Molecular Immunology 47 (2010) 1154–1160

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Short communication

Junctional diversity prevents negative selection of an antigen-specific T cell repertoire

Patrick Kilgannon, Zuzana Novak, Arun Fotedar, Bhagirath Singh*

Department of Microbiology and Immunology, and Robarts Research Institute, University of Western Ontario, London, Ontario N6A 5C1, Canada

ARTICLE INFO

Article history:
Received 30 October 2009
Accepted 3 November 2009
Available online 1 December 2009

Keywords:
T cell repertoire
Junction diversity
T cell receptor
Negative selection
Mammary tumor provirus
Mtv
Superantigen

ABSTRACT

Endogenous mouse mammary tumor proviruses (MMTV; Mtv loci) deletes Vβ6 expressing T cells in the thymus of Mtv-7$^+$ DBA/2 (H2^d) mice through negative selection. We found that in Mtv-7$^-$ BALB/c (H2^d) mice, Vβ6 is a dominant V gene used in T cell responses to an 18 amino acid long peptide antigen: EYKEYAEYAEYAEYAEYA [abbreviated as K5 or EYK(EYA)$_5$]. It was therefore surprising to find that despite the deletion of Vβ6+ T cells, vigorous K5 specific T cell responses that use Vβ6 can be raised in DBA/2 mice. Sequence analysis of Vβ6 junctional diversity in K5 specific T cell lines revealed that the DBA/2 K5 repertoire compensates for the loss of most Vβ6 T cells by overusing and amplifying Vβ6+ T cells escaping central deletion and peripheral tolerization. In order to address the inability of some Vβ6 T cells to recognize Mtv-7$^+$ we analyzed a panel of BALB/c Vβ6 expressing T cell hybridomas. This data supported the argument that certain Vβ6 junctional sequences preclude Mtv recognition and allows their escape from central deletion in DBA/2 mice. These cells are not anergic and can be activated with cognate peptide antigen in periphery. We suggest that junctional diversity at the V region of some of the T cell receptors does not allow these cells to recognize self-superantigens with high enough affinity and thus they escape negative selection in the thymus. These results for the first time provide a molecular explanation of how the immune system compensates for "hole in the repertoire" caused by deletion of the majority of T cells carrying certain V region segments.

1. Introduction

The acquisition of self-tolerance is central to the generation of a functional peripheral T cell repertoire (Pullen et al., 1989). A number of systems have been used effectively to demonstrate negative selection of T cells in the thymus (Kappler et al., 1987a, 1988; MacDonald et al., 1988). Some of these systems make use of the observation that specific Vβ's on the T cell receptor (TCR) appear to confer reactivity to superantigens (SAg) (Kappler et al., 1987b; MacDonald et al., 1988). Mice with endogenous superantigens encoded by mouse mammary tumor proviruses (MMTV; encoded by Mtv loci) are known to delete thymocytes that express Vβ genes that correlate with reactivity (Acha-Orbea and MacDonald, 1995; Hodes and Abe, 2001). For example reactivity to Mtv protein results in deletion of Vβ6, Vβ8.1 and Vβ9 expressing T cells in Mtv-7$^+$ mice (Kappler et al., 1988; MacDonald et al., 1988), while Mtv-6$^+$ is recognized by TCR bearing Vβ3 (Abe et al., 1988; Acha-Orbea et al., 2007). These reactivities are unique in that the role of TCRα chain and the junctional diversity in the β chain appear to have little influence on the interaction with antigen. There are exceptions that provide insight into the limits and parameters of T cell recognition in these model systems. In this paper we address the mechanism that prevents central deletion and allows peripheral repertoire to harbor T cells with self-reactive Vβ's as sampled by an 18 amino acid long peptide antigen: EYKEYAEYAEYAEYAEYA [abbreviated as K5 or EYK(EYA)$_5$] specific T cell response (Novak et al., 1992b; Singh et al., 1980).

Since DBA/2 mice carry Mtv-7$^+$ SAg, they are known to delete Vβ6 cells during thymic maturation (Acha-Orbea and MacDonald, 1995; Hodes and Abe, 2001; Kappler et al., 1987b, 1988; MacDonald et al., 1988). This is in contrast to BALB/c mice that are Mtv-7$^-$ and consequently do not delete their Vβ6 T cells. H2^d strain (BALB/c and DBA/2) mice are high responders to K5 peptide antigen (Singh et al., 1980). This provides an excellent model system to explore if antigen-specific Vβ6 bearing T cells exist in the periphery of DBA/2 mice. We therefore analyzed K5 reactive T cells in BALB/c and DBA/2 mice for Vβ6 usage. However, simply finding Vβ6+ T cells in the periphery of DBA/2 mice would not address the immunological competence of such cells. Therefore, we amplified Vβ6 cells that

Abbreviations: K5 or EYK(EYA)$_5$, EYKEYAEYAEYAEYAEYA; CFA, complete Freund's adjuvant; PPD, purified protein derivative; MMTV or Mtv, endogenous mouse mammary tumor proviruses; V, variable region gene; J, junctional region gene; C, constant region gene; TCR, T cell receptor; SAg, superantigen.

* Corresponding author at: Department of Microbiology & Immunology, Room 3014, Dental Sciences Building, University of Western Ontario, London, Ontario N6A 5C1, Canada. Tel.: +1 519 661 3228; fax: +1 519 661 4226.
E-mail address: bsingh@uwo.ca (B. Singh).

0161-5890/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2009.11.006

escape deletion in DBA/2 thymus by immunization with K5 antigen. This allowed us to work with immunologically competent cells, removing the chance of isolating anergic clones. We used polymerase chain reaction (PCR) to specifically amplified and cloned Vβ6 sequences from K5 reactive bulk T cell lines derived from DBA/2 and BALB/c mice. We assessed the Vβ6 junctional diversity and J region usage associated with the inability to recognize Mtv-7 protein.

2. Materials and methods

2.1. Mice

BALB/c ($H2^d$, Mtv-7^-), DBA/2J ($H2^d$, Mtv-7^+) and CBA/J ($H2^k$, Mtv-7^+) mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

2.2. Peptides

Peptides were synthesized in our laboratory as previously described by using FMOC chemistry on Applied Biosystems 431 Peptide Synthesizer (Novak et al., 1992b; Singh et al., 1980). HPLC purified peptides were analyzed by mass spectrometry and were dissolved in saline, pH was adjusted to 7.2 with 0.1N NaOH and filter sterilized through 0.22 μM filter.

2.3. Lymph node T cell proliferation assay

Six- to eight-week-old female BALB/c and DBA/2J mice (5 mice/group) were injected with 25 μg of K5 antigen in 25 μl saline emulsified with an equal volume of CFA in each hind footpad (total of 50 μg/mouse). Eight days later the popliteal lymph nodes were removed, and a single cell suspension was prepared in RPMI 1640 (Invitrogen/GIBCO, Carlsbad, CA, USA). Cultures were set up in triplicate with 4×10^5 viable lymph node cells in 100 μl of medium containing K5 or control PPD antigen and incubated at 37 °C for 3 days. Each culture was then pulse for 24 h with 0.7 mCi of [methyl-^{3}H]-thymidine (2 Ci/mmole) in 25 μl of medium. The cells were then harvested onto glass filter and the incorporation of ^{3}H-thymidine was measured using a liquid scintillation counter (Packard Tri-Carb scintillation spectrometer). Results were expressed as cpm ± SD (Table 1).

2.4. T cell lines

Six- to eight-week-old BALB/c and DBA/2J mice were immunized in hind footpads with 50 μg of K5 in complete Freund's adjuvant (CFA). Popliteal lymph node T cells were used to generate K5 specific T cell lines as previously described (Fotedar et al., 1985; Novak et al., 1992a). Briefly 7–9 days after immunization lymph node T cells were nylon wool separated and $2–4 \times 10^5$ T cells/ml were cultured with 3×10^6 irradiated (3000 R) spleen cells/ml and 10 μg K5 peptide/ml in RPMI 1640 (Invitrogen/GIBCO, Carlsbad, CA, USA) containing 10% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol, 10 mM Hepes, 2 mM glutamine and penicillin–streptomycin. T cell lines from were carried in the presence K5 and syngeneic irradiated spleen cells for 1–4 months prior to fusing with BW5147 thymoma cells. The antigen reactivity of the T cell lines was tested every week with PPD (purified protein derivative) as control antigen. For this purpose K5 specific T cell lines were co-cultured with 4×10^5 BALB/c or DBA/2J ($H2^d$) irradiated (3000 R) spleen cells as APC and 10 μM antigen in 300 μl of T cell line medium. Cultures were pulsed 3 days later with 1 μCi of ^{3}H-thymidine/well and harvested 16 h later. The ^{3}H-thymidine incorporation was determined by scintillation counting (Table 2).

Table 2
Proliferation assay of DBA/2 and BALB/c K5 specific bulk T cell lines.[a].

T cell line	APC	PPD	K5	cpm ($\times 10^3$)
DBA/2				
+	–	–	–	1
+	+	+	–	1
+	+	–	+	79.2 ± 16.9
BALB/c				
+	–	–	–	1
+	+	+	–	1
+	+	–	+	95.7 ± 1.8

[a] Antigen-specific proliferation of representative K5 specific T cell lines from DBA/2 and BALB/c mice was measured as described in Section 2. T cell lines were generated 2–6 times as described in Section 2 and similar proliferation data was obtained.

2.5. Generation and characterization of T cell hybridomas

K5 specific BALB/c T cell hybridomas were generated by fusing the BALB/c T cell lines described above with the AKR thymoma line BW5147 (Fotedar et al., 1985; Novak et al., 1992a). We have previously reported the characterization of these T cell hybridomas (Fotedar et al., 1985; Novak et al., 1992a,b) and hybridomas used in this study are listed in Table 3. The antigen specificity of T cell hybridomas was tested by incubating T cell hybridoma (10^5) cells with BALB/c irradiated (3000 R) spleen cells (10^6) with 10 μM antigen in 300 μl RPMI 1640 and 10% FCS for 24 h. The supernatants were diluted at 1:2 in 200 μl and tested for the presence of IL-2 by their ability to support the growth of 10^4 IL-2-dependent CTLL cells, as measured by ^{3}H-thymidine incorporation (Novak et al., 1992a).

Table 1
Dose specific T cell proliferative respone of K5 peptide in BALB/c and DBA/2 mice.[a].

Priming antigen (50 μg/mouse)	Challenge antigen (μg/ml)	BALB/c	DBA/2 (cpm ± SD)
K5	Medium	4,399 ± 1,550	3,531 ± 3,557
	PPD		
	50	67,501 ± 17,630	75,417 ± 8,291
	K5		
	1	29,625 ± 4,459	32,618 ± 3,888
	3	35,105 ± 6,998	37,251 ± 5,277
	10	46,015 ± 7,371	36,859 ± 9,918
	30	66,372 ± 8,158	54,983 ± 4,738
	100	64,841 ± 3,249	69,923 ± 10,172

[a] BALB/c and DBA/2 mice were injected in each hind footpad with 25 μg of K5 peptide dissolved in 25 μl saline and emulsified with 25 μl of CFA. Eight days later, drained lymph nodes were harvested. Triplicate single cell suspension cultures were set up with 4×10^5 cells/well and incubated with various amount of K5 peptide as described in Section 2. The results are presented as incorporation of ^{3}H-thymidine by lymph node T cells.