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In vivo elimination of T cells expressing specific T-cell receptor Vβ chains in mice susceptible to collagen-induced arthritis

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SUMMARY

Type II collagen-induced arthritis (CIA) is believed to be dependent on T cells expressing a limited number of Vβ chains. Two different methods were used to selectively eliminate T cells expressing a certain T-cell receptor (TcR) Vβ chain in mouse strains susceptible to CIA. *In vivo* treatment with monoclonal anti-Vβ6 or anti-Vβ8.1,2 antibodies did not alter CIA, despite a reduction of the major part of the Vβ6$^+$ or Vβ8.1,2$^+$ lymph node cells (LNC), as measured by flow cytometric (FACS) analyses. The reduction was not due to complete elimination of Vβ6$^+$ or Vβ8.1,2$^+$ cells, since part of the Vβ6 and Vβ8.1,2 expressing cells returned later, even in mice that had been thymectomized first to prevent maturation of new T cells. In contrast, treatment with antibodies against CD4 efficiently abrogated development of CIA. In the (CBA × DBA/1J)F_1 and the (BALB/c × DBA/1J)F_1 mice, where Mls1^a was combined with expression of I-E, the Vβ6$^+$ LNC were deleted. In spite of the deletion, both F_1 strains were highly susceptible to CIA.

INTRODUCTION

Collagen-induced arthritis (CIA) in mice, which is induced by immunization with type II collagen (CII), is a T-cell dependent and major histocompatability complex (MHC)-restricted disease. It is not possible to induce CIA in athymic nude mice (Holmdahl, 1990), and treatment with anti-CD4 (Ranges, Sriram & Cooper, 1985) or anti-MHC class II antibodies (Wooley *et al.*, 1985) inhibits induction of the disease. Furthermore, only mouse strains with certain MHC haplotypes, such as H-2^q, are susceptible to CIA (Wooley *et al.*, 1981; Holmdahl *et al.*, 1988a). More precisely, susceptibility to CIA is genetically dependent on the expression of specific structures in the class II Aβ chain (Holmdahl *et al.*, 1989b), which is most likely of critical importance for the presentation of certain CII-peptides to arthritogenic T cells. A recent very challenging finding is that T-cell receptor (TcR) polymorphism may also influence the susceptibility to CIA (Banerjee *et al.*, 1988). Thus, certain mouse strains may not be susceptible to CIA due to genomic deletions of some, and allelic differences of other, TcR Vβ genes. In the SWR strain, 50% of the Vβ genes, including β8.1 and 8.2, are absent in the genome, and other V genes, such as β6, are allotypically different from corresponding genes in CIA-susceptible strains (Behlke *et al.*, 1986). More specifically, it has recently been suggested that T cells expressing certain allotypes of Vβ6 are critical for the induction of CIA, on the basis of backcross experiments between SWR and C57BL/10 mice (Banerjee *et al.*, 1988; Haqqi *et al.*, 1988).

The issue of a restricted usage of certain Vβ TcR genes in autoimmune diseases was recently highlighted by the demonstration of successful treatment with antibodies against TcR Vβ8.2 (F23.2) to prevent development of experimental allergic encephalomyelitis (EAE) induced with the N-terminal peptide of basic protein (Acha-Orbea *et al.*, 1988).

In the present study the technique of treatment with monoclonal antibodies (mAb) specific for T cells expressing TcR Vβ6 (44.22.1), as well as Vβ8.1 and Vβ8.2 (KJ16), was applied to the CIA model. Moreover, the susceptibility for CIA was studied in crosses that were made between inbred mouse strains, where Mls1^a was combined with expression of the MHC molecule I-E, a combination which lead to a specific deletion of Vβ6 and a reduction of Vβ8.1 lymph node cells.

Abbreviations: Ab, antibody; CFA, complete Freunds adjuvant; CIA, collagen-induced arthritis; CII, type II collagen; EAE, experimental allergic encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; H+L, heavy plus light chain; LNC, lymph node cells; mAb, monoclonal antibody; MHC, major histocompatibility complex; TcR, T-cell antigen receptor.

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MATERIALS AND METHODS

Mice

DBA/1J mice, originally obtained from Jackson Laboratories Inc., Bar Habor, ME, and the crosses were bred and kept at the Biomedical Center in Uppsala. CBA/Ca and BALB/c mice were obtained from ALAB, Stockholm.

Monoclonal antibodies

The hybridoma 44.22.1, a rat IgG2a specific for mouse TcR Vβ6, was a kind gift of Hans Hengartner, Institute for Pathology, Zurich (Acha-Orbea, Zinkernagel & Hengartner, 1985). KJ16, a rat IgG which recognizes mouse TcR Vβ8.1 and TcR Vβ8.2 mAb (Haskins *et al.*, 1984), F23.1, a mouse anti-TcR Vβ8.1,2,3 (Staerz *et al.*, 1985), and F23.2, a mouse anti-TcR Vβ8.2 mAb (Kappler *et al.*, 1988), were generously provided by Dr P. Marrack, University of Colorado, Denver, CO. GK 1.5 is a rat IgG2b specific for mouse CD4 (Dialynas *et al.*, 1983) and MAR 18.5 is a mouse IgG2a to rat kappa chain (Lanier *et al.*, 1982).

For the fluorescence staining, culture supernatants were used, except for F23.2 which was affinity purified on protein A–Sepharose (Pharmacia AB, Uppsala) prior to biotinylation (Holmdahl *et al.*, 1986b). For the *in vivo* treatments, the rat antibodies were concentrated by ammonium sulphate precipitation of culture supernatants and the concentrations of specific mAb estimated by ELISA with purified standards, as described earlier (Holmdahl *et al.*, 1986c). MAR18.5 was purified by affinity chromatography on protein A–Sepharose (Pharmacia AB) and the protein content calculated from the absorbance at 280 nm.

In vivo *mAb treatments*

The mAb were injected intraperitoneally as single doses of 100 μg per mouse. MAR18.5 was administrated as a secondary antibody 1 hr after the primary antibody in the dose of 200 μg. In the CIA experiment the mAb were given immediately before immunization. Thymectomy was done 2 weeks before the start of treatments.

Flow cytometry (FACS)

FACS analyses were done as described earlier (Goldschmidt, Holmdahl & Klareskog, 1988). In brief, cell suspensions were prepared from inguinal, popliteal, axillary, brachial and cervical lymph nodes from individual mice. Aliquots of 50 μl containing approximately 10^6 cells were stained with the different mAb as hybridoma supernatants followed by fluorescence (FITC)-conjugated goat anti-rat IgG(H+L) Ab (Kirkegaard and Perry Laboratories, Gaithersburg, MD). As a negative control, normal rat Ig (NRIG) was used. In the case of staining with the mouse anti-mouse mAb F23.2, the mouse Ab was biotinylated. Binding of biotinylated Ab was visualized by a phycoerythrin-conjugated streptavidin (Becton-Dickinson Immunocytometry System, Mountain View, CA). The cells were then analysed in a FACstar (Becton-Dickinson). Lymphocytes were selected by forward and right angle scattering and the viability was checked by exclusion of propidium iodide. In each sample 10^4 gated cells were counted, except for the samples in Table 1, which were stained with KJ16 or biotinylated F23.2 where subtraction mathematics was carried out and 3×10^4 counted.

Induction and evaluation of CIA

Mice were injected intradermally in the base of the tail with 100 μl emulsion containing 50 μg of rat CII emulsified 1:1 in complete Freund's adjuvant. Rat CII was prepared by pepsin digestion of a rat chondrosarcoma and subsequent purification with selective salt precipitation and ion-exchange chromatography on DEAE–Sepharose (Miller & Rhodes, 1982). The mice were castrated 2 weeks prior to immunization. The castration procedures have been described in detail elsewhere (Holmdahl, Jansson & Andersson, 1986a). Arthritis development of the four paws was followed by a macroscopic scoring system ranging from 0 to 3 (1 = swelling and/or redness of one toe or finger joint, 2 = two or more joints involved and 3 = severe arthritis in the entire paw).

Measurement of antibodies in serum

For the quantification of anti-CII-reactive autoantibodies in serum, a modified standard ELISA technique was used (Holmdahl *et al.*, 1986b). Micro-ELISA plates (Dynatech, Plochingen, FRG) were coated overnight at 4° with 50 μl/well of phosphate-buffered saline (PBS) containing 10 μg/ml of rat CII. All subsequent incubations were made in a volume of 50 μl/well. Washings were done with Tris-buffered saline (pH 7·4) containing 0·05% Tween 20 (Tris-Tween). The sera were diluted in Tris-Tween. All tests were carried out in duplicate. The amount of bound antibody was estimated after incubation with an anti-mouse IgG Fc antiserum coupled to alkaline phosphatase (Jackson Immunoresearch Lab. Inc., Bar Habor, ME). The subsequent quantification of bound enzyme was performed with a paranitrophenol-containing substrate buffer in a Titertek multiscan spectrophotometer. To estimate the amount of anti-CII-reactive antibodies present in the serum samples, purified DBA/1 anti-CII-reactive antibodies were used as standards and titrated in parallel with the unknown serum samples in ELISA plates. For each serum sample the dilution that gave 50% absorbance compared with the maximum value obtained for the standard antibodies was related to the concentration (in μg/ml) of standard antibodies required to give the same 50% absorbance value. By this method all calculations were performed with data from the steep portion of the slope, where the sample titration curves were parallel to the standard titration curves.

For the quantification of rat antibodies in serum, micro-ELISA plates (Dynatech) were coated with goat anti-rat Ig (H+L) which had been mouse serum absorbed (Jackson Immunoresearch Lab. Inc.) and the serum titrated as described above. Bound rat mAb were subsequently detected by alkaline phosphatase-conjugated, mouse serum-absorbed, goat anti-rat Ig(H+L) (Jackson Immunoresearch Lab. Inc.). Purified rat monoclonal IgG2a and IgG2b were used as standards and the calculations were performed as described above for anti-CII antibodies.

RESULTS

Anti-TcR Vβ6 and Vβ8.1,2 mAb treatment of normal DBA/1 J mice reduced the respective target LNC

In vivo treatment with mAb can be used to specifically affect subsets of T cells. In this case the purpose was to deplete T cells bearing TcR Vβ6 or Vβ8.1,2 with 44.22.1 and KJ16 mAb, respectively. It has been shown that the depletion capacity *in vivo* by a rat anti-mouse T-cell mAb can be enhanced by addition of a mouse anti-rat kappa mAb (MAR18.5) as a secondary antibody (Goldschmidt *et al.*, 1988). This method was also employed in the current experiment. The effect of the mAb treatments was detected by flow cytometric analyses on (LNC) at Day 6. It was shown that treatment with 44.22.1 reduced their target cells both if given alone or together with the secondary Ab (MAR18.5) (Fig. 1a). Treatment with KJ16 resulted in a limited reduction of the $KJ16^+$ LNC, to about 50%

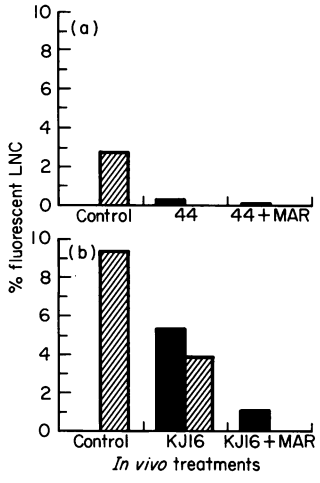

Figure 1. Flow cytometry analyses of LNC from DBA/1J male mice 6 days after *in vivo* treatment with (a) anti-Vβ6 mAb (44.22.1) or (b) anti-Vβ8.1,2 mAb (KJ16), with or without the secondary Ab MAR18.5. Results, mean of two individually analysed mice, are presented as percentage fluorescent LNC after *in vitro* staining with either normal rat Ig, NRIG (■), or the respective anti-Vβ mAb (■), (a) 44.22.1 and in (b) KJ16.

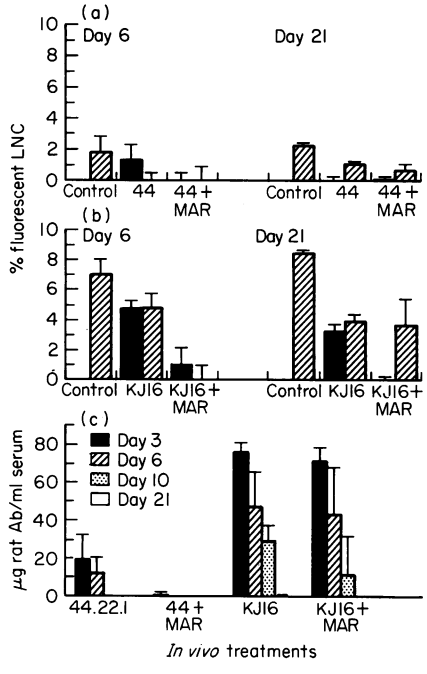

Figure 2. (a) Flow cytometry analyses of LNC from thymectomized DBA/1J female mice on Day 6 or Day 21 after *in vivo* treatment with anti-Vβ6 mAb (44.22.1) or (b) anti-Vβ8.1,2 mAb (KJ16) with or without the secondary Ab MAR18.5. Results, mean of three individually analysed mice, are presented as percentage fluorescent LNC after *in vitro* staining with either normal rat Ig, NRIG (■), or the respective anti-Vβ mAb (■) (a) 44.22.1 and (b) KJ16. (c) Concentrations of rat mAb in mouse serum from thymectomized DBA/1 female mice bled at different point of times after *in vivo* rat mAb treatment. Days 3 and 6, $n=6$, Days 10 and 21, $n=3$; serum from the 44+MAR (44.22.1+MAR18.5)-treated mice on Days 6, 10 and 21 was not examined.

of the untreated control, if given alone, whereas addition of the secondary mAb (MAR 18.5) enhanced the reduction to an undetectable frequency (Fig. 1b). The LNC from KJ16-treated mice also showed a background staining when only NRIG and FITC-conjugated GARIG were added in the *in vitro* staining procedure. This demonstrated the presence of KJ16 from the *in vivo* injection remaining bound to the cell surface (Fig. 1b). The addition of KJ16 *in vitro* did not increase the staining intensity, which showed that the TcR were saturated by mAb from the *in vivo* treatment (data not shown).

Thus, these findings show that the vast majority of Vβ6, Vβ8.1 or Vβ8.2 expressing T cells in the lymph nodes were either eliminated or had the TcR blocked by bound antibodies 6 days after mAb treatment.

The reduction of TcR-expressing LNC was due both to a transient down-regulation of TcR and a more long-lasting reduction

The reduction of the respective TcR Vβ-expressing LNC seen 6 days after treatment did not indicate whether the cells were eliminated or if the TcR were modulated from expression on the cell surface. To clarify the circumstances underlying the reduction, the mice were thymectomized prior to mAb treatment, to prevent maturation of new T cells as described earlier (Goldschmidt *et al.*, 1988) and analysed 6 and 21 days after treatment (Fig. 2). The purpose was to make it possible to conclude whether certain T cells re-expressed their TcR after the mAb had been cleared from the circulation.

Firstly, in accordance with the results obtained with treatment of naive mice, the thymectomized mice showed severe depletion of TcR Vβ6 and TcR Vβ8.1,2-expressing cells after treatment with specific antibodies combined with MAR18.5. At Day 21 after antibody treatment there was a partial reappearance of TcR Vβ6 and TcR Vβ8.1,2 cell populations. Fifty to 70% of the TcR Vβ8.1,2 and TcR Vβ6 cell populations were still absent at Day 21, which demonstrates that at least these frequencies of cells had been specifically eliminated and that the others either had modulated their target receptors or expanded from a small number of cells insensitive to antibody treatment.

Secondly, the mice treated with anti-TcR antibodies only showed a similar degree of long-term depletion of TcR Vβ6 and TcR Vβ8.1,2 target cells. In addition, after KJ16 treatment the target cells were still covered by rat antibodies 21 days after treatment. This correlates most probably to the presence of circulating mAb (Fig. 2c).

No effects on the development of CIA were seen after *in vivo* treatment with mAb directed to TcR Vβ6 or Vβ8.1,2

In order to investigate the dependency of TcR Vβ6$^+$ and Vβ8.1,2$^+$ T cells for development of CIA, thymectomized and castrated DBA/1J mice were treated with 44.22.1 and KJ16, using the same protocol as described above, and subsequently immunized with CII. As shown in Fig. 3, mAb-treated mice developed arthritis with similar incidence and severity as their untreated controls and no significant differences in anti-CII titre in serum were found (Table 1).

In contrast, treatment of DBA/1J mice with an anti-CD4 mAb, GK1.5, using a similar protocol as used for treatment with anti-TcR antibodies, severely inhibited development of disease (Fig. 4). The latter experiment showed that it was possible to affect CIA with an *in vivo* anti-T-cell treatment given at the time of immunization, provided that the mAb was directed to a critical T-cell population.

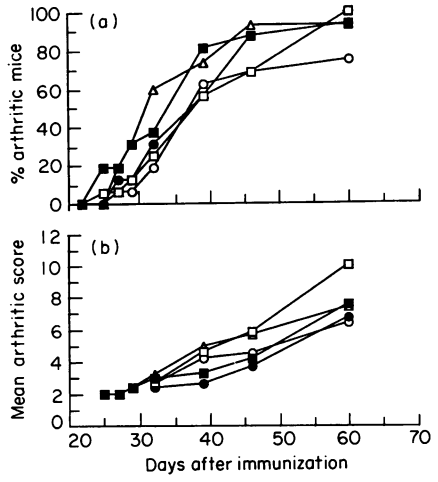

Figure 3. Macroscopic evaluation of CIA in thymectomized and castrated DBA/1J mice after *in vivo* mAb treatment and subsequent immunization with rat CII emulsified in CFA on Day 0. No mAb treatment (△), 44.22.1 (anti-Vβ6) (□), 44.22.1+MAR18.5 (■), KJ16 (anti-Vβ8.1,2) (○) and KJ16+MAR18.5 (●). (a) Incidence is expressed as percentage arthritic mice and (b) severity as mean arthritic score for a minimum of three arthritic mice. $n=15$ for the 'no mAb treatment' group and $n=16$ for the different mAb-treated groups.

Table 1. Anti-CII antibody titre in DBA/1J serum 60 days after treatment with different anti-TcR Vβ mAb and immunization with rat CII

Treatment	*n*	Serum levels of anti-CII Ab (μg/ml±SD)
Untreated control	12	724±526
KJ16	15	354±156
KJ16+MAR18	14	404±125
44.22.1	16	395±254
44.22.1+MAR18	13	486±216

n, number of individually analysed mice.

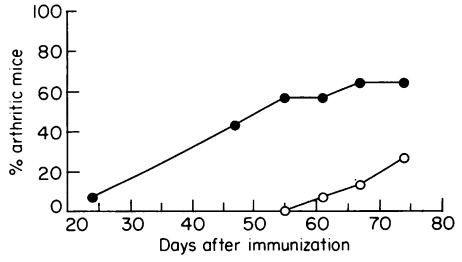

Figure 4. Macroscopic evaluation of CIA in thymectomized and castrated DBA/1J mice after *in vivo* anti-CD4 mAb treatment and subsequent immunization with rat CII emulsified in CFA on Day 0. No mAb treatment (●), GK1.5 (anti-CD4) treatment (○). Incidence is expressed as percentage arthritic mice. $n=14$ for the 'no mAb treatment' group and $n=15$ for the GK1.5-treated group. One of two experiments with similar results is shown.

Crossing of CBA × DBA/1J and BALB/c × DBA/1J resulted in a F_1 generation that was deleted of TcR Vβ6$^+$ lymph node cells

Although it was attempted to optimize the antibody treatment protocol to eliminate and/or block T cells expressing certain TcR Vβ elements, small numbers of mAb-resistant T cells were left. To avoid refractory T cells, another approach was used for deletion. It has recently been shown that T cells bearing the TcR Vβ6 and Vβ8.1 are intrathymically deleted in Mls1^{a+} mouse strains that also expresses I-E (MacDonald *et al.*, 1988; Kappler *et al.*, 1988). Similar to most other CIA-susceptible mouse strains, the DBA/1J strain is of H-2^q haplotype in which I-E is not expressed. Thus, although DBA/1J mice express Mls1^a they have normal levels of T cells with TcR Vβ6 and Vβ8.1 (Table 2). It was found that crossing of DBA/1J with CBA/Ca (H-2^k) or BALB/c (H-2^d) strains, which expresses functional I-E, led to elimination of T cells expressing TcR Vβ6. However, the Vβ8.1$^+$ T cells were only reduced to about 50% of the number in the parental strains, but not to the extent described earlier for I-E$^+$, Mls1^a strains (less than 0·6% of purified lymph node T cells) (Kappler *et al.*, 1988). Both sexes of DBA/1J were analysed and no differences were found (data not shown).

The (CBA/Ca × DBA/1J)F_1 was susceptible to CIA despite the loss of TcR Vβ6$^+$ LNC

To investigate if the selective deletions of Vβ6$^+$ lymph node T cells found in the (CBA/Ca × DBA/1)F_1 mice affected the susceptibility to CIA, the F_1 mice were castrated and 2 weeks later immunized with CII. The castration procedure was done since sex hormones modulate the susceptibility to arthritis to different extents in different mouse strains (Holmdahl *et al.*, 1989a). It was found that the F_1 hybrids were highly susceptible to CIA (Table 3). The incidence and mean score did not differ from that of castrated DBA/1J mice. Furthermore, no deviation in the levels of serum autoantibodies to CII could be found. In concordance with the current data the (BALB/c × DBA/1J)F_1 mice, that also had the Vβ6$^+$ T cells deleted (Table 2), neither showed any impaired CIA nor any differences in anti-CII antibodies, as has been reported earlier (Holmdahl *et al.*, 1986a). It was concluded that the susceptibility to CIA in H-2^q mice is not critically dependent on the presence of TcR Vβ6$^+$ T cells.

DISCUSSION

In this work the intention was to investigate if there is a critical role for T cells expressing TcR using certain Vβ genes in the development of CIA. Two approaches were taken; firstly mAb therapy with antibodies specific for certain TcR Vβ elements was used, and secondly F_1 hybrid mice, in which T cells expressing certain Vβ genes were eliminated or reduced, were analysed. In these initial studies, the role of Vβ8.1, Vβ8.2 and Vβ6 genes, which have been proposed to have an important role for development of CIA, were investigated.

In vivo treatment with mAb specific for either Vβ6 (44.22.1) or Vβ8.1,2 (KJ16) TcR was performed. Treatment of DBA/1J mice with 44.22.1 antibodies depleted the majority of the Vβ6$^+$ LNC for at least 6 days, while only 50% of the Vβ8.1,2$^+$ LNC disappeared after treatment with KJ16. To increase the depleting capacity of KJ16, the previously published method was used, where the depleting capacity of rat anti-mouse T cell mAb was enhanced by the addition of an autologous mouse anti-rat

Table 2. Flow cytometric analysis of LNC from (CBA/Ca × DBA/1J)F_1 and (BALB/c × DBA/1J)F_1 mice

				In vitro staining (% fluorescent LNC)			
	H-2	I-E	Mls1[a]	44.22.1 (Vβ6)	KJ16 (Vβ8.1,2)	B-F23.2 (Vβ8.2)	KJ16−B-F23.2§ (Vβ8.1)
DBA/1J*	q	−	+	2·8	11·3	7·2	4·1
CBA/Ca†	k	+	−	9·9	16·3	9·8	6·5
BALB/c†	d	+	−	7·6	15·8	10·3	5·5
(CBA/Ca × DBA/1J)‡	k/q	+	+	0·6	12·8	11·3	1·5
(BALB/c × DBA/1J)‡	d/q	+	+	0·6	13·7	11·6	2·1

* $n=2$.
† $n=1$.
‡ $n=1$ and also repeated with older mice with similar result.
§ The approximate number of $V\beta8.1^+$ was calculated by subtracting the $F23.2^+$ from the $KJ16^+$ cells.

Table 3. CIA in castrated (CBA/Ca × DBA/1J)F_1 and (BALB/c × DBA/1J)F_1 mice

Mouse strain	Sex	*n*	Incidence of arthritis (%)	Severity of arthritis (mean score)	Serum levels of anti-CII antibodies (μg/ml ± SD)
DBA/1J	Male*	20	80	4·94	ND
(CBA × DBA/1J)	Female	26	62	3·38	126 ± 22†
(CBA × DBA/1J)	Male	19	74	4·36	110 ± 17†

* Castrated female DBA/1J showed the same degree of arthritis as castrated male mice did, published in Holmdahl *et al.* (1986a).
† Serum bled 36 days after immunization.
ND, not determined.

kappa mAb (MAR18.5) as secondary Ab, which in this case bound to the Vβ TcR-specific primary Ab. Addition of the secondary Ab was shown to further reduce the number of Vβ8.1, 2^+ LNC. Furthermore, mAb treatment was also combined with thymectomy to prevent maturation of new T cells. Such mice had a chronic extensive reduction of Vβ6 and Vβ8.1,2 cells, indicating that a large part of the cells had been eliminated by mAb treatment. However, the reappearance of some of the Vβ6 and Vβ8.1,2 cells also indicated that the initially observed reduction was partly due to modulation of the TcR or expansion from a small number of cells resistant to antibody treatment and not to elimination of the cells. In addition, some cells, especially after injection of KJ16 antibodies without subsequent injection of MAR18.5 mAb, had rat antibodies bound to their TcR even as long as 21 days after injection of the antibodies. In either case in which the target TcR was blocked or down-regulated or the cell was eliminated, the mAb treatment must have severely inhibited the function of T cells with TcR encoded from the particular Vβ elements for at least 6 days after treatment. In spite of the efficiency of the mAb treatment on the elimination and/or blocking of their respective target T cells, no effect was seen on the development of CIA. In contrast, a similar treatment with GK.1.5 (anti-CD4) efficiently inhibited development of CIA. These findings indicate that T cells expressing TcR Vβ6, Vβ8.1 or Vβ8.2 played no crucial role for the induction of CIA.

However, mAb treatment experiments must be interpreted with some caution since small numbers of T cells might have been resistant to the treatment. Interestingly, it has been shown that activated T cells are relatively more resistant to mAb treatment (Ranges *et al.*, 1988). It has been postulated that CII-autoreactive T cells might be activated naturally (Holmdahl *et al.*, 1987) and these cells might therefore be resistant to mAb treatment. On the other hand, T cells specific for heterologous epitopes on the rat CII are activated due to immunization (Holmdahl *et al.*, 1988b) and therefore are likely to be depleted by the mAb treatment if given prior to immunization. Activation of these heterologous CII-reactive T cells is most likely responsible for the strong anti-CII antibody production and the severity of arthritis seen after rat CII immunization. Probably all these cells express CD4, but not necessarily TcR Vβ6, Vβ8.1 or Vβ8.2.

In order to further substantiate the possible importance of T cells using certain Vβ elements for the development of CIA, a method other than mAb treatment was looked for, for elimination of these cells *in vivo*. For that purpose F_1 hybrids of (CBA/Ca × DBA/1J) and (DBA/1 × BALB/c) were made. In these F_1 hybrids, Mls1[a] was combined with I-E, which in the present F_1 combinations led to elimination of T cells expressing Vβ6 during thymus ontogeny, as has recently been described in detail by other groups (MacDonald *et al.*, 1988; Kappler *et al.*,

1988). However, it was not possible to reproduce the deletion of Vβ8.1$^+$ cells found parallel to the Vβ6 deletions by these groups. In this study, only a 50% reduction of the Vβ8.1$^+$ cells was found. There is no evidence that this Mls1^a-based negative selection is biased towards any given fine specificity of the deleted T cells. However, since both F_1 hybrid strains developed severe arthritis, it is concluded that T cells expressing TcR Vβ6 are not of crucial importance for the development of CIA.

Although no correlation was found between the expression of Vβ6 and the susceptibility to CIA, there is other evidence arguing for a role of particular TcR Vβ elements in CIA. The SWR mouse is of the CII high responder H-2^q haplotype, but does not develop CIA after immunization with CII. This non-responsiveness has been attributed to both a genetic deficiency of C5 production in the SWR strain (Watson & Townes, 1985; Fujita *et al.*, 1989) and to TcR polymorphism (Banerjee *et al.*, 1988). TcR genes in the SWR are different from TcR genes in the CII high responder DBA/1 (H-2^q) strain, both by a large genomic deletion of a number of Vβ genes (5.1, 5.2, 5.3, 8.1, 8.2, 8.3, 9, 11, 12, 13) and by allotypic differences in some of the remaining Vβ genes (1, 3, 6, 10) (Behlke *et al.*,1986; Chou *et al.*, 1987). Backcross experiments with strains having TcR haplotypes of the 'DBA/1'-type and to C5 competent strains demonstrated that TcR polymorphism had a critical influence but that C5 competence only influenced the severity and onset of arthritis (Banerjee *et al.*, 1989). From these experiments it was suggested that Vβ6 or possibly Vβ8.1 elements were the most critical genes to be used in the induction of CIA. The finding of a critical influence of the expression of certain TcR Vβ genes was further confirmed using H-2^r haplotype strains with an even larger genomic deletion in the Vβ region (S. Banerjee, personal communication). Thus, from the above described findings it may be possible to conclude that the arthritogenic T cells in CIA express a limited number of Vβ elements. Another clearly TcR Vβ-restricted autoimmune model in mice is experimental allergic encephalomyelitis (EAE) induced with the acetylated 1-10 N-terminal peptide of basic protein. Most of the isolated encephalitogenic T-cell clones express TcR with TcR Vβ8.1,2 and Vα3.2 elements (Acha-Orbea *et al.*, 1988). Furthermore, treatment of mice with antibodies reactive with Vβ8.1,2 protein structure (F23.2) abrogates development of EAE both prophylactically and therapeutically. The present finding on the lack of a critical role of T cells expressing TcR Vβ6 in the development of CIA does not exclude an important influence of TcR with a restricted number of Vβ genes in CIA. The influence of TcR polymorphism on autoimmune disease is challenging, but may not be applicable to the complex immunopathology in CIA. The role of other TcR V elements in the development of CIA needs to be explored further.

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