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CDR3 sequence preference of TCRBV8S2^{+} T cells within the CNS does not reflect single amino acid dependent avidity expansion

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Abstract

To investigate the influence of antigen and restricting MHC class II molecule on the T cell repertoire, we varied the peptide source by immunizing either with myelin basic protein (MBP) $_{RAT}$63-88 or MBP$_{GUINEA\ PIG\ (GP)}$63-88, which differ in the core region of the peptide binding site at position 79 by a single exchange of threonine (T) to serine (S) and by altering the MHC by immunizing MHC congenic LEW (RT1^{l}) and LEW.1W (RT1^{u}) rats. In both MHC haplotypes both peptides lead to oligoclonal dominance of TCRBV8S2 expressing T cells within the central nervous system (CNS) as assessed by complementary determining region 3 (CDR3) spectratyping. In contrast cytofluorometric analysis indicated that only MBP$_{GP}$63-88 in context with the RT1^{l} haplotype of the LEW rat lead to strong expansions of TCRBV8S2 expressing T cells within the CNS. Importantly, the small conservative change from S to T at position 79 within MBP63-88 had a strong influence both on the encephalitogenic potential of the peptide and on the number of TCRBV8S2^{+} T cells infiltrating the CNS. These results demonstrate that even minor changes in only one side chain of an amino acid within an (auto)antigen can dramatically alter TCR avidity for certain MHC class II/peptide complexes.

Keywords: T cells; Autoimmunity; EAE/MS; MHC; T cell receptors

1. Introduction

A better understanding of the way in which the pathogenic T cell repertoire is shaped in the context of an autoimmune disease might lead to better therapeutical strategies based on the manipulation of this pathogenic repertoire. Animal models of experimentally induced autoimmune diseases have been very useful for investigating exactly this kind of questions. Experimental autoimmune encephalomyelitis (EAE) is an animal model which mimics the human demyelinating central nervous system (CNS) disease multiple sclerosis (MS). It can be actively induced in susceptible animals by immunization with components of the myelin sheath in complete Freund's adjuvant. Especially in EAE models in mice and rats, the pathogenic T cell repertoire has been extensively studied by the analysis of encephalitogenic T cell lines and clones (Acha-Orbea et al., 1988; Burns et al., 1989; Gold et al., 1991; Sun et al., 1992; Urban et al., 1988) and more recently by the analysis of T cells infiltrating the CNS (Kim et al., 1998; Sun et al., 1994). In acute EAE induced in LEW (RT1^{l}) rats with the immunodominant myelin basic protein (MBP)$_{GUINEA\ PIG\ (GP)}$ 63-88 peptide, several groups reported a frequent usage of TCRBV8S2 chains by in vitro established encephalito-

Abbreviations: CDR, complementary determining region; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; FACS, fluorescence activated cell sorter; GP, guinea pig; MBP, myelin basic protein; RT1, MHC of rat; TCR, T cell receptor; TCRBV, T cell receptor variable beta.

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genic T cell clones. Sequencing of complementary determining region 3 (CDR3) regions of these TCRBV8S2$^+$ T cells revealed the presence of a short common CDR3 motif which consisted of a marked dominance of aspartic acid (D) and serine (S), DS, in the first two amino acid positions after the common cysteine (C), alanine (A), serine (S), serine (S), CASS, motif associated with multiple Jβ elements and few or no N region nucleotide additions in the CDR3 region (Burns et al., 1989; Gold et al., 1991; Zhang and Heber-Katz, 1992). Moreover, a large amount of T cells infiltrating the CNS were equally found to be TCRBV8S2$^+$ (Kim et al., 1998; Weissert et al., 1998).

Interestingly, in this rat model preferential expansion of TCRBV8S2$^+$ T cells is strictly dependent on the usage of the heterologous MBP_{GP}63-88 peptide: immunization with the homologous MBP_{RAT}63-88 peptide, where threonine (T) is substituted for serine (S) in the core region of the peptide, was not followed by dominance of TCRBV8S2$^+$ T cells in CNS as measured by FACS (Weissert et al., 1998). Another study showed that short term cultures with the 'self' peptide did not support selection of TCRBV8S2$^+$ T cells 'in vitro' (Mor et al., 2000).

In the present study, we wanted to investigate the impact of the peptide source (homologous vs. heterologous) and of the MHC class II haplotype on the complete CNS infiltrating T cell repertoire. Due to the limited amount of available rat TCRBV specific antibodies, a PCR-based method was adopted. CDR3 size spectratyping allows the qualitative assessment of the oligoclonality of a T cell response in regard to the expression of certain TCRBV chains (Pannetier et al., 1993). This technique allows the analysis of T cells directly 'ex vivo' out of the periphery and the CNS, thus overcoming the need for establishing T cell lines and clones which may not always reflect the 'in vivo' situation.

2. Materials and methods

2.1. Rats

Female rats, 8–12 weeks of age, were used in all experiments. All strains have been described (Weissert et al., 1998). LEW (RT1^l) and LEW.1W (RT1^u) were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany). Animals were scored for clinical signs of EAE and weighed daily.

2.2. Synthetic peptides

MBP_{gp}63-88 (*AA*RTTHYGSLPQKSQR*S*QDENPVVHF) and MBP_{rat}63-88 (*HT*RTTHYGSLPQKSQR*T*QDENPVVHF) were synthesized by Fmoc/HBTU strategy (Dr. Å. Engström, Dept. of Medical and Physiological Chemistry, University of Uppsala, Sweden). Peptides were purified by reversed-phase chromatography and, subsequently, analysed by plasma desorption mass spectroscopy. The degree of purity of the used peptides was >99%.

2.3. Immunization protocol and scoring of EAE

Rats were injected intradermally at the base of the tail with a total volume of 200 μl inoculum containing 100 μg of peptide in saline mixed (1:1) with CFA. One hundred microliter of CFA consisted of IFA (Sigma-Aldrich, St. Louis, MO) and 500 μg of heat-inactivated *Mycobacterium tuberculosis* (strain *H37* RA; Difco Laboratories, Detroit, MI). The clinical scoring was as follows: 0=no illness, 1=tail weakness or paralysis, 2=hind leg paraparesis or hemiparesis, 3=hind leg paralysis or hemiparalysis, 4=tetraparesis or moribund.

2.4. Extraction of CNS cells

After disease onset rats were perfused with cold PBS and brains and spinal cords were dissected out under deep anaesthesia. Subsequently, brains and spinal cords were mechanically dissociated through a stainless steel sieve and homogenized in 10 ml 50% Percoll (Amersham Biosciences, Freiburg, Germany)/0,1% BSA/ 1% glucose containing 500 U DNase type I (Invitrogen, Karlsruhe, Germany). Ten milliliter of 50% Percoll were added to each sample after homogenization. A discontinuous Percoll gradient was obtained by adding 7 ml of 63% Percoll below and 20 ml of 30% Percoll above the sample. Samples were centrifuged for 40 min at $1000 \times g$ at 4 °C. Lymphocytes were collected from the 63/50% Percoll interface. The cells were subsequently washed twice in 15–25 ml PBS with centrifugation at $600 \times g$ for 15 min at 4 °C and flushed through a 70 μm plastic strainer (BD Biosciences, Hamburg, Germany).

2.5. FACS analysis of CNS-cells

The monoclonal antibodies R78-FITC (TCRBV8S2), CD4-PE and appropriate isotype controls were purchased from Becton Dickinson (BD Pharmingen Heidelberg, Germany). Flow cytometric analysis was performed with a FACScalibur (BD Biosciences) system using CellQuest software for data acquisition. Cells were gated on the lymphocyte population in the FSC-SSC scatterplot.

2.6. cDNA synthesis and PCR amplification

Total RNA was extracted from CNS cells using Trizol (Invitrogen). Subsequently, cDNA was synthesized by reverse transcription with MMLV-RT (Invitrogen) and random pdN6 primers (Amersham Biosciences) in the presence of Rnasin (Promega, Mannheim, Germany). cDNA was amplified by polymerase chain reaction (PCR) using a set of 23 different TCRBV primers together with a 6-FAM tagged CB primer (Table 1). All