Developmental and Comparative Immunology 46 (2014) 171–179

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Diversity of immunoglobulin lambda light chain gene usage over developmental stages in the horse

Rebecca L. Tallmadge *, Chia T. Tseng, M. Julia B. Felippe

Equine Immunology Laboratory, Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, United States

ARTICLE INFO

Article history:
Received 26 February 2014
Revised 31 March 2014
Accepted 1 April 2014
Available online 12 April 2014

Keywords:
Equine
Immunoglobulin
Lambda
Light chain
Diversity
Development

ABSTRACT

To further studies of neonatal immune responses to pathogens and vaccination, we investigated the dynamics of B lymphocyte development and immunoglobulin (Ig) gene diversity. Previously we demonstrated that equine fetal Ig VDJ sequences exhibit combinatorial and junctional diversity levels comparable to those of adult Ig VDJ sequences. Herein, RACE clones from fetal, neonatal, foal, and adult lymphoid tissue were assessed for Ig lambda light chain combinatorial, junctional, and sequence diversity. Remarkably, more lambda variable genes (IGLV) were used during fetal life than later stages and IGLV gene usage differed significantly with time, in contrast to the Ig heavy chain. Junctional diversity measured by CDR3L length was constant over time. Comparison of Ig lambda transcripts to germline revealed significant increases in nucleotide diversity over time, even during fetal life. These results suggest that the Ig lambda light chain provides an additional dimension of diversity to the equine Ig repertoire.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Immunoglobulins (Igs) are membrane-bound or secreted proteins produced by B lymphocytes that can bind pathogens to mediate clearance, killing, and neutralization. As a major component of the adaptive immune response, an individual Ig may achieve high affinity for a single antigen, but the spectrum of Ig antigen specificities that can be attained in a host is immense. Generation of the vast Ig repertoire is accomplished through a multifactorial process of combinatorial diversity (selection of single variable, diversity, and joining gene segments from the multiple germline loci and recombination to create one Ig transcript), junctional diversity from imprecise gene recombination and non-templated or palindromic nucleotide additions, and somatic hypermutation. Ig molecules are heterodimers of heavy and light chains in vertebrates other than sharks and, in some instances, camels (De Genst et al., 2006; Dooley and Flajnik, 2006). The sequence of both heavy and light chain Igs encode variable 'complementarity-determining regions' (CDRs), which together form the antigen-binding site, and are flanked by conserved framework regions (Near et al., 1990; Kabat and Wu, 1991; Kirkham et al., 1992). Clinically, the loss of Ig or B lymphocyte production results in humoral immunodeficiency (Park et al., 2008; Flaminio et al., 2009).

During B lymphocyte differentiation, heavy chain gene rearrangement precedes light chain gene rearrangement (Alt et al., 1981). Two Ig light chains, kappa and lambda, are used in mammalian species and until recently it was accepted that the lambda light chain gene locus only rearranged if the kappa rearrangement was unsuccessful, although the lambda gene locus may be active before kappa in pigs (Hieter et al., 1981; Sun et al., 2012). It is intriguing that the relative abundance of kappa (κ) and lambda (λ) light chain varies among species: 95% κ/5% λ in mice, 66% κ/34% λ in humans, 52% κ/48% λ in pigs, 9% κ/91% λ in cows, 8% κ/92% λ in horses, and lambda light chain is the only one expressed in chickens and ducks (Hood et al., 1967; Gibson 1974; Kessler et al., 1981; Reynaud et al., 1985; Magor et al., 1994; Arun et al., 1996). Subsequently, it has been suggested that the predominant light chain used may be attributed to the number of germline V genes present in that species (Almagro et al., 1998). Additional mechanisms have been shown to affect light chain usage in mice, such as higher recombination frequency at the Ig kappa locus, antigen selection, and increased self-reactivity in Ig kappa-deficient mice implies that Ig lambda may be subject to negative selection (Haughton et al., 1978; Ramsden and Wu, 1991; Knott et al., 1998). This striking lack of conservation in Ig light chain gene rearrangement order and range of usage is an interesting phenomenon of comparative immunology.

Abbreviations: CDR, complementarity-determining region; Ig, immunoglobulin; IGLV, immunoglobulin lambda variable gene; IGLJ, immunoglobulin lambda joining gene; IGLC, immunoglobulin lambda constant chain gene; ORF, open reading frame; MLN, mesenteric lymph node; RACE, rapid amplification of cDNA ends; VDJ, immunoglobulin heavy chain variable diversity and joining regions.

* Corresponding author. Tel.: +1 607 253 3493.
E-mail address: rlt8@cornell.edu (R.L. Tallmadge).

http://dx.doi.org/10.1016/j.dci.2014.04.001
0145-305X/© 2014 Elsevier Ltd. All rights reserved.

Recent studies have greatly advanced our knowledge of the equine Ig lambda genes in terms of genome organization and allelic diversity beyond the initial cDNA characterization (Home et al., 1992). Twenty-seven functional and 5 ORF IGLV gene segments have been annotated on EquCab2 chromosome 8 (NW_001867428), flanking a cluster of 7 IGLJ–IGLC pairs (Sun et al., 2010). The equine IGLV genes are divided into 11 subgroups based on sequence identity, and the expression of subgroup 8 IGLV genes is dominant in the horse spleen, complemented by lesser usage of subgroups 4 and 6 (Sun et al., 2010). Five of the 7 IGLJ–IGLC genes appear to be expressed although some individuals may lack particular genes; differences in Ig lambda gene content between individual genomes have also been reported in pigs (Sun et al., 2010; Hara et al., 2012; Wertz et al., 2013). Alleles of equine IGLV, IGLJ, and IGLC genes have been identified and they differ by up to 4% from the EquCab2.0 reference genome, which may encode distinct serological properties (Sun et al., 2010; Hara et al., 2012).

Early Ig repertoire studies reported restricted diversity during fetal life, for both Ig heavy and light chains. The murine and human fetal repertoire has been characterized by biased Ig gene segment usage and restricted CDR3 length (Schroeder and Wang, 1990; Shiokawa et al., 1999). Unlike humans and horses, fetal mice do not express TdT/DNTT and thus lack non-templated (N) additions (Li et al., 1993; Tallmadge et al., 2009). The human fetal Ig lambda repertoire is generated from distinct and non-random patterns of Ig lambda gene rearrangements in contrast to adults, and it expresses identical $V_{\lambda}J_{\lambda}$ junctions with unique Ig heavy chains (Lee et al., 2000). Analysis of fetal piglet Ig lambda repertoire revealed that 70% of the repertoire is derived from 3 IGLV genes, similar to the IGHV repertoire in adult pigs, although CDR3L diversity is very limited in contrast to the heavy chain (Sun et al., 1998; Butler et al., 2000; Wertz et al., 2013).

Because the equine placenta does not facilitate Ig or cell transfer to the fetus *in utero*, it is possible to investigate the intrinsic humoral system development in the fetus and pre-suckle neonate. Investigation of B lymphocyte markers and Ig heavy chain isotype expression paired with analysis of IGHV, IGHD, and IGHJ gene usage and diversity revealed that equine fetal B lymphocytes express mature and co-stimulatory B lymphocyte markers and most Ig heavy chain isotype transcripts (Tallmadge et al., 2009). In addition, a similar complement of Ig variable genes is used during equine fetal and post-natal life stages and Ig sequence diversity increases during fetal life (Tallmadge et al., 2013). Given the Ig VDJ diversity detected in equine fetal life and findings of other studies of the fetal Ig repertoire cited above, we hypothesized that little diversity would be present in equine fetal Ig lambda chain transcripts. Herein we describe the repertoire of expressed Ig lambda chain sequences spanning equine development from fetal life to adulthood.

2. Materials and methods

2.1. Tissue samples

These experiments were approved by the Cornell University Center for Animal Resources and Education and Institutional Animal Care and Use Committee for the use of vertebrates in research. Tissue samples from a healthy induced-abortion equine Thoroughbred × Warmblood fetus without uterine disease (102 days of gestation), a neonate Warmblood foal (pre-suckle, <1-hour-old), a 2 month-old Warmblood foal, and a Thoroughbred adult horse were archived and available for this study from research investigations performed by us and other investigators over the years at Cornell University College of Veterinary Medicine. None of the donors were related or had history of infections, and were maintained in Cornell University research herds with open housing conditions and vaccinated as recommended by the American Association of Equine Practitioners. Tissue samples were collected within 1 h of euthanasia, snap frozen in liquid nitrogen, and stored at −80 °C until use.

2.2. RACE library construction, Ig lambda chain amplification, and cloning

RNA was isolated from snap-frozen tissues following homogenization by QIAshredder (Qiagen, Valencia, CA) as directed by the RNeasy kit (Qiagen) including on-column digestion of contaminating genomic DNA. RNA was quantified with a Nanodrop (Thermo Fisher Scientific, Inc., Waltham, MA) and one microgram was used to generate a 5′-rapid amplification of cDNA ends (RACE) library with the SMARTer™ RACE cDNA Amplification Kit (Clontech Laboratories, Inc., Mountain View, CA). The same RACE libraries were used to assess Ig heavy chain diversity (Tallmadge et al., 2013). The gene-specific primer was designed to amplify all immunoglobulin lambda light chain constant genes. Amplification reactions contained 1× iProof high fidelity buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, the provided universal forward primer with 0.5 μM gene-specific reverse primer 5′ CTCTGAGGGGGACAGTTTCTTCTCCAC 3′ (Integrated DNA Technologies, Coralville, IA), and 0.02 U iProof DNA polymerase (error rate of 4.4×10^{-7}, Bio-Rad Laboratories, Hercules, CA). Thermal cycling parameters were 5 cycles of 98 °C for 30 s and 72 °C for 1 min; 5 cycles of 98 °C for 30 s, 70 °C for 30 s and 72 °C for 1 min; and 27 cycles of 98 °C for 30 s, 68 °C for 30 s and 72 °C for 1 min. Amplification products were run on 1% agarose gels and stained with GelGreen nucleic acid stain (Phenix Research Products, Candler, NC) for visualization. PCR products of approximately 750 bp were excised from the gel, purified with GeneJET gel extraction kit (Thermo Fisher Scientific, Inc.), and cloned with the CloneJET PCR cloning kit (Thermo Fisher Scientific, Inc.). Individual colonies were expanded in LB broth with ampicillin and plasmid DNA was purified with the GeneJET plasmid miniprep kit (Thermo Fisher Scientific, Inc.).

2.3. Amplification of Ig lambda genes from genomic DNA

Genomic DNA was isolated from liver samples of each horse as directed by the DNeasy Blood & Tissue Kit (Qiagen). Gene-specific primers were used in PCR reactions with 50 ng genomic DNA, 1× iProof GC buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM primers, and 0.02 U iProof DNA polymerase (Bio-Rad). Thermal cycling parameters included initial denaturation at 98 °C for 3 min; 35 cycles of 98 °C for 10 s, 60 °C for 10 s, and 72 °C for 1 min; followed by final extension at 72 °C for 10 min. Primers to amplify IGLJ1–IGLC1, IGLJ4–IGLC4, and IGLJ7–IGLC7 were published by Sun et al. (2010). The IGLC4 locus was also amplified from genomic DNA with primers 5′ TGAGAAGGATTTGGGCGGAG 3′ and 5′ GACTTGACGGTGAGCTGGAA 3′, generating a 320 base pair product. Primer sequences were designed for VL15 (5′ CAAAGGAAGCAGCTGACACG 3′ and 5′ GGGGCTGTGATTTGCATGTG 3′, amplified a 705 base pair product), VL17 (5′ CAAAGGAAGCAGCTGACGTG 3′ and 5′ CTCAGCTTTCCGTGAGGGTT 3′, amplified a 855 base pair product), and VL26 (5′ GGGCTTTGGAGACCTGAGAC 3′ and 5′ GAGGGCACAGCAGGTTTTTG 3′, amplified a 869 base pair product).

PCR products were visualized, cloned, and sequenced as described above.

2.4. Sequence analysis

Clones were sequenced at the Cornell University Institute of Biotechnology, Ithaca, NY. Sequence content analysis was performed with Geneious software version 6.1.6 (Biomatters, Ltd.,

Download English Version:

https://daneshyari.com/en/article/2429131

Download Persian Version:

https://daneshyari.com/article/2429131

Daneshyari.com