ANALYSIS OF T CELL RECEPTOR Vβ GENE EXPRESSION IN B CELL DEFICIENT MICE AFTER EXPERIMENTAL HERPES SIMPLEX VIRUS KERATITIS

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Summary. – To examine the importance of B cells in the regulation of the T cell response to herpes simplex virus (HSV) infection, we have analyzed the selection of the T cell receptor (TCR) repertoire in C.B-17 mice that lack B cells (B^- mice) compared with age-matched immunocompetent C.B-17 mice, usually resistant to herpes simplex keratitis (HSK). TCR Vβ transcripts used by these mice were analyzed by polymerase chain reaction (PCR) with variable gene-specific primers. Clinical examination showed that the incidence of HSK was significantly different between untreated (control) and anti-μ antibody (Ab)-treated mice ($p < 0.0001$). Passive transfer of anti-HSV Ab into B^- mice, before infection, prevented HSK; transfer of naive B cells allowed HSK to evolve in 50% of these mice. At the level of gene expression, we demonstrated that the anti-μ Ab treatment altered TCR Vβ gene expression in eyes, spleen, thymus and lymph nodes (LN) of C.B-17 mice. Preferential utilization of a single TCR Tb gene was not detected in the course of the disease except in LN, although in resistant mice there were different patterns of mRNA induction in T cells expressing specific TCR Vb elements that were not seen in susceptible mice, namely the lack of expression of Vβ8.1, Vβ8.2 and Vβ8.3 in eyes, the expression of Vβ7 in spleen, and the lack expression of Vβ6 and Vβ13 in thymus. These observations together with previous findings suggest that at the level of protein production, anti-HSV Ab not only can provide protection against HSK but is also a critical component for protection against HSV in normally resistant C.B17 mice, and that a dysregulation of the immune system in B^- mice is manifested by dramatic changes in TCR Vβ usage at the molecular level.

Key words: anti-μ antibody; herpes simplex keratitis; T cell receptor; Vβ chain

Introduction

T cells recognize antigens (Ag) through TCR molecules present on the T cell surface. In the TCR two clonotypic chains (α, β or γ, δ) are specifically associated with the γ, δ, ε, ζ and η components of the CD3 complex (Ashwell and Klausner, 1990). This TCR-CD3 complex responds to Ag producing changes in gene expression leading to increased mRNA synthesis (Ullman *et al.*, 1990). Genes for α, β, γ and δ chains are dispersed as separated DNA segments in the genome and are brought together by somatic recombination to generate the diversity required to recognize the large number of antigens present in nature (Kronenberg *et al.*, 1986). The structural and functional relationship of the TCR to T cells has been studied, and limited or preferential TCR Vβ usage responding to certain antigens has been reported (Choi *et al.*, 1989; Tomai *et al.*, 1991; Bell *et al.*, 1993).

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Abbreviations: Ab = antibody; Ag = antigen; CTL = cytotoxic lymphocytes; DTH = delayed type hypersensitivity; HSK = herpes simplex keratitis; HSR = herpes simplex retinitis; HSV = herpes simplex virus; IL-2 = interleukin-2; i.p. = intraperitoneal(ly); LN = lymph nodes; MEM = Eagle's Minimal Essential Medium; NK = natural killer; PCR = polymerase chain reaction; p.i. = post infection; RT = reverse transcription; TCR = T cell receptor; Vβ = variable β chain

Our laboratory has studied a murine model of HSK in order to understand the immune mechanisms and genetic pattern involved in this pathology (Foster *et al.*, 1987). Igh-1 disparate BALB/c congenic mice show striking differences in HSK susceptibility; C.AL-20 (Igh-1^d) mice are susceptible and C.B-17 (Igh-1^b) mice are extremely resistant to destructive keratopathy after corneal infection with HSV. The ability of $CD4^+$ T cells to induce necrotizing stromal keratitis in mice has been subsequently confirmed in several studies. For example, $CD4^+$ T cells are involved in inducing HSK in BALB/c mice (Doymaz and Rouse, 1992); moreover, transfer of either $CD3^+$ or $CD4^+$ T cells isolated from the spleen of C.AL-20 mice immunized with HSV-1 results in the development of severe HSK in recipient athymic *nu/nu* mice (Akova *et al.*, 1993). Furthermore, inflamed corneas of HSK-susceptible C.AL-20 mice are mainly infiltrated by $V\beta8^+CD4^+$ T cells that are not seen in the corneas of resistant C.B-17 mice, as assessed by immunohistological methods (Heiligenhaus *et al.*, 1994). These and various other studies clearly implicate a role for T cells in HSK immunopathology, yet curiously treatment of C.B-17 resistant mice with anti-µ Ab from birth makes them fully susceptible to HSK (Foster *et al.*, 1990).

Given these findings, our goal in the present work was to analyze TCR Vβ usage in B^- mice, to see if B cell Ab perturbations from birth might alter the TCR Vβ repertoire responding to corneal infection with HSV in ordinarily HSK-resistant mice.

Since immunoglobulin has been previously shown to influence TCR repertoires responding to certain Ags, we hypothesized that B cells and Ab play an important role in shaping the TCR repertoire responding to HSV. To test this hypothesis we investigated the TCR Vβ mRNA from organs (cornea, LN, spleen and thymus) of different groups of mice invloved in this study, before and after corneal inoculation with HSV.

The levels of T cell activation markers such as interleukin-2 (IL-2) gene (physiologically active only in T cells that have been stimulated trough the TCR) (Smith, 1984) were also analyzed in some samples of uninfected and infected mice. This was done because we used global T cell populations from all the organs studied without first generating T cell clones; this could result in analyzing TCRs from bystander cells present in the spleen and/or LN but not directly involved in HSK pathogenesis. Despite this limitation, our results demonstrate a predominant, although not exclusive, use of several Vβs in response to HSV infection.

The results of these studies suggest that the extraordinary HSK susceptibility of B cell-modulated C.B-17 mice is due to a disturbance of B cell – T cell ineractions rather than to an absence of B cells exclusively, and provide evidence that B cells play an important and apparently obligatory role in shaping the TCR Vβ repertoire expression in response to HSV corneal infection.

Materials and Methods

Mice. Adult (6-8-week-old) C.B-17 mice (Igh-1^b) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). C.B-17 offspring from parental breeding pairs were raised in our facilities in the Massachusetts Eye and Ear Infirmary. Groups of litter mates (i.e., age-matched) were divided into four experimental groups of 6 mice each, one untreated (control) group (group A) and three anti-µ Ab-treated groups (groups B,C and D). All animals were handled according to animal care guidelines from the National Institutes of Health, Bethesda, MD, USA, and the ARVO resolution on the use of animals research.

Virus. HSV-1 KOS strain was grown in Vero cells (CCL 81, ATCC, Rockville, MD, USA). Prior to experimental use the virus stock was titered using a standard plaque assay technique as previously reported (Foster *et al.*, 1987).

Anti-µ Ab treatment and transfer procedures. These experiments were performed as previously described (Arrunategui-Correa *et al.*, 1994). C.B-17 neonatal mice were injected daily with 0.05 ml (0.3 mg) of anti-µ Ab for the first week of life, 24 hrs after birth, and on alternate days thereafter for 4 more weeks at doses increased by 0.025 ml per week. Splenocytes of representative mice from the untreated group and from the anti-µ Ab-treated groups were analyzed by flow cytometry with a Becton Dickinson FACScan using single colour analysis to determine the completeness of depletion of B cells.

B cells were isolated from naive donor C.B-17 mice (8-week-old) treated intraperitoneally (i.p.) with a single injection of 0.04 ml of asialo-GM-1 antiserum (Waco Chemicals, Inc., VA, USA) one day prior to sacrifice in order to eliminate natural killer (NK) cells. B cell preparations were >96% $B220^+$ and <1.6% Thy 1.2^+, as evaluated by flow cytometry. The group C recipient B^- mice received $15 - 25 \times 10^6$ naive B cells and the group D mice received i.p. 0.5 µg of commercial polyclonal rabbit anti-HSV Ab in 0.5 ml (Dako Corp., Carpinteria, CA, USA). The third group of B^- mice remained without transfer as a control group for B^- mice.

Corneal challenge. Mice were anesthetized and the corneal surface was scratched with a 22-gauge needle and then inoculated with 2.5×10^5 PFU of HSV in 5 ml of Eagle's Minimal Essential Medium (MEM). This dose of the virus was predetermined to be optimal for producing HSK in susceptible C.AL-20 mice as previously reported (Akova *et al.*, 1993). Only one eye per mouse was infected while the contralateral eye served as the control. Each group of mice was housed separately.

Clinical scoring and statistical analysis. All animals were evaluated masked for clinical signs of disease every day, until they were sacrificed (21 days p.i.). An operating microscope was used to determine the severity of keratitis. Clinical scores from 0 to 4+ were assigned based on the size of the affected area, and the incidence of HSK was analyzed by Fischer's exact test as described earlier (Akova *et al.*, 1993; Jayaraman *et al.*, 1993). The experiment was repeated, starting afresh with new mice; the results were identical and therefore the data were pooled for this report.

RNA isolation and cDNA synthesis. Mice were killed 21 days post corneal HSV infection. The corneas, LN, spleen and thymus were harvested, and individual tissues pooled for each experimental group. Total RNA from the different samples was extracted

according to the modified method of Chomczynsky and Sacchi (1987). RNA was assayed spectrophotometrically at 260 nm, and its structural integrity and purity was assessed by formaldehyde RNA gel electrophoresis. cDNA was synthesized from oligo(dT)-primed RNA by reverse transcription (RT). cDNA was diluted 1:10 and used as a template for PCR amplification. A standard curve was generated for each cDNA sample using β-actin primers in order to demonstrate that all samples were reversely transcribed and amplified by PCR at similar efficiencies.

Oligonucleotide primers and PCR amplification. To analyze the TCR Vβ usage, we synthesized 19 different Vβ-specific oligonucleotides for use as 5'-sense primers for PCR and a primer specific for the constant region of TCR β chain (Cβ) for use as a 3'-antisense primer. The oligonucleotide primer sequence for Vβ1-19 were described previously (Arrunategui-Correa *et al.*, 1995).

PCR was performed as previously described (Arrunareguí-Correa *et al.*, 1995). A 20 µl sample of cDNA was amplified using 25 pmol/l both the Vβ- and Cβ-specific primers and 1.0 U of Taq DNA polymerase was added. The mixture was amplified in a Perkin Elmer Thermal Cycler model 9600 (Perkin Elmer Cetus, Norwalk, CT, USA) with the following profile:30 cycles of 45 secs at 94°C, 1 min at 55°C, and 2 mins at 72°C. A final extension for 7 mins was performed at 72°C.

The amplified products were separated on 1.5% agarose gels. In order to improve the sensitivity of fragment detection and examine the specificity of PCR products, Southern blot analysis was performed by blotting to nylon membranes (Boehringer). Hybridization, using digoxigenin-labelled Cβ probes and a detection system (Boehringer), was performed as described (Pedroza-Seres *et al.*, 1995). We present the data as positive or negative based on the presence or absence of a signal following Southern blot analysis. The entire experiment was repeated on additional (separate) mice, beginning with HSV corneal inoculation through mRNA extraction, RT-PCR and Southern blotting; the results of this replicate experiment confirmed the results of the first one, from which the data are shown. The results of Vβ usage induced by HSV coerneal infection and the modulation of same by anti-µ Ab therapy were precisely reproducible.

Results

Keratopathy

To assess more directly the importance of B cells in HSV infection, we modulated HSK-resistant C.B-17 mice with the anti-µ Ab treatment. As shown in Table 1, the incidence of HSK was significantly different between untreated (group A) and anti-µ Ab-treated C.B-17 mice (group B) ($p < 0.0001$). By day 21 post corneal inoculation all anti-µ Ab-treated mice had developed a severe (4+) stromal infiltrate accompanied by corneal ulceration and perforation. In contrast, only 2 of 12 mice in the untreated group developed HSK. In an attempt to determine the mechanisms by which B cells influence the resistance, we reconstituted one group of B$^-$ mice with naive B cells (group C), and another group was given anti-HSV Ab (group D) 24 hrs before corneal HSV infection. Six of the twelve mice belonging to group C developed HSK, in contrast to only 2/12 of group D.

TCR Vβ expression in cornea

We first analyzed the corneas of the different groups of mice in order to investigate the TCR Vβ gene expression at the local site of inflammation (Table 2, Fig. 1). We found that the anti-µ Ab treatment depleted Vβ1, Vβ5.1, Vβ6, Vβ9, Vβ10, Vβ13, Vβ15, and Vβ18 TCR expression and activated Vβ8.3- and Vβ16-expressing TCRs in uninfected mice. These results confirm once more the shaping of the TCR Vβ repertoire due to B cell depletion caused by the anti-µ Ab treatment.

HSV inhibited expression of Vβ8.1 and Vβ8.2 and activated expression of Vβ11, Vβ12, Vβ14, Vβ16, and Vβ19 in HSV-infected C.B-17 mice (Fig. 1A). Vβ8.1, Vβ8.2, and Vβ8.3 mRNAs were present only in susceptible but not in resistant mice (Fig. 1B). In marked contrast, however, HSV activated Vβ5.1, Vβ6, Vβ9, Vβ12-Vβ15, Vβ18 and Vβ19 mRNAs in B$^-$ mice. No appreciable expression of Vβs was noted in B$^-$ Ab-transferred mice, except for Vβ7, Vβ11, and Vβ17 genes. Analysis of B$^-$ B cell-transferred mice revealed that HSV activated Vβ1, Vβ6, Vβ9-Vβ15, Vβ18, and Vβ19 expression.

TCR Vβ gene expression in LN

As shown in Table 3, LN of uninfected C.B-17 mice actively expressed mRNA from the majority of the TCR Vβ gene families. However, after B cell modulation, several Vβ gene families, namely Vβ1, Vβ5.1, Vβ6, Vβ9-Vβ15, and Vβ17-Vβ19, failed to be expressed.

The HSV infection did not change the overall pattern of TCR Vβ expression in LN of CV.B-17 mice at day 21 after corneal challenge, except for a pronounced decrease of Vβ17 and Vβ18 (Table 3). We reasoned that if the patho-

Table 1. Comparison of HSK incidence in untreated and anti-µ Ab-treated (B$^-$) mice after inoculation of HSV in cornea

Groups	HSK incidence
Untreated	
Group A: C.B-17 mice	2/12
Anti-µ Ab-treated	
Group B: B$^-$ mice	12/12
Group C: B$^-$ B cell-transferred mice[a]	6/12
Group D: B$^-$ Ab-transferred mice[b]	2/12

[a]Each mice received 15 – 25 x 10^6 B cells.
[b]Each mice received 0.5 µg of commercial anti-HSV Ab.

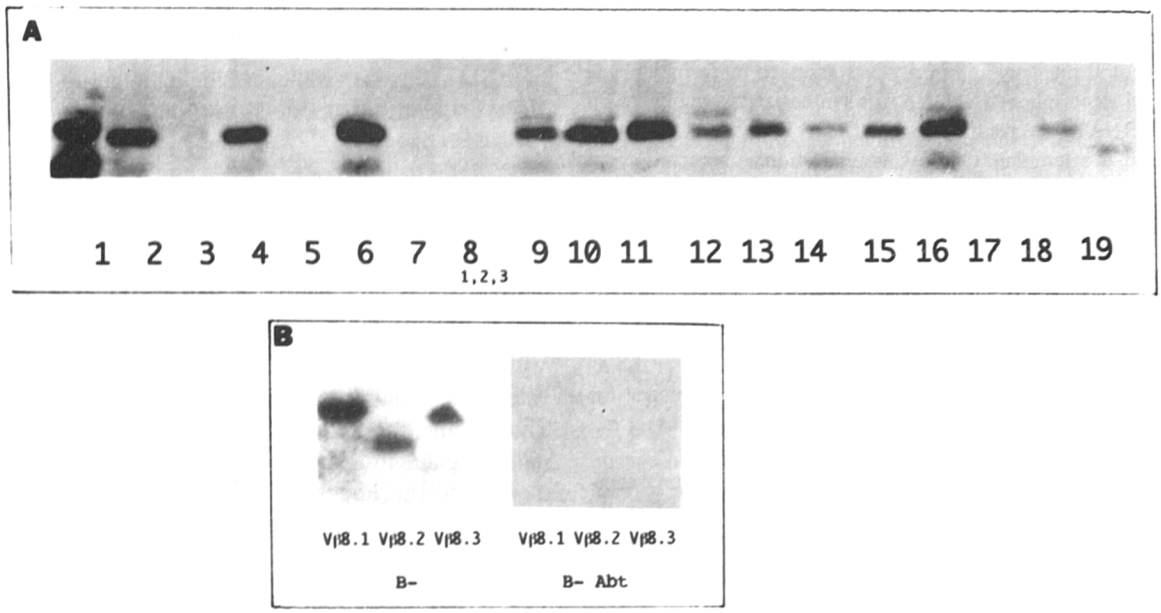

Fig. 1
PCR amplification of TCR Vβ mRNA in cornea of C.B-17, B⁻ and B⁻ Ab-transferred mice 21 days after inoculation of HSV in cornea
A: C.B-17 mice. B: B⁻ mice. Lanes 1-19 represent Vβ1-Vβ19.

Table 2. T cell receptor Vβ family gene expression in cornea

Mice	Vβ gene family																				
	Vβ1	Vβ2	Vβ3	Vβ4	Vβ5.1	Vβ5.2	Vβ6	Vβ7	Vβ8.1	Vβ8.2	Vβ8.3	Vβ9	Vβ10	Vβ11	Vβ12	Vβ13	Vβ14	Vβ15	Vβ16	Vβ17	Vβ18	Vβ19
C.B–17																						
U	+	+	–	+	+	–	+	–	+	+	–	+	+	–	–	+	–	+	–	–	+	–
I	+	+	–	+	+	–	+	–	–	–	–	+	+	+	+	+	+	+	+	–	+	+
B⁻																						
U	–	+	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–
I	–	+	–	+	+	–	+	–	+	+	+	+	–	–	+	+	+	+	+	–	+	+
Ab[a]	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
B[b]	+	+	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+

U = uninfected; I = infected.
[a]Infected, Ab-transferred mice.
[b]Infected, B cell-transferred mice.
The data are expressed as present (+) or absent (–) for each sample. Only data of one of two similar experiments are shown.

genic T cells in HSK were dependent on a limited number of specific TCRs, B⁻ mice that failed to express 13 of 22 Vβs and suffered pronounced keratitis after HSV infection would provide useful information. When the expression of Vβs was examined in infected B⁻ mice, HSV appeared to increase the percentage of Vβ gene transcipts. Fig. 2 shows a comparison between HSK-resistant C.B.-17 (top row) and B⁻ Ab-transferred (bottom row) mice, 21 days p.i. Strong expression of Vβ1 and Vβ13 was seen in B⁻ Ab-transferred mice. In B⁻ B cell-transferred mice, only Vβ1, Vβ6, Vβ8.1 and Vβ10 messages were expressed.

TCR Vβ gene expression in spleen

The expression of Vβ transcripts was examined in the spleen under identical experimental conditions, and the results are shown in Table 4.

The same proportion of homology as found in the cornea between untreated and anti-μ Ab-treated C.B-17 mice (55.5%) was found in the case of the spleen.

We have previously reported the Vβ11 gene expression in the spleen of resistant mice in the herpes simplex retinitis (HSR) model (Arrunategui-Correa *et al.*, 1995). Table 4

Table 3. T cell receptor Vβ family gene expression in LN

Mice	Vβ gene family																					
	Vβ1	Vβ2	Vβ3	Vβ4	Vβ5.1	Vβ5.2	Vβ6	Vβ7	Vβ8.1	Vβ8.2	Vβ8.3	Vβ9	Vβ10	Vβ11	Vβ12	Vβ13	Vβ14	Vβ15	Vβ16	Vβ17	Vβ18	Vβ19
C.B–17																						
U	+	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I	+	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+
B⁻																						
U	–	+	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–
I	+	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ab[a]	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+
B[b]	+	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–

U = uninfecte; I = infected.
[a]Infected, Ab-transferred mice.
[b]Infected, B cell-transferred mice.
The data are expressed as present (+) or absent (–) for each sample. Only data of one of two similar experiments are shown.

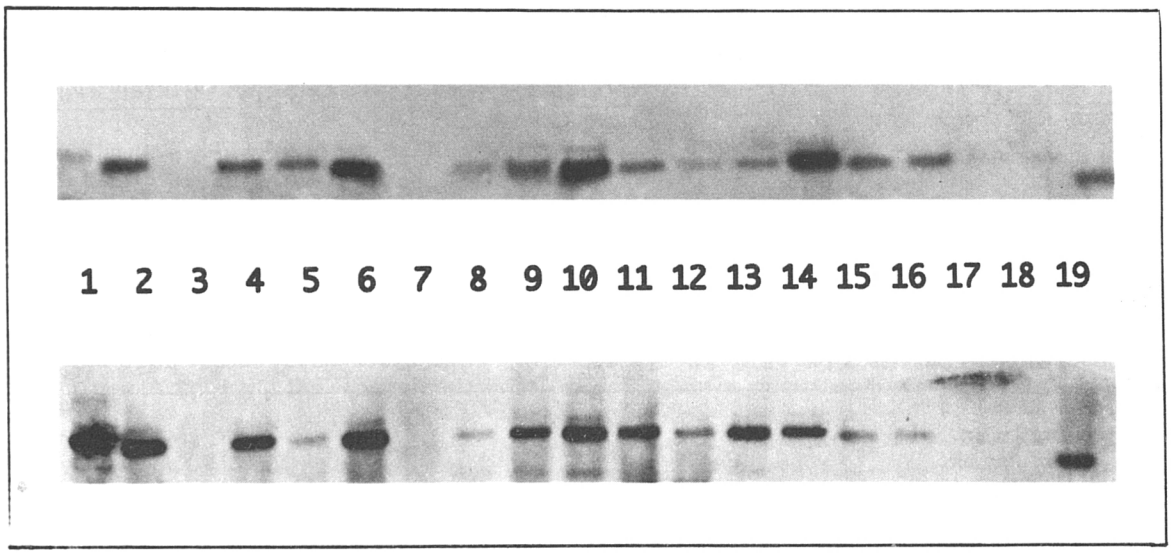

Fig. 2
PCR amplification of TCR Vβ mRNA in LN of C.B-17 and B⁻ Ab-transferred mice 21 days after inoculation of HSV in cornea
Top: C.B-17 mice. Bottom: B⁻ Ab-transferred mice. Lanes 1-19 represent Vβ1-Vβ19.

shows the results of Vβ gene excpression in spleens collected from untreated and B⁻ mice. As can be seen, Vβ5.1, Vβ5.2, Vβ13-Vβ15, abd Vβ18 gene expression could not be detected in the 3 different groups of B⁻ mice studied. The frequency of utilization of the 19 Vβ gene families at day 21 p.i. was somewhat different between untreated and B⁻ mice, but there were major differences between B⁻ and B⁻ Ab- and B⁻ cell-transferred mice. The observation that the Vβ7 gene was expressed in resistant but not in susceptible mice raises the possibility that the TCR Vβs involved in HSK pathology are distinct from those leading to HSR pathology.

TCR Vβ gene expression in thymus

We examined the TCR Vβ gene expression in the thymus. T cell precursors develop from pluripotent hemopoietic stem cells, which in the adult animal arise in the bone marrow and migrate to the thymus, one of the major sites of T lymphocyte differentiation (Gause *et al.*, 1988).

In the preceeding experiments we have demonstrated than the anti-μ Ab treatment disturbs 45.5% of the TCR Vβ gene repertoire in the cornea and spleen. The fraction of disturbed gene expression in the thymus was similar as shown in Table 5.

Table 4. T cell receptor Vb family gene expression in spleen

Mice	Vβ gene family																					
	Vβ1	Vβ2	Vβ3	Vβ4	Vβ5.1	Vβ5.2	Vβ6	Vβ7	Vβ8.1	Vβ8.2	Vβ8.3	Vβ9	Vβ10	Vβ11	Vβ12	Vβ13	Vβ14	Vβ15	Vβ16	Vβ17	Vβ18	Vβ19
C.B–17																						
U	+	+	–	+	+	–	+	–	+	+	–	+	+	–	–	+	–	+	–	–	+	–
I	–	+	–	+	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–
B																						
U	–	+	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–
I	–	–	–	+	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	+	–	+
Ab[a]	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	+	–	–
B[b]	+	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–

U = uninfected; I = infected.
[a]Infected, Ab-transferred mice.
[b]Infected, B cell-transferred mice.
The data are expressed as present (+) or absent (–) for each sample. Only data of one of two similar experiments are shown.

Table 5. T cell receptor Vβ family gene expression in thymus

Mice	Vβ gene family																					
	Vβ1	Vβ2	Vβ3	Vβ4	Vβ5.1	Vβ5.2	Vβ6	Vβ7	Vβ8.1	Vβ8.2	Vβ8.3	Vβ9	Vβ10	Vβ11	Vβ12	Vβ13	Vβ14	Vβ15	Vβ16	Vβ17	Vβ18	Vβ19
C.B–17																						
U	+	+	–	+	+	–	+	–	+	+	–	+	+	–	–	+	–	+	–	–	+	–
I	–	–	–	+	+	+	–	+	+	+	+	–	–	+	+	–	+	–	+	–	–	–
B																						
U	–	+	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–
I	+	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	–
Ab[a]	–	+	–	–	+	–	–	–	–	–	–	+	+	+	+	–	+	+	+	+	–	+
B[b]	+	+	–	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	–	–	+

U = uninfected; I = infected.
[a]Infected, Ab-transferred mice.
[b]Infected, B cell-transferred mice.
The data are expressed as present (+) or absent (–) for each sample. Only data of one of two similar experiments are shown.

HSV infection affected 14 Vβ genes in the thymus of C.B.-17 mice, of which 7 were expressed and 7 were not expressed after HSV encounter. Transcripts derived from 10 Vβ families were altered in B$^-$ mice after HSV infection. Vβ11, Vβ12 and Vβ14 families that were not expressed in uninfected C.B-17 and B$^-$ mice were expressed in both kinds of mice after HSV infection; Vβ6, Vβ9, Vβ10, Vβ13, Vβ15, and Vβ18 families were expressed in uninfected C.B-17 but not in B$^-$ mice. Vβ6 and Vβ13 TCRs were not expressed in uninfected C.B-17 and B$^-$ Ab-transferred mice, both HSK-resistant, but were expressed in infected B$^-$ and B$^-$ B cell-transferred mice, both HSK-susceptible. Fig. 3B shows pronounced differences in Vβ5.1, Vβ5.2, Vβ8.1, and Vβ8.3 mRNAs between resistant and susceptible mice.

Seventeen of twenty-two Vβ genes were expressed in the B$^-$ B cell-transferred group at day 21 p.i., of which 12 were not expressed in B$^-$ mice, suggesting that repopulation of B cells had an impressive effect on the TCR repertoire of those mice.

Discussion

Necrotizing stromal HSK develops primarily as the result of an exuberant immunopathologic response to herpes infection of the cornea with certain murine strains. T lymphocytes play a central role in the ocular immune response to HSV by killing virus and infected cells, regulating inflammatory responses and helping B lymphocytes to produce Abs (Lanzavecchia *et al.*, 1993).

An anti-μ Ab treatment of neonatal mice converts C.B-17 mice, usually HSK-resistant, into HSK-susceptible ones, similarly to our prior observations with experimental HSR (Arrunategui-Correa *et al.*, 1994).

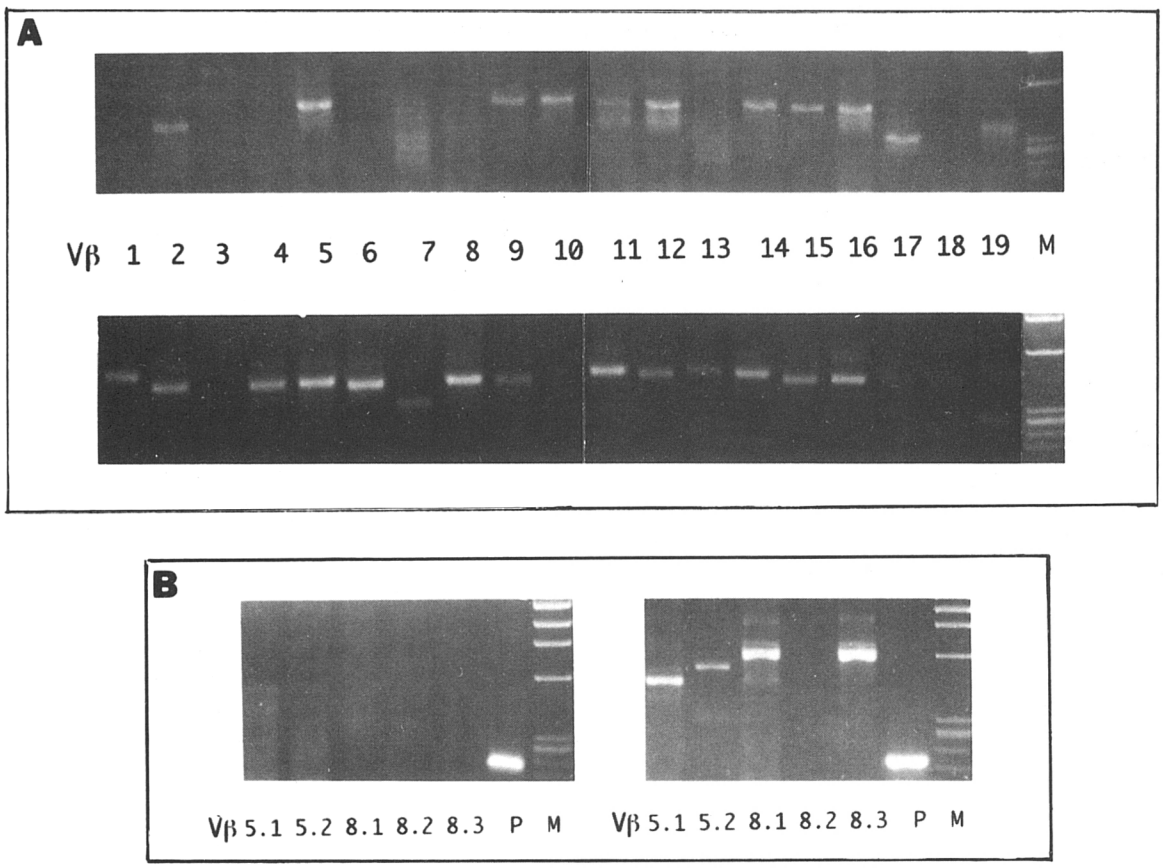

Fig. 3

PCR amplification of TCR Vβ mRNA in thymus of B⁻ Ab-transferred and B⁻ B cell-transferred mice 21 days after inoculation of HSV in cornea

B⁻ Ab-transferred mice (A, top; B, left). B⁻ B cell-transferred mice (A, bottom; B, right). Size marker (lane M). Cb gene as positive control (lane P).

We previously studied the possibility that B cell modulation could alter T cell function; delayed type hypersensitivity (DTH) and cytotoxic lymphocyte (CTL) results for anti-μ Ab-treated and untreated mice were similar (Foster *et al.*, 1990; Arrunategui-Correa *et al.*, 1994), indicating that the anti-μ Ab-induced HSK enhancement was independent of T cell-mediated DTH and CTL responses. But now we find (and report here) that, in C.B-17 mice made B cell-deficient by the anti-μ Ab treatment (B⁻ mice), the TCR Vβ repertoire gene family appears to be severely altered, suggesting that this alteration may be a consequence of deficient B cell participation in early developmental shaping of the TCR repertoire and, further, that such alteration produces a deficiency in regulatory T cells which ordinarily prevent an overly exuberant inflammatory response in C.B-17 mice after HSV inoculation of the corne.

To understand better the effect of the anti-μ Ab treatment on the local immune response, eye samples were analyzed; the results suggest that there are major differences in the Vβ expression of T cells infiltrating the HSV-inoculated eyes between susceptible and resistant mice. Specifically, 21 days p.i., Vβ8.1, Vβ8.2 and Vβ8.3 were expressed in both susceptible groups of mice (B⁻ and B⁻ B cell-transferred) while expression of these Vβs was absent from both resistant groups (C.B-17 and B⁻ Ab-transferred). These extraordinary findings at the level of gene expression in eyes confirm exactly and extend our previous findings at the level of protein production (Jayaraman *et al.*, 1993; Heiligenhaus *et al.*, 1994), once again providing strong evidence for the critical participation of Vβ8⁺ T cells in production of HSK immunopathology.

To examine whether the immune response can be affected in other organs as previously seen in HSR (Arrunategui-Correa *et al.*, 1994), and whether corneal HSV infection induces a systemic T cell response reflecting the T cell population in eyes, we compared TCR Vβ gene expression in the LN, spleen and thymus. This analysis revealed striking differences between C.B.-17 and B⁻ mice in the Vβ expression in the spleen, where Vβ7 seems to be involved in protection; this gene was not expressed in susceptible mice (B⁻ and B⁻ B cell-transferred) in our experiments.

There is now increasing evidence to suggest that an anti-μ Ab treatment blocks B cell development at the pre-

B cell stage, affecting TCR Vβ gene expression and therefore blocking thymocyte development (Palmer *et al.*, 1993). On the basis of these observations, some T lymphocytes become functionally incompetent.

Taken together, available evidence suggests that B cells and their products, anti-HSV Abs, not only can provide protection against destructive inflammation of the cornea after HSV-1 inoculation, but also are critical for protection against HSK in normally resistant C.B-17 mice because of their effects on TCR repertoire shaping, a TCR repertoire different from that of HSK-susceptible mice. The B cell-modified C.B-17 mouse enhanced susceptibility to HSK reflects preferential usage of Vβ TCR genes, with a loss of some TCR Vβ expressing protective T cells and (perhaps) a delay of anti-herpes Ab synthesis.

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