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Stable expression of the extracellular domains of rabbit recombinant CD5: development and characterization of polyclonal and monoclonal antibodies

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Abstract

Previous studies in our laboratory suggested that there was positive selection of B cells during early development in the appendix of normal and V_H mutant (*ali/ali*) rabbits. Preferential expansion and survival of B lymphocytes was affected by the Ig V_H frameworks 1 and 3 sequences expressed on the cell surface. We demonstrated a specific interaction between rabbit CD5 and the V region of rabbit heavy chains and suggested that CD5 is a potential selecting ligand for B-cell surface immunoglobulin framework region sequences. To further investigate the role of CD5 in rabbit B-cell selection and survival we prepared recombinant constructs and obtained stable expression of the three scavenger receptor cysteine-rich (SRCR) extracellular domains of rabbit CD5. Here we describe the production and purification of this expressed recombinant CD5 protein, polyclonal antibody obtained by immunization of a goat and initial production and characterization of specific mAbs against peptides selected from each sequenced SRCR domain.
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1. Introduction

CD5 is a member of subgroup B of the scavenger receptor cysteine-rich (SRCR) superfamily of cell surface molecules and has an extracellular region consisting of three SRCR domains each of which is encoded by a single exon. Whereas SRCR domains of subgroup A are encoded by two exons and each contain six cysteines, most of those in group B contain

eight cysteines. CD5 is found on a small subset of B cells and most T cells in man and mouse. In the rabbit, CD5 is present on the majority of B cells as well as on T cells (Raman and Knight, 1992a, 1992b). Previous studies in our laboratory suggested that there was positive selection of B cells during early development in the appendix of normal and V_H mutant (Kelus and Weiss, 1986) (*ali/ali*) rabbits (Pospisil and Mage, 1995; Pospisil et al., 1995). It appeared that preferential expansion and survival of B lymphocytes was affected by the Ig V_H frameworks 1 and 3 sequences expressed on the cell surface. We subsequently showed that there is a specific interaction between rabbit CD5 and the V_H domain of rabbit heavy chains and suggested that CD5 is a potential selecting ligand for B-cell surface immunoglobulin framework region sequences (Pospisil et al., 1996; Pospisil and Mage, 1998). Based on these observations, the interaction of human CD5 with human immunoglobulins was also investigated and specific interactions were again observed (Pospisil et al., 2000; Mage and Pospisil, 2000). Our studies in both species suggested that CD5 acted like a superantigen in its interaction with V_H and this could be influenced by the presence of particular sequences within the V_H domain. The availability of recombinant molecules with various combinations of the extracellular domains of human CD5, also allowed us to determine that whereas most mAbs raised against human CD5 reacted with domain 1 (Calvo et al., 1999), the interaction of human CD5 with human Igs depended upon the presence of domain 2. To further investigate the role of CD5 in rabbit B-cell selection and survival, we prepared recombinant constructs and expressed the extracellular domains of rabbit CD5. The purified recombinant protein was used to raise polyclonal antibodies in a goat. Peptides selected from each sequenced domain were used to generate specific

mAbs. The need for such reagents became particularly acute when the mAb to human CD5, T1, which cross-reacts with rabbit CD5 and was used extensively in our earlier studies, was no longer commercially available.

2. Materials and methods

2.1. Rabbit CD5 clones

Clones CD52A 5-3 pSSFVgpt and CD52A 3-5 pSSFVgpt containing cloned rabbit CD5 cDNA (GenBank Accession No. AY682087) in two orientations were a gift from Dr. Chander Raman (University of Alabama at Birmingham).

2.2. Rabbits

Rabbits were from the NIAID allotype-defined colony, NIH, Bethesda, MD. In order to determine whether a putative unpaired cysteine encoded by the sequence of extracellular SRCR domain 2 of the rabbit CD5 in the cloned cDNA from Dr. Raman was also present in the CD5 of rabbits in our colony, we used two rabbits from distinct breeding groups: (rabbit number 2XX288-1 with allotypes V_Ha1 and Cκb9, and rabbit number 1WW14-2 with allotypes V_Ha2 and Cκb5) to generate cloned cDNA for sequencing. The characterizations of the anti-CD5 mAbs and polyclonal anti-CD5 described in this report were done using cells and tissues from homozygous rabbits of the V_Ha2 (F–I haplotype).

2.3. Reverse transcriptase PCR

Total RNA was isolated from BGG-immunized spleen tissues of the two different rabbits from distinct breeding groups described above. The primers used

Table 1
Primers used for cloning and sequencing rCD5

Primer name (5′ to 3′)	Primer sequence[a]
RCD5-5′	GGA GAT CTT GGT CCA GCT GGG ATG AGC CAG G
RCD5-3′	GGA CCG GTG TTT GGG TCC TGG CAT GTG ACA AAC ACC C
RCD5A	TGG GCC TGG TCT GCT TAG
RCD5B	GTC GAG GCT GCG GGG GAG CT
RCD5C	ATG CCC CAT CGG ATT GGC AGA
RCD5D	TGT TCC TGG CAC AGC TCC T

[a] The restriction sites in RCD5-5′ (*Bgl*II) and RCD5-3′ (*Age*I) are underlined.