THY-1 HETEROGENEITY OF MOUSE THYMOCYTES

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Received 5 October 1979

1. Introduction

The Thy-1 molecule (formerly called theta) is the major membrane antigen of mouse and rat thymocytes [1,2]. Large amounts are also found in brain tissue of these species and to a lesser extent on bone marrow cells of the rat, but not of the mouse [3]. On the other hand, Thy-1 antigen is expressed on peripheral T-lymphocytes only of the mouse [4] and is widely used for serological differentiation of T- and B-lymphocytes of the mouse. Following neonatal thymectomy a dramatic decrease of the proportion of Thy-1 positive peripheral lymphocytes is observed together with a decrease in cell-mediated immunity [5].

Furthermore physiological maturation of potential T-lymphocytes by passage through the thymus, a process which might depend on soluble thymic factors, can be followed by appearance of Thy-1 antigen [6]. Despite the obvious importance of this molecule, little is known of the chemical nature of mouse Thy-1 which is still a matter of controversy [7].

In the rat, Thy-1 has been described as a glycoprotein with mol. wt 18 000 [8], and it seems likely that the corresponding molecule of the mouse is of similar nature [7].

The function of Thy-1 is still unclear but a possible role in cell–cell interactions, involving the carbohydrate part, has been discussed. Antisera with specificity for Thy-1 antigen do not necessarily detect differences in carbohydrate constitutents of glycoproteins. By use of lectins, with defined specificity for different sugar derivatives, heterogeneity in the carbohydrate part of Thy-1 from rat has been found [9]. Mouse Thy-1 has not, so far, been shown to reveal such diversity. The possible existence of Thy-1 heterogeneity of mouse thymocytes is challenging and might have functional implications. This paper describes such a heterogeneity.

2. Materials and methods

2.1. *Cell and Thy-1 preparation*

Thymus glands from 2–4 month old C57BL mice (expressing Thy-1.2 antigen) were used as the source of thymocytes. Cells were washed twice in Hank's medium and lysed in 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5% Na-deoxycholate. This concentration of detergent has been found not to interfere with the assay for quantitative determination of Thy-1 [10]. The sample (corresponding to ~40×10^6 cells/ml) was sonicated twice for 60 s at +4°C and left for 1 h at +4°C with slight agitation. After centrifugation 50 min at 100 000 × *g* the supernatant was collected.

2.2. *Antiserum*

The antiserum was raised in rabbits against mouse brain homogenate [11] and was shown to be specific for Thy-1 after appropriate absorption [10].

2.3. *Thy-1 assay*

Thy-1 was quantitated as in [10]. Briefly, the material to be assayed was diluted in ^{2}log dilutions in Hanks' medium complemented with 10% bovine serum albumin. After addition of antiserum the mixture was incubated at +4°C for 1 h. The amount of anti-Thy-1 antibodies consumed was measured by adding guinea pig serum as source of complement and ^{51}Cr-labelled thymocytes. The mixture was incubated for 1 h at 37°C. The antigen dilution giving 50% lysis was used as a measure of antigen activity.

2.4. *Lectin gels*

Lentil lectin–Sepharose 4B (2 mg lentil/mg gel) and concanavalin A–Sepharose 4B (10 mg con A/mg gel) were purchased from Pharmacia, Sweden. Pasteur pipettes were used as columns, and filled with 0.5 ml gel. Before use, each gel was washed with 10–20 vol. of 0.5% Na-deoxycholate in 0.1 M Tris–HCl buffer (pH 8.0). After sample application, unbound material was washed out with the same buffer. The bound fraction was eluted with 0.5 M α-methyl-D-mannoside in deoxycholate buffer.

3. Results

3.1. *Binding to lentil lectin*

An aliquot of the cell preparation (corresponding to 1.6×10^8 thymocytes) was applied to the lentil lectin–Sepharose 4B column and washed with 5–6 vol. buffer. Bound material was eluted with α-methyl-D-mannoside. Fractions were collected and assayed for Thy-1 activity. As shown (fig.1a) one population of Thy-1 molecules passed through the column unbound, while another population was bound and specifically eluted with sugar. The amount of Thy-1 antigen was about the same in the two peaks. To exclude the possibility of overloading the column, each population was pooled and dialyzed against deoxycholate buffer overnight and rechromatography was performed on new gels under the same conditions as above (fig.1b,c). It was shown that the two pooled fractions performed in the same way as in the first run. The pooled fraction A was again unbound and fraction B was completely bound and eluted.

3.2. *Binding to Con A*

The binding of Thy-1 antigen to con A was carried out in the same way as above. In contrast to lentil lectin all Thy-1 was bound to con A, but only a fraction of the bound material (~30%) could be eluted with α-methyl-D-mannoside, probably due to strong interaction of Thy-1 with con A.

4. Discussion

Here study heterogeneity in the carbohydrate part of Thy-1 molecules from mouse thymocytes is shown

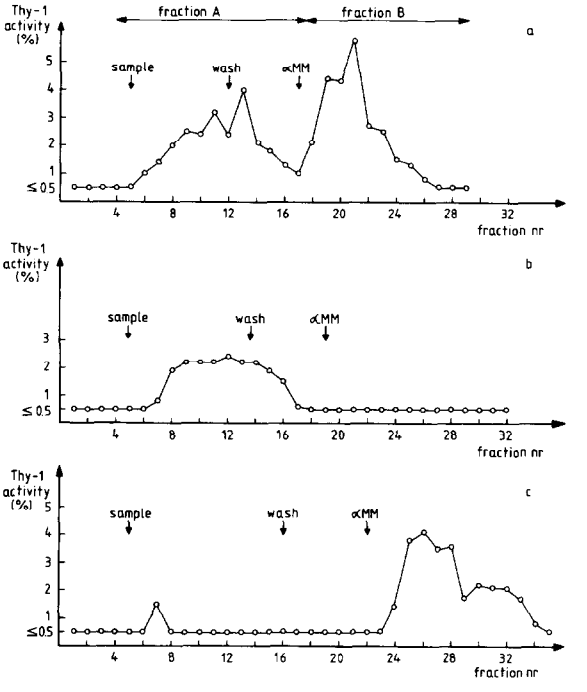

Fig.1. Affinity chromatography on lentil lectin–Sepharose 4B: (a) thymocyte extract (3.5 ml sample); (b) pooled fraction A from expt a (4.5 ml sample); (c) pooled fraction B from expt a (5.5 ml sample). Fractions of 0.5 ml were collected and assayed for Thy-1 activity, which is expressed as percent of the total applied activity. αMM, α-methyl-D-mannoside (0.5 M) in deoxycholate buffer.

for the first time. These cells possess at least two populations of Thy-1 molecules, one which has affinity for lentil lectin and one which has not. The difference between the two forms of Thy-1 can not be detected by any antisera used [1,12–16] and it is suggested that the antigenic determinants exist on the protein part of the molecule [17]. However, in [18] two antigenic specificities of mouse Thy-1 were demonstrated by use of rat anti-Thy-1.1 xenoantiserum. Whether these antigenic determinants are related to the protein or the carbohydrate part is still unclear.

Preliminary results show that the two forms described here are of about the same size, as judged by SDS–polyacrylamide gel electrophoresis [10]. Using a T-cell lymphoblastoid cell line, a homogeneous Thy-1 population has been shown that binds to lentil lectin [19]. Whether the two forms of the Thy-1 molecules now demonstrated exist on the

same or different cells is yet to be elucidated. Both for cell differentiation within one cell line and/or discrimination between subpopulations of thymocytes a heterogeneity of Thy-1 might be of importance. Interestingly, the Thy-1 antigenic specificities reported [18] were identified on different subpopulations of peripheral T-lymphocytes and reflected a maturation process rather than a functional diversity.

The amount of the two forms of Thy-1 antigen described here was about equal, an observation which is in agreement with the findings in [9] for rat thymocytes. This observation indicates similarities between rat and mouse Thy-1 of thymocytes.

Concanavalin A and lentil lectin usually are considered to possess similar sugar specificity [20]. In the present investigation differences in binding pattern however were observed since con A was able to absorb all Thy-1 antigen activity from the thymocyte extract.

Acknowledgements

The present investigation was supported by grants from the Swedish Medical Research Council (no. 4217), Lions Foundation, Umeå University, and the Medical Faculty, University of Umeå. The advice of Dr C. J. Fowler is appreciated, and skillful technical assistance was performed by Eva Dehlin.

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