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Theta Isoantigen as a Marker of Thymus-derived Lymphocytes in Mice

THERE is an obvious need for a marker that will differentiate one type of lymphocyte from another. The need has become urgent in view of recent evidence suggesting that there are at least two populations of lymphocytes, one thymus-derived and one bone marrow-derived, which participate in different ways in the immune response[1]. The theta (θ) isoantigen (θ is determined by a single locus with two alleles: θAKR found in AKR and RF mice and θC_3H present in most other inbred strains of mice tested), described by Reif and Allen[2,3], which is found chiefly in thymus lymphocytes and brain, and to a lesser extent in peripheral lymphocytes in mice, seemed a possible antigenic marker of thymus-derived lymphocytes. To establish that θ is such a marker, it is necessary to demonstrate that there is a discrete population of peripheral lymphocytes which carry the antigen and that these cells are thymus-dependent.

Anti-θC_3H was prepared by injecting CBA thymocytes into AKR mice[3]. The cytotoxic activity of the antiserum was completely absorbed by adult CBA brain, confirming the specificity of the antiserum. ^{51}Chromium cytotoxic testing, as described by Wigzell[4] and modified by Bomford *et al.*[5], was used to detect θ on the surface of lymphocytes. Lymphocyte suspensions free of red blood cells were prepared[5] from adult CBA mice. The cells were labelled with $Na_2{}^{51}CrO_4$ and added to serially diluted anti-θ or normal AKR serum (NMS). After incubating for 30 min at 37° C, the cells were washed to remove the excess antiserum and resuspended in hamster or guinea-pig complement. A detergent ('Triton') was added to another set of tubes to kill all the cells and release their chromium. After a further 30 min incubation the reaction was stopped with cold saline. The tubes were centrifuged, and the supernatants decanted and counted in a well-type scintillation counter.

Fig. 1 shows the results of a representative experiment. The plateaux of cytotoxicity seen with high concentrations of anti-θ suggest that there is a discrete population of θ-bearing lymphocytes. In the case of the thymus, this consists of 97–100 per cent of the cells as judged by trypan blue dye exclusion cytotoxic testing[6]. This agrees with the findings of Wigzell[4] and Sanderson[7] that 70–80 per cent ^{51}Cr release from lymphoid cells corresponds to 100 per cent immune lysis. By extrapolation from ^{51}Cr cytotoxic testing and more directly from dye exclusion cytotoxic testing, approximately 70–80 per cent of lymph node lymphocytes and 30–50 per cent of splenic lymphocytes appear to carry the θ antigen in CBA mice.

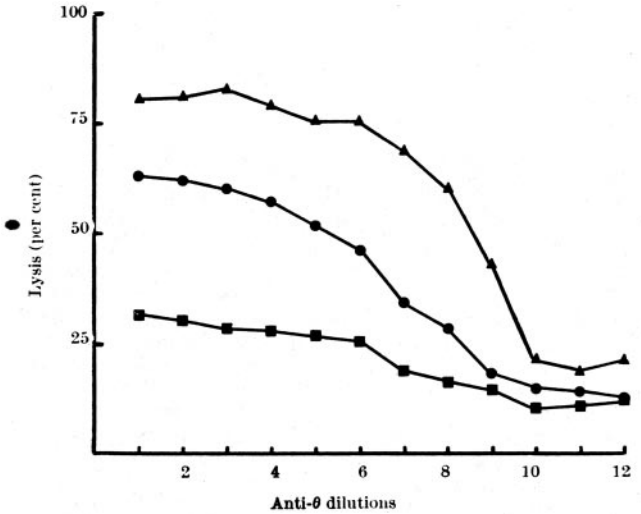

Fig. 1. Cytotoxic activity of anti-θC_3H on CBA thymocytes (▲), lymph node (●) and spleen lymphocytes (■). Tubes 1–12 contained doubling dilutions of anti-θ, beginning with 1 : 1 for lymph node and spleen and 1 : 10 for thymocytes. Per cent lysis = counts released with anti-θ/counts released with 'Triton'. Hamster complement, absorbed with mouse liver, spleen and thymus and diluted 1 : 7, was used with thymocytes. Guinea-pig complement, absorbed with mouse RBC and diluted 1 : 5, was used with lymph node and spleen.

To determine if this population of θ-bearing lymphocytes is thymus-dependent the θ content of lymph nodes and spleen lymphocytes in normal mice was compared with that of mice depleted of thymus-derived lymphocytes by chronic treatment with anti-lymphocyte serum (ALS)[8,9]. To minimize the effects of infection, CBA mice reared in a specific pathogen-free environment (SPF) were used.

One group of mice received ALS prepared according to the method of Levey and Medawar[10] beginning on the day of birth and given twice weekly until they were killed at 6–8 weeks old. Another group of mice received normal rabbit serum (NRS) according to the same schedule and some mice were left untreated.

Cytotoxic testing was carried out as before except the cells were incubated with anti-θ or NMS diluted 1 : 4 and hamster complement was used with all cell types. All tests were done in quadruplicate and the average number of counts released per tube with anti-θ, NMS, and 'Triton' was calculated and a cytotoxic index determined for each cell type by the following formula:

$$\frac{\text{Counts released (CR) with anti-}\theta - \text{CR with NMS}}{\text{CR with 'Triton'} - \text{CR with NMS}} \times 100$$

The results of three separate experiments are outlined

in Table 1. There was an 80–90 per cent reduction in the number of θ-bearing cells in the lymph nodes and spleens of ALS-treated mice when compared with the NRS-treated and untreated controls. There was no significant difference in the θ content of the thymuses in the different groups. Giemsa and methyl green pyronine stained smears of the various cell suspensions showed that in all cases at least 90 per cent of the cells were lymphocytes, mostly of the small variety. All experimental animals were autopsied and the only significant finding was a marked depletion of small lymphocytes in the thymus-dependent areas[11] of the spleen and lymph nodes in the

Table 1. CYTOTOXIC INDICES* (PER CENT) FOR THYMOCYTES, LYMPH NODE AND SPLEEN LYMPHOCYTES FROM CBA MICE TREATED WITH ANTI-LYMPHOCYTIC SERUM (ALS), NORMAL RABBIT SERUM (NRS) OR UNTREATED

	Mice	Thymus	Lymph node†	Spleen
Experiment 1	NRS-treated	89·8	51·4	16·4
	ALS-treated	80·8	7·1	1·7
	Per cent reduction‡	9·9	87	90
Experiment 2	Untreated	84·4	59·4	26·8
	NRS-treated	84	52·4	25·4
	ALS-treated	81·2	10·5	2·5
	Per cent reduction‡	3·35	80	90
Experiment 3	Untreated	70·4	53·3	
	NRS-treated	67·8	50·5	
	ALS-treated	76·3	6·1	
	Per cent reduction‡	—	88	

* Cytotoxic index =

$$\left[\frac{\text{Counts released (CR) with anti-}\theta\ (1:7) - \text{CR with NMS } (1:7)}{\text{CR with 'Triton'} - \text{CR with NMS } (1:7)}\right] \times 100$$

† Pooled mesenteric, inguinal, axillary, brachial, deep and superficial cervical.

‡ $\text{Per cent reduction} = \left(\frac{\text{NRS cytotoxic index} - \text{ALS cytotoxic index}}{\text{NRS cytotoxic index}}\right) \times 100$

ALS-treated mice. Both ALS-treated and NRS-treated mice showed prominent lymph follicles in lymph nodes and spleen with moderate numbers of plasma cells and lymphoblasts.

Thus, in CBA mice, by cytotoxic testing, 70–80 per cent of lymph node lymphocytes and 30–50 per cent of splenic lymphocytes appear to have the θ antigen on their surface. This population of theta-bearing lymphocytes in lymph node and spleen is strikingly reduced in mice which have been depleted of thymus-derived lymphocytes by chronic treatment with ALS. Preliminary experiments with newborn thymectomized mice, adult thymectomized lethally irradiated and foetal liver reconstituted mice, and mice with congenital absence of the thymus[12], have demonstrated a similar reduction of the θ content in lymph nodes and spleen[5]. This suggests that there is a discrete population of lymphocytes in the peripheral lymphoid tissues which carries the θ antigen and that these lymphocytes are thymus-dependent. θ therefore appears to be a useful marker for thymus-derived lymphocytes.

Since first submitting this report, Schlesinger and Yron[13] have reported a fall in the θ content in the lymph nodes of ALS-treated mice. Using dye exclusion cytotoxic testing, they were unable to detect θ-bearing cells in the spleen and did not estimate the relative number of θ-positive cells in the lymph nodes.

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