Immunology 1977 33 721

The influence of antigen presentation on the generation of T-helper cells with different functions

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Received 14 *February* 1977; *accepted for publication* 3 *March* 1977

Summary. Thymocytes adoptively transferred into syngeneic irradiated recipients can be primed with antigen (KLH) to generate two types of helper function termed 'specific' and 'non-specific'. Low doses of KLH given without adjuvant generate high levels of non-specific compared to 'specific' helper T cells. Large doses of KLH given in adjuvant (FCA) generate high levels of both types of helper T cell. Explanations for this observation are discussed.

INTRODUCTION

T cells have been shown to co-operate with syngeneic B cells in two ways. The first of these, referred to as specific co-operation was elucidated from studies with 'hapten-carrier' systems where strict specificity and linked recognition of hapten and carrier were observed (Mitchison, 1971). The second is that in which T cells responding to one antigen are able to facilitate the responses of B cells to other bystander antigens, for example erythrocytes or haptens bound to erythrocytes. This latter type of co-operation is a result of the activity of a mediator non-specific factor (NSF), of mol. wt 30,000–50,000 which is released after T cells are activated by antigen (Waldmann & Munro, 1974a). In order to achieve a proper understanding of how T cells interact with B cells it is important to determine whether both forms of co-operation, specific and non-specific, are mediated by one kind of T-helper cell or whether different sub-populations of T cells determine these effects.

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The clues which suggest these effects may be mediated by different T-cell sub-populations are: (a) the marked difference in antigen concentration requirements to mediate both these effects *in vitro* (Waldmann & Munro, 1974b; Marrack & Kappler, 1976) and (b) the studies performed at limiting dilution of T cells, where it has been shown that it is a rare event to detect a T cell obviously manifesting both helper functions (specific and non-specific) (Marrack & Kappler, 1975; Waldmann, Lefkovits & Feinstein, 1976). The most direct approach to resolve this question would be to generate a population of T cells which would exhibit one property and not the other. In this paper we report experiments based on this approach and demonstrate that it is possible to prime a relatively pure population of T cells *in vivo*, in such a way, that very high numbers of non-specific helpers can be generated, under conditions where the level of specific helpers remains low.

MATERIALS AND METHODS

Sources of B and T cells

B cells for assaying non-specific T helper function were obtained from anti-θ treated spleen suspensions

of adult thymectomized, irradiated, bone marrow reconstituted (ATXBm) CBA mice. Those used for determining specific helper T-cell function were the anti-θ treated spleen cells of CBA mice which had been primed with trinitrophenylated ovalbumin (TNP_3-OVA). The priming regime for this was to prime initially with 500 μg in Freund's complete adjuvant and then to boost 5 weeks later with 100 μg of soluble antigen. Spleen cells from such mice were used 4–8 weeks after the booster injection. Helper T cells were derived from the spleens of CBA mice which had been irradiated (750 rad) 7 days previously and then injected i.v. with 1×10^8 thymocytes and antigen (i.p.). 6 h following irradiation (T_{KLH}) B cells were removed by passage of the spleen cells through nylon wool (Julius, Simpson & Herzenberg, 1973) and contaminating red cells lysed by treatment with ammonium chloride.

Assay of T–B collaboration

T–B collaboration was determined by mixing appropriate combinations of KLH-primed T cells with anti-θ-treated B cells in 10-μl vol. in the wells of Terasaki plates (Lefkovits, 1972). For assessing specific helper function 2 μg/ml (the predetermined optimum) of TNP-KLH was added as challenge antigen. For analysing non-specific helper function 20 μg of TNP-KLH (same batch) and also SRBC (50 μl of 1% ml^{-1}) were used as the challenge antigens. Anti-TNP responses were determined after 5 days incubation of the cells at 37°. This was done by sampling thirty wells from each treatment group for anti-TNP plaque-forming cells (PFC) by conventional techniques using TNP coupled to donkey erythrocytes. Results are displayed as the fraction of non-responding wells, and also the total number of PFC, from the thirty wells sampled. Anti SRBC responses from the second set of trays were determined by use of the spot test where supernatants from cultures which had been incubated for 6 days were placed by a replicator (Lefkovits & Kamber, 1972) onto a plate containing SRBC embedded in agar. After incubation of such plates with added guinea-pig complement for 1 h at 37° the number of lytic spots was determined. Again, results are expressed as the fraction of non-responding wells where 60 wells were sampled for each test group.

RESULTS

The influence of priming conditions on the generation of T-helper cells

Figure 1 shows the results of an experiment where T cells were taken from two groups of mice, one group 'primed' to KLH (200 μg) with Freund's complete adjuvant and the other without adjuvant and these were then assessed for specific and non-specific helper function. The data are displayed in a conventional semilog plot where T-cell input is plotted against the

Table 1. Failure to detect suppressor cells in T_{KLH} from mice primed with soluble KLH without adjuvant

	No. of T cells per well ($\times 10^{-3}$)	T cells from mice primed with 200 μg of: Soluble KLH	KLH/FCA	The anti-hapten response to TNP-KLH: Fraction of non-responding cultures (thirty wells sampled)	Total anti-TNP PFC from thirty wells	The anti-SRBC response with TNP-KLH and SRBC*: Fraction of non-responding cultures (out of sixty)
1	25·0	–	+	0·10	1283	0·27
2	25·0	+	–	0·57	531	0·12
3	8·3	–	+	0·27	1063	0·20
4	8·3	+	–	0·80	272	0·29
5	2·8	–	+	0·30	948	0·43
6	2·8	+	–	0·63	307	0·45
7	0·9		+	NT	NT	0·77
8	0·9	+	–	NT	NT	0·60
9	None	–	–	0·90	51	0·90
10	8·3	+	+	0·23	1318	NT

* The ability of cultures to produce anti-TNP (to measure specific helper function) and anti-SRBC responses (for non-specific helper function) was determined as described in the Materials and Methods section.

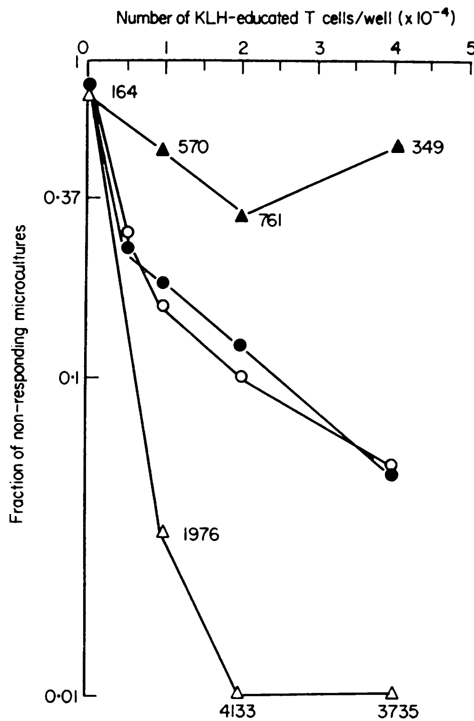

Figure 1. Titration of T_{KLH} from two priming regimes for their ability to co-operate specifically (△) with TNP-OVA primed B cells in the response to TNP-KLH or non-specifically (○) with anti-θ-treated ATXBm spleen cells for an anti-SRBC response. B cells were at $1 \cdot 5 \times 10^5$ per well. T cells were from donors primed with either 200 μg soluble KLH (closed symbols) or 200 μg KLH emulsified in FCA (open symbol).

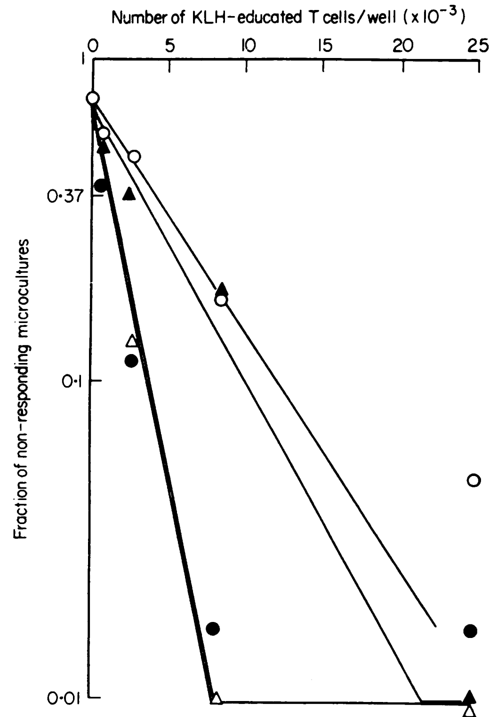

Figure 2. The influence of KLH dose for the priming of thymus cells towards non-specific helper function. Irradiated CBA mice were injected with 1×10^8 thymocytes i.v. and various doses of KLH i.p. without adjuvant. After 7 days the spleen T cells were compared for non-specific helper function by titration in the microculture system. The following priming doses were compared: 100 μg (△); 50 μg (●); 5 μg (▲); 0·5 μg (○).

fraction of non-responding cultures. This allows us to approximate the frequencies of active helper T cells. It can be seen that the frequency estimates of helper cells for non-specific collaboration were identical for both groups since both titration curves were virtually contiguous. In contrast, the FCA/KLH primed T cells contained at least a four-fold greater number of specific helpers (approximately 2×10^{-4}) than did those primed with KLH alone (approximately 4×10^{-5}). This difference in frequency was also substantiated by differences in the anti-TNP-PFC values.

Suppressor T-cell activity cannot account for this result

This marked difference in specific helper function for the group primed with, compared to that without, adjuvant cannot be ascribed to the presence of any hypothetical suppressor cells in the latter. Mixing of T cells from mice given KLH alone with T cells from mice given KLH in FCA did not reduce the response of the latter group (Table 1). Furthermore differences in the sensitivities of the assay systems (i.e. plaque assay *vs* spot lysis) for both types of helper function cannot explain these findings because we have also obtained comparable data using PFC assays to measure the SRBC response.

It is also unlikely that the FCA itself activates a population of cells which synergize *in vitro* with KLH primed T cells because mixing of T cells from mice given FCA alone with those given soluble KLH alone did not increase the number of detectable helper cells of the specific type (unpublished data).

Table 2. Low doses of soluble KLH can prime efficiently for non-specific helper function but poorly for specific helper function

Priming dose for KLH for T_{KLH}		Fraction of non-responding cultures of TNP-KLH (out of thirty)	Total anti-TNP PFC in thirty wells	Fraction of non-responding cultures to SRBC (out of sixty)
1	200 μg in FCA†	0·30	676	0·49
2	None	0·93	34	1·0
3	200 μg soluble‡	0·80	198	0·05
4	20 μg soluble	0·77	151	0·07
5	2 μg soluble	0·90	56	0·09

* The ability of cultures to produce anti-TNP (to measure specific helper function) and anti-SRBC responses (for non-specific helper function) was determined as described in the Materials and Methods section.
† 2×10^4 T_{KLH} per well.
‡ $4{\cdot}5 \times 10^4$ T_{KLH} per well.

The influence of antigen dose

If we examine the dose of soluble KLH required to elicit high frequencies of non-specific helper T cells then it would seem (Fig. 2) that even as little as 0·5 μg i.p. can generate high levels of these cells—that is a frequency of the order of 1×10^{-4}.

With this in mind we modified the priming dose to accentuate differences in post-priming frequencies of both helper cells. In Table 2 it can be seen that T cells from mice educated with 2 μg of soluble KLH without adjuvant contained virtually no specific helpers (less than $2{\cdot}5 \times 10^{-6}$), whereas their content of non-specific helper cells was at least 5×10^{-5}. It would seem, therefore, that in the 'education' of T cells it is possible, by using low concentration of soluble KLH without adjuvant, to generate a population of cells widely endowed for non-specific but impoverished for specific helper function. Recently it has been demonstrated (Marrack & Kappler, 1976) that when very low doses of antigen in FCA are given to conventional mice then again, non-specific helper function is preferentially developed over specific help. This present study shows that indeed adjuvant is not even required to generate high levels of non-specific helper cells, but rather influences the production of specific helpers. The converse situation has so far, not proven possible: we have not been able to reproducibly generate cells having high frequencies of specific helper but low numbers of non-specific helper T cells.

DISCUSSION

Two explanations would be compatible with the available data. Firstly it may be that two entirely different T-cell populations mediate each function. Alternatively, it may be that the non-specific helper cell develops into a specific-helper cell (albeit one still capable of generating the non-specific helper factor (NSF). In this case we must postulate that FCA contributes to the second stage of the pathway. This might be for trivial reasons such as the persistence of antigen in the FCA depot, or alternatively through the activation of a contaminating cell type, within the irradiated recipient, which contributes to the transformation of a T cell having non-specific helper capacity to one having either, specific helper function only, or possessing both properties together. We are currently investigating the means by which a predominantly high content non-specific, low content specific population of helper T cells can be converted to one with high numbers of specific helper cells.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council.

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