6.13 Clustering of monoclonal antibodies recognizing different members of the WC1 gene family

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Abstract

Mouse L cell lines expressing two different bovine WC1 glycoproteins were produced by transfection of the cells with the corresponding cDNAs. The cell lines were used to analyze the reactivities of 67 monoclonal antibodies (mAbs) which recognize bovine γ/δ T cells. The results indicated that preliminary clustering of mAbs can be achieved based on their recognition of epitopes expressed on all gene products, or of epitopes encoded by individual members of the gene family. The studies also showed that at least three members of the WC1 gene family are expressed, although it is not yet known how many can be expressed by individual bovine γ/δ T cells. Final clustering of the WC1 mAbs will not be possible until the exact number of expressed gene products is known, and the reactivities of the mAbs with these products have been analyzed.

Introduction

Monoclonal antibodies (mAbs) recognizing an antigen of M_r 215 kDa which is expressed on the surface of ruminant γ/δ T cells were clustered as WC1 in the First Workshop on leukocyte antigens in cattle, sheep and goats. However, the proportion of γ/δ T cells recognized by each mAb in the cluster varied quite considerably. Possible explanations for this anomalous behaviour include variation in the products of a single gene as a result of alternative splicing or post-translational modification, or the expression of antigens encoded by different members of a gene family.

Recently, the genes encoding two different WC1 glycoproteins have been cloned (Wijngaard et al., 1992). Both cDNAs (WC1.1, WC1.2,) were transfected into mouse L cells to generate stable transfectants which were used to analyze the reactivities of 67 putative anti- γ/δ T cell mAbs (mAbs from the

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First and Second Workshops plus a number of new mAbs). The results are summarized in table form.

Materials and methods

Two bovine cDNAs, encoding different WC1 glycoproteins, were transfected separately into mouse L cells by the calcium phosphate precipitation technique (Alberti et al., 1988). Positive transfectants were selected by fluorescence activated cell sorting using the WC1 specific mAb IL-A29 (Clevers et al., 1990) and cloned by limiting dilution. The panel of mAbs was analyzed by indirect immunofluorescence and flow cytometry for reactivity with the cloned transfectants (Table 1).

Dual fluorescence analysis was carried out using mAbs of different isotype and isotype specific secondary reagents conjugated to either fluorescein isothiocyanate or biotin (Southern Biotechnology, Birmingham, AL, USA). The

Table 1

Analysis of the reactivities of WC1 mAbs on mouse L cells transfected with bovine cDNAs encoding two distinct WC1 products

Second Workshop number	mAb	WC1.1 TX	WC1.2 TX
45	IL.A29	+	+
	CC15	+	+
	11F5	+	+
	SBUT19	+	+
	BAQ4A	+	+
	N8/26	+	+
	CACT28A	+	+
	CACT60A	+	+
	BAQ113A	+	+
	BAQ128A	+	+
	CACTB42A	+	+
	CACTB23A	+	+
	CACT73A	+	+
	CACTB31A	+	+
	N12/345	+	+
	BAQ109D	+/-	+/-
	BAQ159A	+	-
	N7/132	+	-
	CACTB7A	+	-
9,18	CC101	+	-
149	BAQ89A	+	+
150	CACTB7A	+	-

Second Workshop number	mAb	WC1.1 TX	WC1.2 TX
151	BAG25A	+	–
	MAB197	–	+
	N8/121	–	+
	CACTB33A	–	+
	BAQ76A	–	+
	B7A1	–	+
	CACTB15B	–	+
	CACTB32A	–	+
	BAS1A	–	+
	CACTB1A	–	+
30	CC117	–	+
31	CC115	–	+
	CC154	–	+
146	CACTB1A	–	+
147	CACTB15B	–	+/-
	CACT19C	–	+/-
	86D	–	–
	N7/30	–	–
136	CACTB6A	–	–
	BAQ102A	–	–
	CACT47A	–	–
	BAQ136A	–	–
	CACTB10A	–	–
	CACT46A	–	–
	BAQ90A	–	–
	CACT22A	–	–
	BAQ78A	–	–
	CACTB81A	–	–
	CACTB17A	–	–
	BAQ53A	–	–
	CACTB35A	–	–
	CACT41A	–	–
	CACT61A	–	–
	CACT21A	–	–
	CACT16A	–	–
	CACTB21A	–	–
	CACTB14B	–	–
139	CACT18A	–	–
140	CACT61A	–	–
141	CACT71A	–	–
142	CACT16A	–	–
143	CACT17A	–	–
144	CACTB12A	–	–
145	CACTB44A	–	–
148	BAQ72A	–	–

TX, transfectant.

Fig. 1. Two colour fluorescence of bovine PBL with selected workshop mAbs. (A) Two mAbs BAG25A and BAQ159 react only with the WC1.1 transfectant but the contour plot shows that BAG25A has a broader specificity than BAQ159. (B, C) mAbs CC154 and N8/121 are specific for the WC1.2 transfectant but react with different proportions of mAb BAG25A⁺ (WC1.1⁺) cells.

biotinylated reagents were visualized using streptavidin–phycoerythrin (Becton Dickinson, Sunnyvale CA, USA).

Results

Reactivities of mAbs in WC1 with L cells transfected with WC1 cDNAs

Of the 67 mAbs analyzed 15 were positive on both transfectants, seven mAbs reacted with only the WC1.1 transfectant and 13 mAbs reacted with only the WC1.2 transfectant (Table 1). Several other mAbs reacted only weakly with the transfectants (+/–) possibly as a result of antibody concentration; these results were considered equivocal and were ignored for the purpose of this study. mAbs N7/30 and BAQ9OA immunoprecipitated a 215 kDa protein and had similar tissue distribution to the other WC1 mAbs, indicating that they recognize the WC1 antigen, but were negative on both transfectants, suggesting that further WC1 gene products are expressed by bovine γ/δ T cells. The other mAbs that were negative on both transfectants may also recognize further WC1 gene products or unrelated antigens expressed on the surface of bovine γ/δ T cells. In fact a number of these mAbs have been shown to recognize the TCR1 complex.

Dual fluorescence analysis

The reactivities of bovine peripheral blood leukocytes (PBL) with mAbs selected from each group were analyzed by two colour fluorescence. The results revealed that some of the mAbs had broader specificities than others within the same group (Fig. 1). This can be explained by the existence of further WC1 genes such that some mAbs react with only one gene product while others have more public specificities. It is therefore difficult to cluster these mAbs without knowing how many WC1 genes are expressed and the reactivities of the mAbs with each gene product.

Discussion

mAbs recognizing the WC1 specificity can be divided into at least three subgroups based on their reactivities with two WC1 cDNA transfectants. However, the specificities of the mAbs within these subgroups cannot yet be fully defined since the exact number of expressed WC1 genes is not known. Final clustering of the WC1 mAbs awaits the identification and characterization of the remaining genes in this family.

The function of the WC1 antigens is, at present, unknown. The sequence analysis of the WC1.1 cDNA showed that the putative protein has 11 repeats of a cysteine-rich extracellular domain of 110 amino acids, a hydrophobic

transmembrane region and an intracytoplasmic domain. Database searches revealed that this type of cysteine-rich domain is found in a number of glycoprotein structures including CD5, CD6, epidermal growth factor and macrophage scavenger receptor (Wijngaard et al., 1992). Identification of a putative ligand(s) may help in determining the role of these glycoproteins in the cell surface interactions of γ/δ T cells and, thus, elucidate the function of the γ/δ T cell itself.

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