Sequence and diversity of bovine T-cell receptor β-chain genes

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Abstract. The nucleotide sequences of 38 T-cell receptor (Tcr) β-chain cDNA clones which were isolated from a cDNA library (2 × 10⁶ plaques) constructed from bovine peripheral blood lymphocytes were determined. Of 38 cDNA clones, 22 were rearranged and contained the functional variable (V) gene segments. These clones were tentatively divided into nine Tcrb-V gene families which correspond to the human Tcrb-V family. Among them, a Tcrb-V12 gene segment was isolated from 9 out of 22 clones, suggesting that this Tcrb-V family was expressed in the bovine peripheral blood lymphocytes. Two different constant (C) gene segments were found, and both C regions were composed of 178 amino residues. The amino acid sequences of bovine Tcrb-C regions are approximately 80 %-82 %, 78 %, and 78 % similar to those from human, mouse, and rabbit, respectively. To estimate Tcrb-V-associated restriction fragment length polymorphisms (RFLPs), Southern blot analysis was performed using liver DNAs from four bovine breeds, Holstein, Angus, Hereford, and Japanese Black. However, no significant difference was observed among genomic DNAs of Tcrb-V loci from these four breeds.

Introduction

The T-cell receptor (Tcr) is a cell surface heterodimeric glycoprotein composed of α and β chains (Meuer et al. 1983; Chien et al. 1984a; Hedrick et al. 1984; Yanagi et al. 1984). The Tcrs have been known to recognize foreign antigens in association with class I or class II major histocompatibility complex molecules (Shevach and Rosenthal 1973; Zinkernagel and Doherty 1975). The functional Tera and Tcrb genes are assembled by the somatic rearrangement of variable (V), diversity (D), and joining (J) gene segments in a manner similar to immunoglobulin chains except that Tera genes apparently lack D segments (Chien et al. 1984b; Saito et al. 1984b; Harday et al. 1985; Yoshikai et al. 1985). The other Tcr genes consisting of Terg and Tcrd have been isolated and characterized (Saito et al. 1984a; Chien et al. 1987). The Terg and Tcrd genes are also formed by somatic recombinations of V, D, J, and constant (C) gene segments. These rearrangements occur in the thymus during the differential development of T lymphocytes. Much information about human and mouse Tcr genes is now available for the molecular study of T cells. However, little is known about the primary structure of Tcr genes from domestic animals.

Recently, we reported the primary structure of the bovine Tcr α chain (Ishiguro et al. 1990). In this study, we have sequenced and analyzed 38 bovine cDNA clones of β chains derived from bovine peripheral blood T lymphocytes. This report compares the sequence of the Tcrb-V region with those reported for mouse and human. In addition, we described restriction fragment length polymorphisms (RFLPs) of Tcrb genes among bovine breeds.

Materials and methods

Construction and analysis of a cDNA library. Bovine peripheral blood lymphocytes (4 × 10⁸ cells) were obtained from a healthy Holstein cow using the Ficoll-Paque method and stimulated for 24 h with 10 μg/ml concanavalin A (Con A). Total RNA was isolated by the guanidine thiocyanate method and subsequently Poly(A)⁺ RNA was selected with oligo (dT) cellulose columns (Maniatis et al. 1982). Oligo (dT)-primed cDNA was synthesized by the method of Watson and Jackson (1985). Double-stranded cDNA bearing Eco RI linkers was ligated into Eco RI-cut Xgt10 (Stratagene, La Jolla, California). The ligated DNA was packaged in phage heads by an in vitro packaging system (Gigapack-Gold, Stratagene) to make a cDNA library. The cDNA library was screened for the bovine Tcr β chain by mouse Tcrb-C probes (450 base