Genomic sequencing of the bovine T cell receptor beta locus

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Abstract

The T cell, which plays an integral role in the coordination of the immune system, has a heterodimer receptor (TCR) that can exist in one of the two forms: alpha/beta or gamma/delta. Cells displaying the gamma/delta TCR comprise less than 5% of T cell populations in humans and mice. In the bovine system, however, gamma/delta populations can reach as high as 60%. Differences in T cell populations make the bovine system an excellent candidate for genomic TCR sequencing and multi-species comparisons.

In an effort to characterize the bovine TCR loci, a genomic library was screened for the beta TCR gene. A shotgun sequencing library was constructed and preliminary analysis demonstrates that the organization of the bovine TCR beta constant regions is different from both humans and mice. The bovine beta locus appears to have a third constant region. Overall, the genomic characterization of the bovine TCR genes will provide insight into the evolution of T cell receptor. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Multi-species comparisons are useful in many areas of life sciences. From morphological studies in the field of taxonomy to analyses of multigene families, comparison has proved to be an indispensable tool for understanding the relationships between species.

In the field of immunogenetics the immunoglobulin superfamily is a group of structurally related proteins that have been studied extensively in both humans and mice. One member of this family, the T cell receptor, has four different genes that code for the receptor subunits (alpha, beta, gamma, and delta). All the TCR genes have similar organization containing variable (V), joining (J), diversity (D, in beta and delta) and constant (C) regions (Schatz et al., 1992). What makes these loci genetically interesting are the differences in the number of gene segments between loci and between species. This poses the question: Does a difference in gene segment number lead to a difference/alteration in function?

Many researchers have compared heterodimer receptor (TCR) gene segment structure in different species and approximated gene segment numbers through cDNA analysis. Today, with the increased efficiency of DNA sequencing methods, it is important to obtain genomic sequence from species other than humans and mice. Using genomic sequence data to provide information for multi-species comparisons may help support known data, resolve conflicting hypotheses regarding evolutionary relationships in gene families, and answer unresolved questions about immune system function. The present study focuses on genomic sequencing of the bovine T cell receptor

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loci. In this paper we will focus on an interesting new aspect of the bovine T cell receptor beta locus.

2. Cattle and T cell receptors

Cattle are ruminants that digest via fermentation. Inside the rumen there exists a complex ecosystem of many different types of bacteria (including fermenters), protists, and fungi. Comparatively, the ruminant digestive system is different from that of the systems of humans and mice. It has also been demonstrated that the bovine system contains elevated numbers of T cells expressing the gamma/delta receptor (Hein and Dudler, 1997).

These facts leads to a very interesting yet unanswered question: Do gamma/delta T cells have a special function in the ruminant digestive system? As a first step in answering this question a bovine genomic library (kindly provided by Scott K. Davis, Texas A&M University) was screened for bacterial artificial chromosomes (BACs) containing the alpha, beta, and gamma T cell receptor loci. Shotgun sequencing libraries were created and are presently being sequenced (Povinelli and Gibbs, 1993; Rowen and Koop, 1994).

3. Genomic sequencing of the bovine T cell receptor beta locus

It has previously been thought, through cDNA studies, that the bovine TCR beta locus is organized similar to the human locus (Tanaka et al., 1990). The human TCR beta locus contains a string of V (variable) gene segments followed by two tandemly repeated DJC (diversity, joining, constant) regions illustrated in Fig. 1. The genomic sequence of the bovine TCR beta has been obtained and preliminary analysis indicates the existence of a third constant region located between constant region 1 and constant region 2 (Fig. 2).
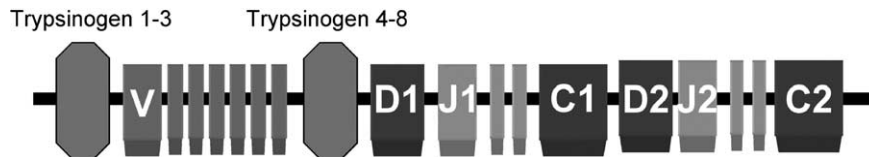

Fig. 1. General organization of the human T cell receptor beta locus. Exact numbers of variable (V), diversity (D), and joining (J) gene segments not shown.
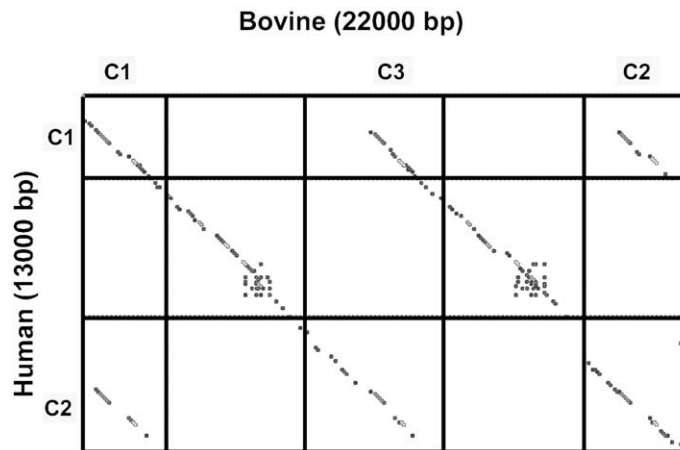

Fig. 2. Human vs. bovine dotplot analysis. Genomic DNA sequence from the human TCR beta constant regions (Genbank accession: U66061) plotted against new bovine genomic sequence constant regions. Dots indicate 75% similarity over a 30 bp window. This dotplot illustrates the existence of three bovine constant regions, with the new constant (constant 3) situated between constant 1 and constant 2. Note: for diagram simplicity joining (J) and diversity (D) gene segments have not been annotated.

3.1. Verification of accurate sequence assembly

Genomic sequence of the bovine TCR beta locus has been obtained to 7.4-fold redundancy (GenBank accession: AF453325). Two assembly programs, Seqman 99 (http://www.dnastar.com) and Phrap (Gordon et al., 1998), constructed identical BAC assemblies suggesting that the sequence obtained is correct.

3.2. Expression of bovine TCR beta constant 3

A transcript of bovine beta constant 3 has been identified in the NCBI EST database (http://www.ncbi.nlm.nih.gov). Running NCBI's BLAST program with the new bovine constant region produced a 100% match over 517 bp with a bovine EST produced from pooled tissue (GenBank accession: BE809613).

3.3. Further studies

Southern blot analysis is presently being performed to provide further evidence of the existence of constant region 3.

4. Conclusion

Genomic sequencing and multi-species comparison are powerful tools for investigating evolutionary relationships between groups of genes. The bovine T cell receptor beta locus has been sequenced and preliminary analysis indicates the existence of a third constant region (constant 3) located between constant region 1 and constant region 2.

Acknowledgements

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