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Effect of T-cell receptor Vβ-specific monoclonal antibodies on cyclophosphamide-induced diabetes mellitus in non-obese diabetic mice

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Summary. The expression of specific T-cell receptor gene segments by T lymphocytes appears to be critically important for the induction of several experimental autoimmune diseases mediated by these cells. We examined whether this situation also applied to non-obese diabetic mice by using various T-cell receptor Vβ-specific monoclonal antibodies. No significant age- or sex-related differences were observed in Vβ usage by peripheral and splenic T lymphocytes. $CD8^+$ T lymphocytes among the islet-derived mononuclear cells isolated from 20-week-old female non-obese diabetic mice showed heterogeneity of their Vβ gene usage. In order to examine the role of T lymphocyte subsets expressing specific T-cell receptor Vβ gene segments in the development of diabetes mellitus, T-cell receptor Vβ-specific monoclonal antibodies were administered to 10-week-old male non-obese diabetic mice treated with cyclophosphamide. None of the antibodies used could significantly diminish the incidence of cyclophosphamide-induced diabetes and the severity of insulitis [anti-Vβ3 (11 of 22 mice became diabetic, 50%), anti-Vβ5 (9 of 14, 64%), anti-Vβ8 (9 of 21, 43%), anti-Vβ11 (12 of 23, 52%), anti-Vβ14 (7 of 12, 58%), and anti-Vβ5 + anti-Vβ11 (6 of 12, 50%)] when compared with control mice (12 of 21, 57%). In addition, there were no significant differences in T-cell receptor Vβ usage between diabetic and non-diabetic cyclophosphamide-treated mice. These results suggest that five T-lymphocyte subsets expressing different T-cell receptor Vβ gene segments, considered to be candidates involved in the pathogenesis of autoimmune diabetes, do not individually contribute to the development of cyclophosphamide-induced diabetes in non-obese diabetic mice.

Key words: Non-obese diabetic mouse, T cell receptor, monoclonal antibody, cyclophosphamide, insulitis.

Non-obese diabetic (NOD) mice develop infiltration of mononuclear cells into their pancreatic islets (insulitis), and some mice progress to develop Type 1 (insulin-dependent) diabetes mellitus [1]. Therefore, the NOD mouse has been accepted as a model of human Type 1 diabetes and the factors producing the pathology in this animal have been intensively investigated. Type 1 diabetes has been shown to be T-lymphocyte mediated [2–4] and a contribution from both $CD4^+$ and $CD8^+$ T lymphocytes seems to be necessary for the development of overt diabetes [5–9]. In the search for selective immunotherapy for Type 1 diabetes, it is important to determine which T-lymphocyte subsets contribute to diabetes in the NOD mouse. In this context, we have previously reported that MHC class I K^d-restricted $CD8^+$ T lymphocytes act as direct effector cells in the destruction of pancreatic beta cells in this mouse strain [10–12]. The limited heterogeneity of T-cell receptor (TCR) usage in experimental allergic encephalomyelitis (EAE) was recently demonstrated [13–15]. EAE is an autoimmune disease of the central nervous system that produces symptoms in animals similar to those seen in humans with multiple sclerosis. It has also been reported that monoclonal antibodies (MoAbs) directed against particular TCR Vβ gene segments can be used to prevent and treat this autoimmune disease [13–15]. Accordingly, investigation of the association between T lymphocytes expressing specific TCR Vβ gene segments and the pathogenesis of diabetes in NOD mice is necessary. However, at present, the TCR Vβ usage in NOD mice is a very controversial topic with divergent results being reported from different groups. In this study, we investigated TCR Vβ gene usage by peripheral and splenic T lymphocytes from untreated and cyclophosphamide (CY)-treated NOD mice, and also studied the same genes in islet-derived $CD8^+$ T lymphocytes [10]. Moreover, we attempted to prevent insulitis and overt diabetes in CY-treated male NOD mice by administering several MoAbs directed against various TCR Vβ gene segments.

Materials and methods

Mice

Male and female NOD mice were obtained from the colony kept at the animal facility of Kobe University School of Medicine [16]. The original colony was kindly supplied by the Aburahi Laboratories of Shionogi Research Institute (Shiga, Japan). In our NOD colony, most of the mice develop insulitis at 6–8 weeks of age and the cumulative incidence of diabetes is 5% in males and 65% in females at 30 weeks of age. Mice aged 12 weeks or less have not developed overt diabetes in our colony. BALB/c mice were purchased from Clea (Osaka, Japan).

Monoclonal antibodies

Hybridoma cells secreting various MoAbs against TCR Vβ gene products were injected into nude mice. The following MoAb hybridomas were used: anti-TCR Vβ3 (KJ-25, hamster IgG, kindly supplied by Dr. J. W. Kappler and Dr. P. Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, Co., USA) [17], anti-Vβ5 (MR9.4, hamster IgG, from Dr. H. Nakauchi, The Institute of Physical and Chemical Research, Tsukuba, Japan), anti-Vβ6 (44-22-1, rat IgG, from Dr. H. Hengartner, University Hospital, Zurich, Switzerland) [18], anti-Vβ7 (TR-310, rat IgG, from Dr. I. L. Weissman, Stanford University School of Medicine, Stanford, Calif., USA) [19], anti-Vβ8 (F23.1, mouse IgG, from Dr. U. D. Staerz, National Jewish Center for Immunology and Respiratory Medicine) [20], anti-Vβ11 (KT11, rat IgG, from Dr. K. Tomonari, MRC Clinical Research Centre, Harrow Middlesex, UK) [21] and anti-Vβ14 (14.2, rat IgM, from Dr. N. S. Liao and Dr. D. Rault, Massachusetts Institute of Technology, Cambridge, Mass., USA). MoAbs were harvested as ascites from the nude mice and partially purified by 50% ammonium sulphate precipitation and dialysis in phosphate-buffered saline (PBS, 120 mmol NaCl, 2.7 mmol KCl and phosphate buffer salts, 10 mmol; pH 7.4). These MoAbs were biotinylated for flow cytometric analysis.

Isolation of peripheral and splenic T lymphocytes

Peripheral and splenic T lymphocytes were obtained from orbital venous blood and spleen cells, respectively. T-lymphocyte-rich fractions were extracted from these cells by Ficoll-Hypaque gradient centrifugation and the nylon wool technique, and the fractions obtained had a T lymphocyte content greater than 80%. These fractions were used for the flow cytometric analysis of peripheral and splenic T lymphocytes.

Isolation of islet-infiltrating mononuclear cells

Pancreatic islets with infiltrating mononuclear cells were isolated from 20-week-old female NOD mice using collagenase and the Ficoll gradient method as described previously [10]. These islets were cultured in 24-well plates with 10 U/ml of human recombinant interleukin-2 (rIL-2; Takeda Chemical Co, Osaka, Japan) in 1 ml of RPMI 1640 medium containing 10% fetal calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin and 50 μmol 2-mercaptoethanol. After 4 days, 10 U/ml of rIL-2 in 1 ml of the above-mentioned medium was added to the culture. After 7 days of culture, islet-derived mononuclear cells were obtained by Ficoll-Hypaque gradient centrifugation. Flow cytometric analysis revealed that 10–30% of these cells were $CD4^+$ T lymphocytes and 70–90% were $CD8^+$ T lymphocytes.

Flow cytometry

To determine TCR Vβ gene usage by both peripheral or splenic T lymphocytes from NOD mice and control BALB/c mice, cell surface staining was carried out by incubation with the seven biotinylated-anti-Vβ MoAbs mentioned above, followed by incubation with phycoerythrin-conjugated streptavidin (Vector Laboratories, Burlingame, Calif., USA). Furthermore, peripheral and splenic T lymphocytes were stained with fluorescein-conjugated anti-Thy1.2 (Becton Dickinson Immunocytometry Systems, Mountain View, Calif., USA) and the percentage of cells expressing each particular Vβ gene segment among whole T lymphocytes was calculated. In CY-treated mice, peripheral and splenic T lymphocytes were subjected to flow cytometric analysis on day 28. To investigate the percentage of each type of TCR Vβ-positive cell among islet-derived $CD8^+$ T lymphocytes, the islet-derived mononuclear cells were double-stained with biotinylated anti-Vβ MoAb followed by phycoerythrin-conjugated streptavidin and also a fluorescein-conjugated anti-CD8 (Lyt2) MoAb (Becton Dickinson). The percentage of positive cells was determined with a FACS 440 flow cytometer (Becton Dickinson).

Effect of MoAbs on CY-induced diabetes

Ten-week-old male NOD mice were injected with CY (Asta, Frankfurt, FRG) at a dose of 200 mg/kg body weight on days 0 and 14. The mice were assigned to seven groups, and received either PBS (control), anti-Vβ3, anti-Vβ5, anti-Vβ8, anti-Vβ11, or anti-Vβ14 MoAb or a combination of both the anti-Vβ5 and anti-Vβ11 MoAbs. The control PBS (200 μl) and the five types of anti-Vβ MoAbs (1 mg in 200 μl PBS) were administered intraperitoneally on days 0 and 14. The mice were tested for glucosuria two times per week until day 28 using Tes-Tape (Eli-Lilly, Indianapolis, Ind., USA). In mice showing a positive result with the Tes-Tape, the non-fasting level was determined by the glucose oxidase method. A glucose level greater than 16.7 mmol/l was taken to indicate diabetes. On day 28, the mice were killed for histological examination and the depletion of T lymphocytes expressing corresponding Vβ gene products was checked by flow cytometric analysis.

Histological examination

On day 28, the pancreases removed from CY-treated mice injected with PBS or the anti-Vβ MoAb(s) were fixed in Bouin's solution. Paraffin-embedded sections were stained with haematoxylin and eosin and the severity of insulitis was assessed using the scoring system of Charlton et al. [9]: (0), islets with no peri-islet mononuclear cells; (1), focal peri-islet aggregates affecting less than 25% of the islet circumference; (2), more extensive peri-islet aggregates; (3), intra-islet infiltration with preservation of the beta cells; (4), extensive intra-islet infiltration with obvious beta-cell damage and gross architectural distortion. The scores were converted to a percentage, with 100% indicating total islet destruction.

Statistical analysis

The significant differences in the incidence of diabetes was determined with the chi-square test. Statistical analysis of the insulitis scores was done using the non-parametric Mann-Whitney U-test, while the other results were statistically evaluated by Student's *t*-test. All data are presented as the mean ± SD.

Table 1. Flow cytometric analysis of T-cell receptor (TCR) Vβ gene usage in peripheral T lymphocytes, splenic T lymphocytes, islet-derived $CD8^+$ T lymphocytes from non-obese diabetic (NOD) mice and from BALB/c mice

		Percentage of positive cells						
	n[a]	Vβ3	Vβ5	Vβ6	Vβ7	Vβ8	Vβ11	Vβ14
Peripheral T lymphocytes (NOD)								
5-week-old male	4	13.3 ± 1.9	2.2 ± 0.3	6.0 ± 0.9	7.4 ± 2.2	15.4 ± 6.3	20.6 ± 3.5	8.3 ± 2.7
5-week-old female	4	12.9 ± 1.4	1.7 ± 0.6	6.8 ± 1.3	5.7 ± 2.2	16.2 ± 3.2	20.5 ± 3.0	7.6 ± 1.2
10-week-old male	4	11.3 ± 3.5	1.8 ± 1.0	5.9 ± 1.5	5.1 ± 2.0	16.5 ± 2.2	16.9 ± 2.2	7.8 ± 1.2
10-week-old female	4	11.3 ± 3.2	1.8 ± 1.0	5.8 ± 1.0	5.4 ± 2.5	15.0 ± 2.9	15.1 ± 5.5	6.2 ± 2.3
24-week-old male	4	11.3 ± 2.3	1.9 ± 0.4	6.2 ± 0.7	5.9 ± 1.0	18.0 ± 2.9	17.4 ± 3.2	8.0 ± 0.9
24-week-old female	4	13.8 ± 4.9	1.7 ± 0.9	7.5 ± 1.1	6.0 ± 1.5	15.0 ± 4.5	17.9 ± 1.2	7.4 ± 2.1
Splenic T lymphocytes (NOD)								
10-week-old male	4	5.7 ± 0.8	2.3 ± 0.4	4.4 ± 0.6	6.4 ± 1.1	9.1 ± 1.0	14.3 ± 2.4	10.7 ± 1.1
10-week-old female	4	5.7 ± 1.3	2.1 ± 0.3	4.6 ± 0.7	6.1 ± 1.3	9.0 ± 1.4	14.9 ± 1.9	10.8 ± 2.1
Islet-derived $CD8^+$ T lymphocytes	3	11.8 ± 1.8	2.5 ± 0.5	4.8 ± 1.4	6.4 ± 2.1	15.7 ± 6.7	9.8 ± 4.2	5.4 ± 3.2
Peripheral T lymphocytes (BALB/c)								
10-week-old male	4	0.9 ± 0.2	0.7 ± 0.3	12.2 ± 1.4	15.4 ± 1.4	23.3 ± 1.8	1.0 ± 0.2	10.6 ± 1.5
10-week-old female	4	1.0 ± 0.2	0.5 ± 0.4	12.4 ± 1.7	14.4 ± 2.4	23.0 ± 2.4	1.0 ± 0.4	9.5 ± 2.0

Values are means ± SD. There were no significant differences in TCR Vβ gene usage in relation to age or sex.
[a] n is the number of mice examined except for islet-derived $CD8^+$ T lymphocytes where n is the number of experiments performed

Table 2. Cumulative incidence of cyclophosphamide (CY)-induced diabetes and insulitis score on day 28 in male non-obese diabetic mice injected with several anti-T-cell receptor Vβ gene segment monoclonal antibodies

	Incidence of diabetes (%)		Insulitis score	
			n[a]	%
CY + PBS (control)	12/21	(57)	10	57 ± 22
CY + Anti-Vβ 3 MoAb (KJ-25)	11/22	(50)	8	48 ± 21
CY + Anti-Vβ 5 MoAb (MR9.4)	9/14	(64)	8	55 ± 26
CY + Anti-Vβ 8 MoAb (F23.1)	9/21	(43)	9	45 ± 25
CY + Anti-Vβ11 MoAb (KT-11)	12/23	(52)	9	46 ± 23
CY + Anti-Vβ14 MoAb (14.2)	7/12	(58)	8	54 ± 27
CY + Anti-Vβ 5 MoAb (MR9.4) and Anti-Vβ11 MoAb (KT-11)	6/12	(50)	8	50 ± 23

Insulitis scores are means ± SD.
[a] n is the number of mice examined. *PBS*, phosphate-buffered saline; *MoAb*, monoclonal antibody

Results

We first investigated which TCR Vβ gene segments are expressed by peripheral and splenic T lymphocytes in NOD mice using various TCR Vβ-specific MoAbs. Table 1 shows that the pattern of expression of Vβ gene segments by peripheral T lymphocytes did not change with age and no differences were seen between the sexes. Splenic T lymphocytes from 10-week-old NOD mice also showed no significant sex difference in TCR Vβ usage (Table 1). It is notable that T lymphocytes expressing the Vβ5 and Vβ11 gene segments existed in NOD mice, which do not have MHC class II I-E molecules. On the other hand, Vβ5 and Vβ11 positive T lymphocytes were almost depleted in I-E positive BALB/c mice.

We isolated mononuclear cells from the pancreatic islets of 20-week-old female NOD mice. The $CD8^+$ T lymphocytes among these mononuclear cells were previously shown to destroy pancreatic islet cells in a MHC class I K^d-restricted manner in vitro [10]. Therefore, we investigated whether particular TCR Vβ gene segments were expressed by islet-derived $CD8^+$ T lymphocytes. Table 1 also shows the percentage of $CD8^+$ T lymphocytes expressing each Vβ gene segment. A remarkable heterogeneity was observed in TCR usage by these cells.

To further examine the role of T-lymphocyte subsets expressing specific TCR Vβ gene segments in the development of diabetes in these mice, we attempted to prevent CY-induced diabetes by administering MoAbs against five different TCR Vβ gene segments. As shown in Figure 1, CY-treated mice injected with PBS or with the anti-Vβ MoAb(s) developed diabetes after day 10 and the incidence continued to increase until day 28. The flow cytometric analysis showed the depletion of T lymphocytes expressing corresponding Vβ gene products (data not shown). Table 2 summarizes the cumulative incidence of diabetes on day 28. It was not reduced significantly by any of the MoAb(s), although anti-Vβ8 treated mice had a lower incidence of diabetes than the control mice. Similarly, the severity of insulitis was not significantly diminished by any of the MoAb(s). Although some of the CY-treated mice developed diabetes very rapidly, no significant changes were observed in their TCR Vβ gene usage on day 28 when compared with that of untreated mice (Fig. 2).

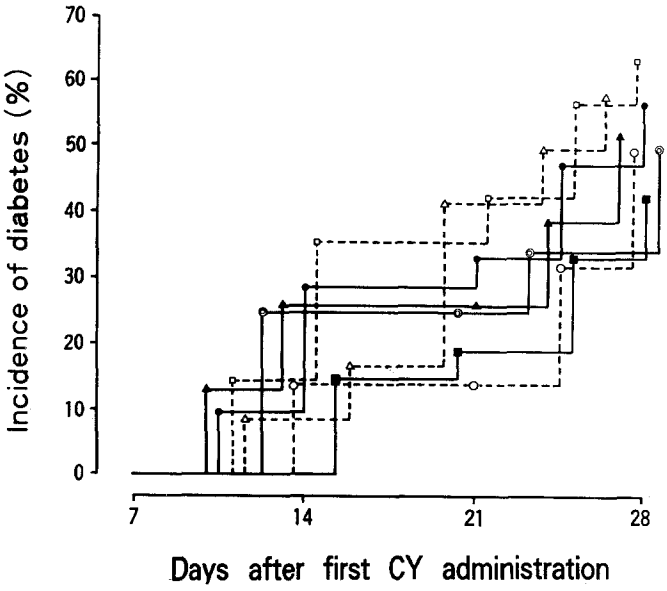

Fig. 1. Incidence of diabetes in cyclophosphamide (CY)-treated male non-obese diabetic mice that were injected with either phosphate-buffered saline (control, ●, $n = 21$), or monoclonal antibodies: anti-Vβ3 (○, $n = 22$), anti-Vβ5 (□, $n = 14$), anti-Vβ8 (■, $n = 21$), anti-Vβ11 (▲, $n = 23$), anti-Vβ14 (△, $n = 12$) or the combination of both the anti-Vβ5 and Vβ11 (◎, $n = 12$)

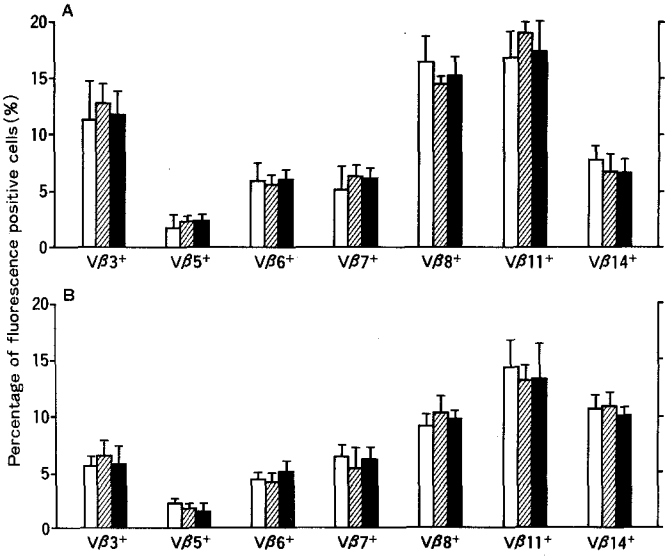

Fig. 2 (A, B). Flow cytometric analysis of peripheral (**A**) and splenic (**B**) T lymphocytes from untreated non-obese diabetic mice (□, $n = 4$), cyclophosphamide (CY)-treated diabetic mice (▨, $n = 4$) or CY-treated non-diabetic mice (■ columns, $n = 3$)

Moreover, there were no significant differences between CY-treated diabetic mice and CY-treated non-diabetic mice with regard to TCR Vβ usage (Fig. 2).

Discussion

Recently, the structure of the TCR has been intensively studied and much attention has been given to the relationship between the pathogenesis of autoimmune disease and TCR V gene usage. In particular, the limited usage of TCR Vβ gene segments in EAE [13–15], a disease which shows symptoms similar to those of multiple sclerosis, suggested the possibility that similar restricted TCR usage might occur in other models of autoimmune disease. In such cases, the administration of anti-TCR MoAbs would have the potential to be used for selective immunotherapy. However, whether the findings observed in EAE are applicable to NOD mice is not known. It has been reported that T lymphocytes expressing either the Vβ5, Vβ11, Vβ12, Vβ16, or Vβ17a gene segments are depleted in mice which have I-E molecules [22–25]. In addition, the prevention of insulitis and diabetes in I-E-positive transgenic NOD mice [26, 27] suggested that a lack of I-E molecule might lead to an inability to deplete the diabetogenic T lymphocytes expressing certain Vβ gene segments. On the other hand, Reich et al. [28, 29] have reported that both the $CD4^+$ and $CD8^+$ clones of islet-specific T cells derived from the islets of recently diabetic NOD mice, expressed TCR Vβ1 gene segments. Bacelj et al. [30] reported that the administration of an anti-Vβ8 MoAb could prevent CY-induced diabetes in NOD mice and that $V\beta8^+$ T lymphocytes appeared to have a role in the development of disease, similar to the situation with EAE. In contrast, Nakano et al. [31] reported that islet-reactive T-cell clones derived from NOD mice showed remarkable heterogeneity in their TCR Vα and Vβ usage. Lipes et al. [32, 33] introduced a rearranged TCR Vβ chain transgene derived from a T-lymphocyte hybridoma specific to chicken ovalbumin into the NOD background by progressive backcrossing. Although the transgene segments replaced the normally expressed Vβ gene segments, the transgenic NOD mice developed autoimmunity similar to control littermates. These results suggested that the TCR Vβ gene repertoire alone does not determine the susceptibility of NOD mice to autoimmune diabetes.

Therefore, to examine the implications of T lymphocytes expressing a particular TCR Vβ gene segment in the pathogenesis of diabetes in NOD mice, we first determined the TCR Vβ gene usage by peripheral and splenic T lymphocytes in NOD mice by using seven different anti-TCR Vβ MoAbs. In NOD mice, insulitis became noticeable in the majority of mice at age 6–8 weeks, and was greatly enhanced by 10–15 weeks, with the incidence of diabetes showing a large sex-related difference. Accordingly, we examined the changes of TCR Vβ usage during advancing age as well as comparing it between the sexes. However, no significant age- or sex-based differences in TCR usage were found. Our flow cytometric data with some anti-Vβ antibodies varies from the results with these same antibodies from other laboratories [31, 34]. This discrepancy may be due to the differences in the NOD colonies or of sources of T cells examined (peripheral blood cells, splenic cells, and lymph node cells) or both.

Rapidly progressing overt diabetes was induced by CY in approximately 60% of the 10-week-old male NOD mice, most likely by inhibiting T suppressor cell activity [35]. Furthermore, it has already been reported that CY administration caused a rapid decrease of the proportion of Thy1.2, $CD4^+$ and $CD8^+$ cells among splenocytes [8, 11]. Therefore, we investigated whether diabetogenic ef-

fector cells expressing a particular Vβ gene segment increased dramatically after CY administration. However, it was found that CY injection caused no significant changes in TCR Vβ gene usage.

Next, we isolated islet-infiltrating mononuclear cells from 20-week-old female NOD mice. As we reported previously [10], these cells are mainly $CD8^+$ T lymphocytes and are capable of destroying pancreatic beta cells in an MHC class I K^d-restricted manner in vitro. Therefore, we investigated whether the $CD8^+$ T lymphocytes among these cells have a restricted usage of Vβ gene segments. We found that these $CD8^+$ T lymphocytes actually showed a marked heterogeneity of TCR Vβ usage. It is not clear whether $CD8^+$ T lymphocyte populations established from NOD islets are really a mixture of islet reactive T lymphocytes or a heterogeneous population containing both specific and non-specific T lymphocytes. These $CD8^+$ islet-derived T lymphocytes, however, appear to be mainly composed of beta-cell specific cytotoxic T lymphocytes. This was demonstrated morphologically by the rapid and intense cytolysis of beta cells caused by these T lymphocytes [12]. Therefore, at present, we are unable to establish that a single T-lymphocyte subset with a special Vβ gene segment is associated with beta-cell destruction in NOD mice. In addition, O'Reilly et al. [36] have reported that T lymphocytes expressing the Vβ8, Vβ6 and Vβ11 gene segments were detected by immunohistochemical analysis in the infiltrates of spontaneously diabetic female NOD mice, non-diabetic male NOD mice, and recipients of spleen cells from diabetic NOD donors, again suggesting heterogeneity of TCR usage by these cells.

We attempted to shorten the time until the onset of overt diabetes using CY [35, 37]. We selected the anti-Vβ3, Vβ5, Vβ8, Vβ11, and Vβ14 MoAbs for this experiment for the following reasons: (1) $V\beta5^+$ and $V\beta11^+$ T lymphocytes are absent in $I\text{-}E^+$ mice, (2) $V\beta8^+$ T lymphocytes are reported to be implicated in EAE, and (3) $V\beta3^+$ and $V\beta14^+$ T lymphocytes are common among the peripheral and splenic T lymphocytes of NOD mice. None of the five MoAbs specific for TCR Vβ gene segments (including the combination of anti-Vβ5 and anti-Vβ11 MoAbs) were found to be capable of significantly reducing the severity of insulitis and the incidence of CY-induced diabetes. However, since the present analysis of T-cell subsets did not involve nearly one-half of the TCR and other Vβ TCR could be the key elements in disease expression, the analysis of the T-cell repertoire that were not tested here could be important.

Our findings suggest that none of five T lymphocyte subsets expressing Vβ3, 5, 8, 11, and 14 are likely to play a crucial role independent of other subsets in the pathogenesis of diabetes in NOD mice. Our conclusion is apparently contrary to the published report of Bacelj et al., [30] in which administration of anti-Vβ8 MoAb could prevent CY-induced diabetes. This discrepancy may be due to the differences in the NOD colonies, the difference in animal age at CY administration (10 weeks in our study, 100~120 days in Bacelj's study) or the dose and interval of CY administration (200 mg/kg on days 0 and 14 in our study, 350 mg/kg on day 0 in Bacelj's study). Some recently published backcross-intercross studies [38, 39] have bred NOD mice to mouse strains that congenitally lack some of the conventional TCR Vβ alleles. Backcross-intercross animals with NOD genetic background could develop insulitis and diabetes, although they lacked natural T lymphocytes expressing Vβ5, 8, 9, 11, 12 and 13 gene segments. These data appear to support our conclusions.

Candeias et al. [40] recently reported that the sequences of the TCR α and β chains from NOD derived islet-specific clones were quite heterogeneous, a finding which also supports our conclusions. In contrast to EAE, in which a single peptide is injected artificially, the antigens associated with the pathogenesis of spontaneously developing diabetes in NOD mice are probably far more complex. As Candeias et al. [40] noted, however, the T lymphocytes which recognize the actual disease-initiating antigen at an early stage of diabetes may show limited heterogeneity of TCR usage. However, once the disease cascade has started, multiple T-lymphocyte subsets that recognize other antigens could also participate in the development of overt diabetes. Thus, examination of the TCR heterogeneity of T lymphocytes isolated from the pancreases of very young NOD mice is of interest.

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