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Development of transgenic mice expressing a coronavirus-specific public CD4 T cell receptor

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ABSTRACT

Mice that are transgenic (Tg) for T cell receptor (TCR) expression are used extensively to analyze longitudinal T cell responses during effector and memory phases of the T cell response. Generation of TCR Tg mice generally requires T cell stimulation and cloning *in vitro* prior to amplification, processes which introduce biases into selection of the TCR that is ultimately chosen for TCR Tg mouse generation. Here we describe an alternative approach that involves no T cell stimulation or propagation *in vitro*. We generated mice that were transgenic for a TCR responding to a CD4 T cell epitope (epitope M133) that is immunodominant in mice infected with a neurotropic coronavirus, the JHM strain of mouse hepatitis virus. The CD4 T cell response to epitope M133 is of particular interest because it may be pathogenic, protective or regulatory, depending upon the physiological setting. We applied an iterative process in which we identified a TCR-β chain expressed by all mice that were examined ('public sequence'). This TCR-β chain was introduced into bone marrow cells with a lentivirus vector, generating TCR-β retrogenic mice. A TCR-α chain that paired with this TCR-β was then identified and used to generate a second set of TCR (α/β) retrogenic mice. After demonstrating that these cells were functional and responded to epitope M133, these TCR chains were used to generate an epitope M133-specific TCR Tg mouse. This method should be generally useful for engineering TCR Tg mice without introduction of bias caused by *in vitro* manipulation and propagation.

1. Introduction

The T cell response is critical for pathogen clearance and tumor eradication and is mediated by interactions between the T cell receptor and MHC class I or II/peptide (MHCp) complex. Multiple factors, including the kinetics and affinity of the interaction, size of the T cell precursor pool, kinetics of peptide presentation and affinity of the peptide for the MHC antigen are critical for understanding the T cell response in a given physiological or pathological setting. Further, the response to a single epitope is generally complex, involving T cells with a variety of affinities for the MHCp complex. In addition, expansion of a specific T cell in a single immune response is partly stochastic, depending on access of the T cell in question to the MHCp. Finally, the number of epitope-specific cells in the naïve T cell pool is small, ranging from less than 100 to 1000 cells (Pewe et al., 2004; Moon et al., 2007; Obar et al., 2008; La Gruta et al., 2010; Zhao et al., 2011), making it difficult to monitor endogenous T cell responses at early times after stimulation.

Many of these problems are circumvented by the use of T cell receptor (TCR) transgenic (Tg) mice. While in some cases only the TCR-β or TCR-α chain is engineered (e.g., (Turner et al., 1996), most commonly, both the alpha and beta chains of the TCR are fixed in TCR Tg mice, increasing the fraction of cells in the naïve pool that recognize the epitope in question (Barnden et al., 1998). The majority of the T cells in these mice express the Tg T cell receptor, making them an excellent

Abbreviations: CNS, central nervous system; p.i, post infection; tg, transgenic.

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source for large numbers of clonotype-specific cells. Transgenic TCR T cells generally recognize MHCp complexes and function similarly to polyclonal T cells recognizing the same epitope. Development of transgenic mice is most often preceded by the generation of T cell clones specific for the epitope under study. Epitope-specific T cell clones are isolated after repeated stimulation *in vitro* and limiting dilution; it is well established that the conditions under which T cells are expanded contribute to the selection of T cells with varying affinity for the MHCp. Thus, CD8 T cells selected in the presence of low levels of antigen exhibit high affinity for MHCp whereas exposure to high levels of antigen results in the outgrowth of cells with low affinity (Alexander-Miller et al., 1996). T cells with high versus low affinity for MHCp will behave differently in an infected or cancerous animal and neither type of cell may represent a cell with average functionality in the general population of T cells responding to an epitope.

Mice infected with the neurotropic JHM strain of mouse hepatitis virus (JHMV) develop acute and chronic encephalitis and demyelination (Stohlman et al., 1998). We and others showed previously that demyelination is T cell mediated, involving virus-specific CD4 and CD8 T cells (Wang et al., 1990; Wu et al., 2000). Further, clinical disease in mice with acute encephalitis is largely CD4 T cell-driven (Anghelina et al., 2006). Thus, mutation of the immunodominant CD4 T cell epitope (M133, spanning residues 133–147 of the transmembrane M protein, TVYVRPIIEDYHTLLT) recognized in C57Bl/6 mice resulted in a loss of virulence in this strain, but not BALB/c mice (H-2^d) or in RAG1$^{-/-}$ mice, which lack T or B cells. Virulence could be restored if another immunodominant CD4 T cell epitope (from Listeria monocytogenes) was introduced into the M133-mutated virus, indicating that the CD4 T cell response was pathogenic. On the other hand, our results also demonstrated that the memory CD4 T cell response was protective. In another report, we showed that a population of regulatory CD4 T cells in the brains of mice infected with a neuroattenuated variant of JHMV (rJ2.2) recognized epitope M133 (Zhao et al., 2011). Understanding the relationship between effector, memory and regulatory CD4 T cells responding to a single epitope would be facilitated by the availability of an M133-specific TCR Tg mouse.

Here, we describe an approach to developing TCR Tg mice that does not require *in vitro* cell culturing or limiting dilution. The beta chain of an M133-specific CD4 T cell receptor was initially selected on the basis of its expression in 3/3 JHMV-infected mice. The alpha chain was then selected using infected single chain TCR retrogenic mice in which the beta chain identified in the initial studies was fixed. Subsequent analyses showed that these M133-specific TCR Tg cells, upon transfer to mice prior to infection with rJ2.2, proliferated and trafficked to the infected brain.

2. Materials and methods

2.1. Mice

Specific pathogen-free 6 week old C57BL/6 (B6) and Thy1.1 congenic mice were purchased from the National Cancer Institute. Mice were maintained in the animal care facility at the University of Iowa. After viral inoculation, mice were examined and weighed daily. Clinical evaluation was based on the following scoring system: 0, asymptomatic; 1, limp tail or slightly hunched; 2, wobbly gait or hunched and ruffled fur; 3, hindlimb paresis or moderate wasting; 4, hindlimb paralysis or severe wasting; 5, moribund. All animal studies were approved by the University of Iowa Animal Care and Use Committee (Iowa City, IA).

2.2. Virus

Neurovirulent JHMV and its neuroattenuated variant, rJ2.2 (a recombinant version of the J2.2-V-1 virus) were grown and titered as described (Pewe et al., 2005). B6 mice were infected intraperitoneally with 1.5×10^5 PFU JHMV or intracranially with 600–700 PFU of rJ2.2.

2.3. IFN-γ capture assay

Cells were harvested from the spleens of JHMV-infected B6 mice 7 days after infection. To isolate epitope M133-specific CD4 T cells, 1×10^7 unfractionated splenocytes/ml were stimulated 1:2 with CHB3 cells for 4 h with 1 μM of M133 peptide (TVYVRPIIEDYHTLT) (Xue et al., 1995). After stimulation, M133-specific CD4 T cells were identified using a mouse IFN-γ secretion assay kit (Miltenyi Biotec, Auburn, CA) following the manufacturer's protocol for frequencies of IFN-γ-secreting T cells <2%. IFN-γ^+ cells were detected with PE-conjugated anti-IFN-γ antibody. Cells were additionally stained with FITC-conjugated anti-CD4 Abs. IFN-γ-expressing CD4 T cells were sorted with a FACS DiVa (BD Biosciences, San Jose, CA). A total of 40,000–50,000 epitope M133-specific cells were obtained from each sort. Under our sorting conditions, very few IFN-γ-expressing CD4 T cells were detected when cells were stimulated in the absence of added peptide (Fig. 1).

2.4. Isolation of RNA from lymphocytes and TCR-β sequence analysis

RNA was isolated from lymphocytes using an RNeasy Mini Kit (Qiagen, Valencia, CA). cDNA was synthesized from each entire sorted lymphocyte RNA preparation in a 60 μl reaction, as described (Pewe et al., 1996). A total of 5 μl of cDNA was used for PCR. Vβ8 was the commonly used Vβ element in the M133-specific CD4 T cell response (Haring et al., 2001). For synthesis of Vβ8-specific PCR products, a Vβ8-specific forward primer (5′-CATGGGCTGAGGCTGATCCATT-3′) was used with a common Cβ reverse primer (5′-GCAATCTCTGCT TTTGATGGCTC-3′) and Taq polymerase (AmpliTaq Gold DNA Polymerase; Applied Biosystems, Foster City, CA). Amplification was conducted for 25 cycles, as described previously (Pewe et al., 1996). The PCR error rate was 0.12% (Pewe et al., 2004). PCR products were cloned into plasmid vector pCR2.1-TOPO, as described by the manufacturer (Invitrogen, Carlsbad, CA). Colonies containing inserts were identified and sequenced with an automated ABI Prism 3700 Sequencer (Applied Biosystems).

2.5. Antibodies

The following antibodies were used in this study: CD4-PerCP-Cy5.5 or -FITC (RM 4–5), TCRVβ8.3-FITC or -PE (1B3.3) (BD Pharmingen, San Diego, CA); CD8-APC (53-6.7), CD16/CD32-biotin (93), IFN-γ–allophycocyanin (APC; XMG1.2),