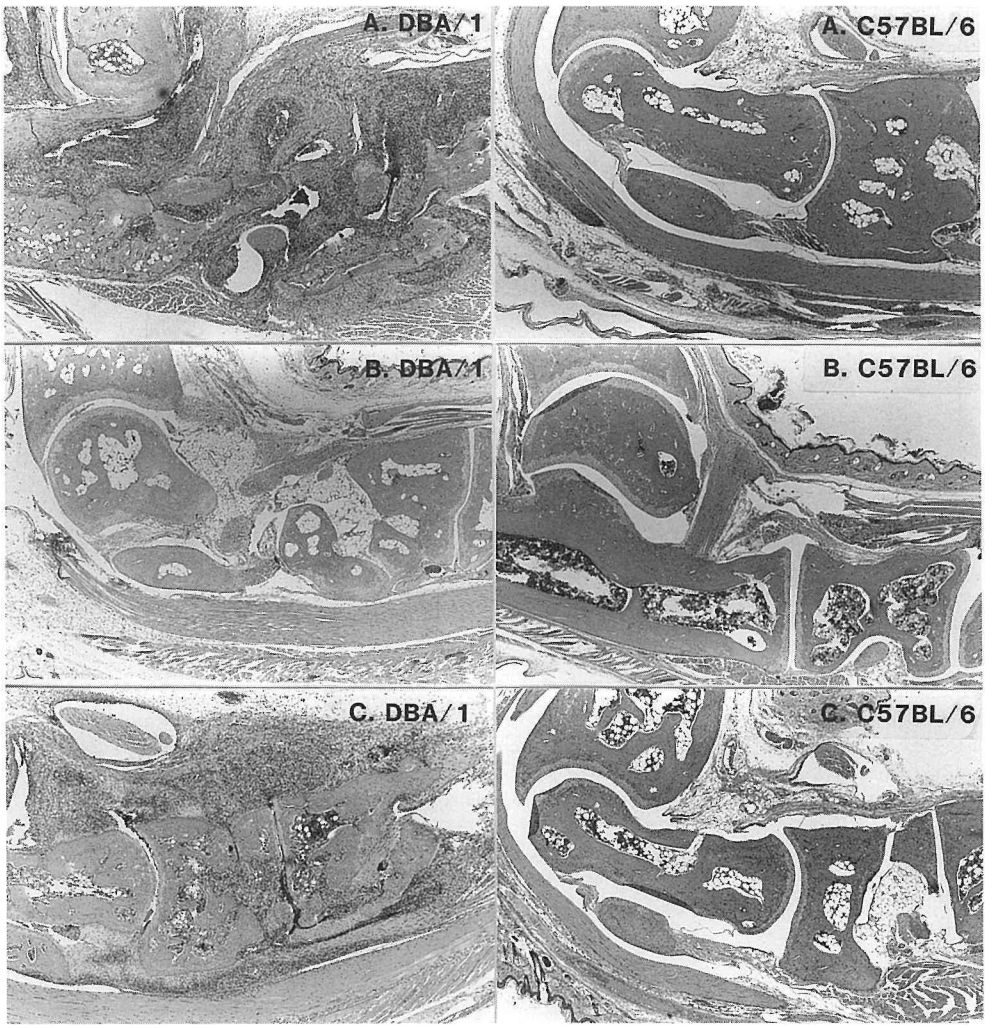

Fig. 4. Light micrographs of joints. Photographs A, B, and C correspond to Groups (panels) A, B, and C in Figure 3. H & E Staining, x 10.

Strain difference in the induction of cell-mediated immunity required for development of clinical arthritis

We finally investigated whether genetic difference of anti-collagen L3T4$^+$ T cell-mediated immunity is crucially related to the high or low incidence of arthritis induction. High (DBA/1) or low (C57BL/6) responder strains of mice were allowed to generate selectively L3T4$^+$ T cell-mediated immunity by sensitization with heat-denatured collagen. These mice received two rounds of injection of cocentrated serum which was prepared from native collagen-immunized DBA/1, C57BL/6, or (C57BL/6 x DBA/1)F1 mice. The results are summarized in Fig. 3. The results clearly demonstrate that arthritis is induced in high responder mice only when they are inoculated with concentrated serum prepared from high or (high x low)F1 responder mice. The histopathological change of

joint from each group of mice is also shown in Figure 4. These results confirm that synergy between humoral and L3T4$^+$ T cell-mediated immunities is required for inducing a chronic arthritis and illustrate that the generation of L3T4$^+$ T cell-mediated immunity responsible for the clinical manifestation of the chronic arthritis is under the genetic control(Fig. 4).

DISCUSSION

Susceptibility to collagen-induced arthritis is associated with MHC genes in mice (Wooley et al., 1981; Griffiths et al., 1981a; Griffiths et al., 1981b; Wooley et al., 1981; Huse et al., 1984; Holmdahl et al., 1986). The association with a specific phenotype of MHC has also been documented in the development of human rheumatoid arthritis (Sasazuki, 1983; Aho et al., 1986). Therefore, analysis of cellular and molecular mechanisms underlying the collagen-induced experimental arthritis could contribute to a better understanding of the pathogenesis of rheumatoid arthritis.

We have demonstrated that the induction of a typical, chronic arthritis requires synergy between antibody and L3T4$^+$ T cell-mediated immunity (Seki et al., 1988). This indicates that the genetic difference in the induction of arthritis should be analyzed at both aspects of antibody production and induction of L3T4$^+$ T cell-mediated immunity.

The results obtained in this study illustrate: (1) the induction of each component of humoral and cell-mediated immunities required for the development of arthritis is under the genetic control; (2) the potentiality of anti-collagen antibody response by B cells themselves from low responders is almost comparable to that from high responder DBA/1 mice; (3) there exists the genetic difference in responsiveness of anti-collagen L3T4$^+$ T cells including the induction of DTH response and production of various lymphokines; (4) genetic difference in the inducibility of cell-mediated immunity required for the development of a clinical arthritis is also demonstrated in a model in which antiserum is injected into high or low responders to heat-denatured collagen.

The antibody production of normal splenic B cells stimulated with LPS has been shown for a number of autoantigens such as DNA and bromelain-treated erythrocytes (Theofilopoulos & Dixon, 1985). We have also demonstrated that the stimulation in vitro of DBA/1 splenic B cells with LPS resulted in the production of appreciable anti-collagen reactivity in the culture supernatant. The present study further demonstrates that the magnitude of anti-collagen reactivity by low responder B cells was almost comparable to that obtained by B cells from high resonder DBA/1 mice.

The generation of anti-collagen reactivity in culture supernatants after polyclonal activation of B cells with LPS might be interpreted by either of the following two possibilities: (1) anti-collagen cross-reactivity of autoantibodies expressing the characteristics of "multispecificity",and (2) the detection of anti-collagen antibody as a single component among a mixture of autoantibodies with each specificity. To further characterize the above two possibilities, experiments were conducted to test whether a specific autoantibody activity can be absorbed with the relevant autoantigens. Since anti-collagen and anti-thyroglobulin antibody activities were selectively absorbed with the respective antigens, anti-collagen reactivity detected in ELISA was ascribed to the generation of collagen-specific antibody. Thus, taken collectively, the results demonstrate that splenic B cells from high and low responder mice have a potential to

mount comparable levels of anti-collagen antibody response. However, it remains to be determined whether the epitope specificity of anti-collagen antibody produced in high responders is the same as that obtained in low responders. Additional analyses will be, therefore, required to more accurately make the comparison of the epitope specificity of anti-collagen antibody produced *in vitro* as well as *in vivo* between high and low responders.

The fact that genetic control is not detected at B cell level despite differential abilities of various strains of mice to produce anti-collagen antibody after in vivo immunization suggested the localization of the genetic defect in the anti-collagen antibody response at the helper T cell level. This was supported by the detection of genetic difference in abilities of lymphoid cells from high and low responders to produce IL-6 (Table 2) and IL-5 (unpublished observation) which represent the molecular basis for the function of helper T cells assisting antibody response. Thus, it is more likely that the genetic control in anti-collagen humoral immunity is expreesed at the level of the helper T cells but not of B cells.

Since anti-collagen cell-mediated immunity has been demonstrated to be an essential component part of immune response to collagen, the present study has also attempted to investigate the existence of genetic control in the induction of $L3T4^+$ T cells responsible for the mediation of cellular immunity. As shown in the preceding papers, $L3T4^+$ T cell-mediated immunity which induces arthritis by senergy with antibody can be generated by sensitizing DBA/1 mice with heat-denatured collagen. Inasmuch as such $L3T4^+$ T cell-mediated immunity is monitored by DTH response *in vivo* and the production of IL-2 (Table 2) and γ-IFN (unpublished observation), a clear distinction was observed in the DTH inducibility and the levels of these lymphokines produced between $L3T4^+$ T cells from high and low responders. However, it is possible that $L3T4^+$ T cell-mediated immunity involves additional molecular mechanisms. Therefore, further studies are required to investigate the genetic difference in the induction of the overall $L3T4^+$ T cell-mediated immunity. This was approached by injecting antiserum from high or low responder mice into high or low responder mice which had been sensitized with heat-denatured collagen. The results demonstrated that arthritis can be induced solely by using the antiserum from high responders [DBA/1 or (C57BL/6 x DBA/1)F1] only when injected into high responder strains. The above analyses illustrated that generation of the overall $L3T4^+$ T cell-mediated immunity required for arthritis induction is also under genetic control.

The mechanisms by which $L3T4^+$ T cells from low responders exhibit marginal capacities of DTH response and the production of various lymphokines have not been determined in the present study. It could be that there exists the defect of these $L3T4^+$ T cell functions at the level of $L3T4^+$ T cells themselves or antigen-presenting cells that are required for activating $L3T4^+$ T cell subset. Further studies will be required to determine more accurate cellular site(s) for the genetic defect in the anti-collagen $L3T4^+$ T cell activation. Such analyses are currently being approached by examining the activation of $L3T4^+$ T cells from (high x low)F1 mice by collagen in association with antigen-presenting cells from high or low responder mice.

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REFERENCES

Aho,K., M.Koskenvuo, J.Tuominen, and J.Kaprio (1986) Occurence of rheumatoid arthritis in a nationwide series of twins. *J. Rheumatol.*13: 899–902.

Baker,P.E., S.Gillis, and K.A.Smith (1979) Monoclonal cytolytic T-cell lines. *J. Exp. Med.* 149: 273–278.

Fujitsu,T., S.Sakuma, N.Seki, H.Senoh, J.Mori, and H.Kikuchi (1986) Effect of auranofin on autoimmune disease in a mouse model. *Int. J. Immunopharmacol.* 8: 897–910.

Griffiths,M.M., and C.W.DeWitt (1984) Genetic control of collagen-induced arthritis in rats: the immune response to type II collagen among susceptible and resistant strains and evidence for multiple gene control. *J. Immunol.* 132: 2830–2836.

Griffiths,M.M., E.J.Eichwald, J.H.Martin, C.B.Smith,and C.W.DeWitt (1981a) Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis Rheum.* 24: 781–789.

Griffiths,M.M., and C.W.DeWitt (1981b) Immunogenetic control of experimental collagen-induced arthritis in rats. II. ECIA susceptibility and immune response to type II collagen (CALF) are linked to RT1. *J. Immunogenet.* 8: 463–470.

Holmdahl,R., L.Klareskog, M.Andersson, and C.Hansen (1986) High antibody response to autologous type II collagen is restricted to H-2^q. *Immunogenetics* 24: 84–89.

Huse,A., P.H.Wooley, H.S.Luthra, J.M.Sturt, and C.S.David (1984) New recombinants confirm mapping of susceptibility to type II collagen-induced arthritis to the I-A subregion in mice. *Fed. Proc.* 43: 1820.

Ogata,M., S.Sato. H.Sano, T.Hamaoka, H.Doi, K.Nakanishi, Y.Asano, T.Itoh, and H.Fujiwara (1987) Thymic stroma-derived T cell growth factor (TSTGF). I. Functional distinction of TSTGF from interleukin 2 and 4 and its preferential growth-promoting effect on helper T cell clones. *J. Immunol.* 139: 2675–2682.

Rogers,M.P., D.E.Trentham, and W.J.McCune (1980) Effect of psycological stress on the induction of arthritis in rats. *Arthritis Rheum.* 23: 1337–1342.

Sasazuki,T. (1983) Immunogenetic analysis of rheumatoid arthritis. *J. Rheumatol. Suppl.* 10: 54–58.

Seki,N., Y.Sudo, T.Yoshioka, S.Sugihara, T.Fujitsu, S.Sakuma, T. Ogawa, T.Hamaoka, H.Senoh, and H.Fujiwara (1988) Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. *J. Immunol.* 140: 1477–1484.

Stuart,J.M., and F.J.Dixon (1983) Serum transfer of collagen-induced murine in mice. *J. Exp. Med.*158: 378–392.

Sugihara,S., Y.Izumi, T.Yoshioka, H.Yagi, T.Tsujimura, O.Tarutani, Y.Kohno, S.Murakami, T.Hamaoka, and H.Fujiwara (1988) Autoimmune thyroiditis induced in mice depleted of particular T cell subsets. I. Requirement of Lyt-1^{dull} L3T4 ^{bright} normal T cells for the induction of thyroiditis. *J. Immunol.* 141:105–113.

Takai,Y., N.Seki, H.Senoh, T.Yokota, F.Lee, T.Hamaoka, and H. Fujiwara (1989) Enhanced production of interleukin 6 in mice with type II collagen-induced arthritis. *Arthritis Rheum.* 32: 594–600.

Takai,Y., A.Kosugi, T.Yoshioka, S.Tomita, H.Fujiwara, and T. Hamaoka (1985) T-T cell interaction in the induction of delayed-type hypersensitivity (DTH) responses: vaccinia virus-reactive helper T cell activity in enhanced in vivo induction of DTH responses and its

application to augmentation of tumor-specific DTH responses. *J. Immunol.* 134: 108–113.
Theofilopoulos,A.N., and F.J.Dixon (1985) Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37: 269–390.
Wooley,P.H., A.M.Dillon, H.S.Luthra, J.M.Sturt, and C.S.David (1983) Genetic control of type II collagen-induced arthritis in mice: factors influencing disease susceptibility and evidence for multiple MHC-associated gene control. *Transplant. Proc.* 15: 180–185.
Wooley,P.H., H.S.Luthra, J.M.Stuart, and C.S.David (1981) Type II collagen-induced arthritis in mice. I.Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* 154: 688–700.

著者： 関 信男，〒532 大阪市淀川区加島2-1-6，藤沢薬品工業株式会社新薬研究所，電話番号 (06) 390-1145