

DEVELOPMENT OF NOVEL RABBIT MONOCLONAL ANTIBODY TO CD3 (SP7) AND IMMUNOHISTOCHEMICAL EVALUATION WITH SOME COMMERCIALY AVAILABLE ANTIBODIES

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ABSTRACT

The CD3 molecule consists of five different polypeptide chains: gamma, delta, epsilon, zeta and eta. The CD3 complex is closely associated at the lymphocyte cell surface with the T cell antigen receptor (TCR). It is believed that the CD3 complex is involved in signal transduction to the T cell interior following antigen recognition. The CD3 antigen is first detectable in early thymocytes and its appearance probably represents one of the earliest signs of commitment to the T cell lineage. Antibodies that label CD3 epsilon have been useful tools for the identification of T cells and related neoplasms. Having a better CD3 antibody with higher affinity and sensitivity has always been a challenge. Rabbit has been known to be a good source for generating high affinity antibodies. Novel rabbit monoclonal antibody to CD3 (clone SP7) was developed by using hybridoma technology and immunohistochemical evaluation of this clone was performed in formalin-fixed, paraffin-embedded (FFPE) reactive spleen, in FFPE bone marrow (BM) biopsies of acute T-lymphoblastic leukemia (T-ALL) and T-lymphoblastic lymphoma (TLB) cases, in epoxy resin embedded BM sample from a T-ALL case as well as in 2 cases of FFPE lymph node biopsy from T-lymphoblastic lymphoma in comparison with CD3 mouse monoclonal antibody (clone F7.2.38), CD3 epitope specific rabbit antibody as well as CD5 epitope specific rabbit antibody. Clone SP7 and CD3 epitope specific rabbit antibody gave exactly same reaction pattern in every sample tested with stronger intensity when clone SP7 was used. CD3 mouse monoclonal (clone F7.2.38) however demonstrated heterogeneous staining pattern usually with weaker intensity at the dilution recommended. The results indicated that the clone SP7 has higher affinity and is more sensitive than the other CD3 antibodies especially the mouse monoclonal (clone F7.2.38).

INTRODUCTION

The CD3 epsilon is an important marker for the identification of T cells and related neoplasms. Having a better CD3 antibody with higher affinity and sensitivity has always been helpful. Rabbit has been known to be a good source for generating high affinity antibodies. Novel rabbit monoclonal antibody to CD3 (clone SP7) was developed by using hybridoma technology and immunohistochemical evaluation of this clone was performed in several formalin-fixed, paraffin-embedded (FFPE) tissues that express CD3 in comparison with some commercially available antibodies.

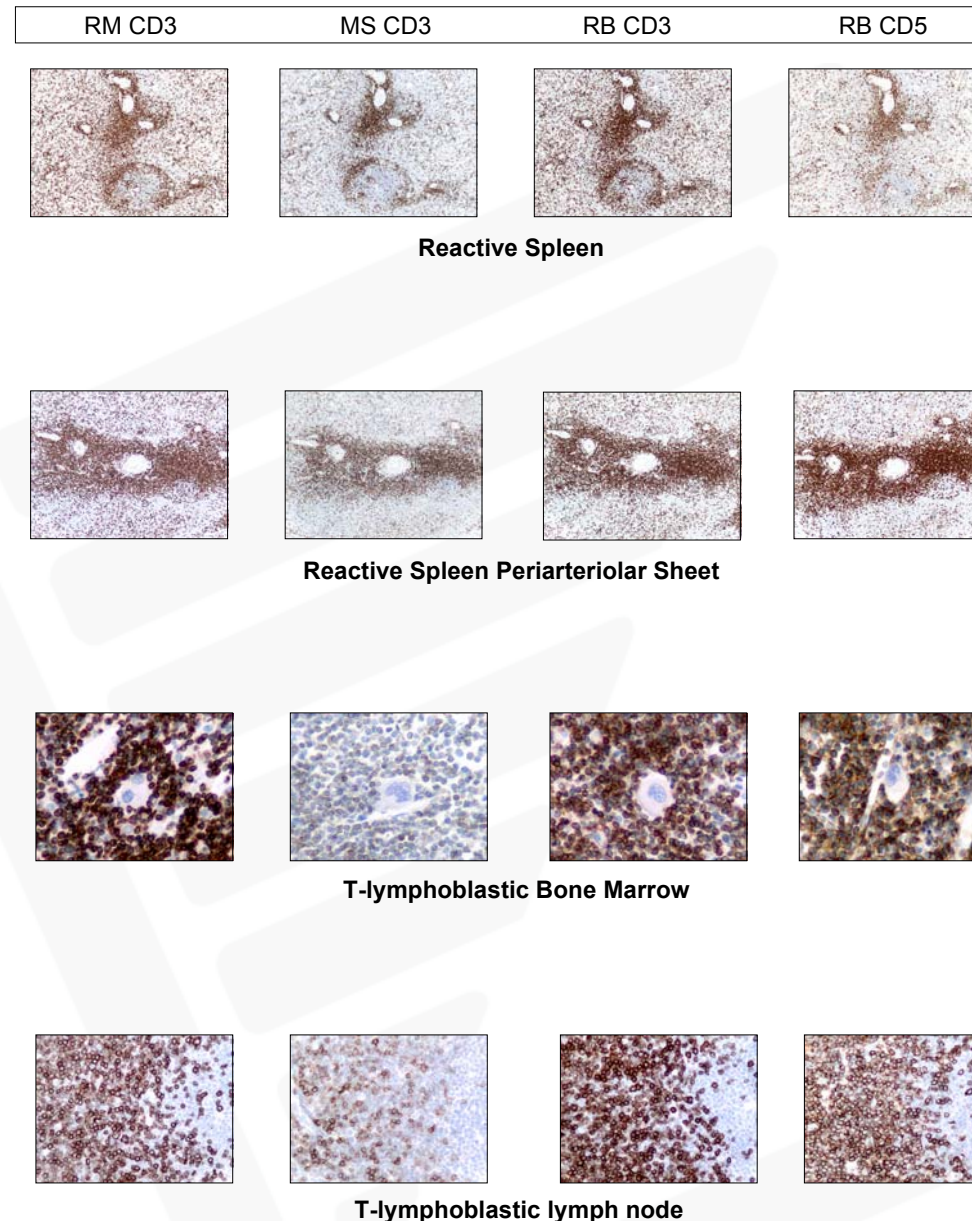
METHODS

Development of the Rabbit Monoclonal Antibody to CD3 (SP7):

A peptide representing the C-terminal of human CD3 epsilon chain protein was synthesized and covalently conjugated with keyhole limpet haemocyanin (KLH). New Zealand White rabbits were immunized. The sera were tested by immunoassay and immunohistochemical staining. The rabbit with the best titer in the immunoassay and IHC was selected for a final intravenous boost four days before removal of the spleen. Fusions were performed using lymphocytes from an immunized rabbit and the fusion partner. Supernatants were tested for the presence of antibody, specific for the immunogen, by ELISA. Immunohistochemistry and western blotting was also used as screening assays. The hybridomas were sub-cloned by limit dilution. The final antibody is produced from hybridoma culture supernatants using serum free media with no further purification. The antibody is diluted in 10mM phosphate buffered saline with 0.3% carrier protein and 0.05% sodium azide.

Immunohistochemical Evaluation with Some Commercially Available Antibodies:

Clone SP7 together with CD3 mouse monoclonal (clone F7.2.38 from Dako Cytomation), CD3 epitope specific rabbit antibody and CD5 epitope specific rabbit antibody (Lab Vision Corporation) were tested on reactive spleen, T-ALL bone marrow, T-lymphoblastic bone marrow, T-ALL bone marrow/resin and T-lymphoblastic lymph node (all are formalin-fixed, paraffin-embedded tissues).



Results

Results are shown in Table 1.

Table 1	RM CD3 (SP7)	MS CD3 (F7.2.38)	RB CD3	RB CD5
Reactive spleen				
Periarteriolar sheet	3+	2+ to 3+	3+	3+
Red pulp T-cells	3+	2+ to 3+	3+	3+
T-ALL bone marrow	3+ cytoplasmic perinuclear	1+ cytoplasmic perinuclear	3+ cytoplasmic perinuclear	3+ membranous
T-lymphoblastic bone marrow	1+ perinuclear	negative	1+ perinuclear	negative
T-ALL bone marrow/resin	3+ cytoplasmic perinuclear	1+ perinuclear	3+ cytoplasmic perinuclear	negative
T-lymphoblastic lymph node	3+ predominantly perinuclear	1+ perinuclear	3+ predominantly perinuclear	3+ membranous
T-lymphoblastic lymph node	1+ to 3+ perinuclear	negative	1+ to 3+ perinuclear	negative to 3+ membranous
			1+ weak; 2+ moderate; 3+ strong	

Conclusion

- Rabbit monoclonal CD3 (SP7) and rabbit CD3 antibody gave exactly the same reaction pattern in every sample tested. SP7 gave lower background.
- Mouse monoclonal CD3 (F7.2.38) demonstrated heterogeneous staining pattern usually with weaker intensity at the dilution recommended.
- Cytoplasmic CD3 epsilon chain detected by these antibodies occurs early in T-cell precursors, preceding CD5 expression.
- CD5 is more heterogeneous in T-ALL/lymphoblastic lymphomas and shows surface/membranous staining.
- Surface CD3 epsilon expression in lymphocyte sized cells is typical for mature/peripheral T-cells.