Brief Definitive Reports

SPECIFIC HETEROLOGOUS ENHANCEMENT OF IMMUNE RESPONSES

II. Immunological Memory Cells of Thymic Origin

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Cellular cooperation between different lymphoid cell populations is a characteristic feature of many immune responses (1, 2). The classes of lymphoid cells which have been shown to collaborate have been divided into two major groups: thymus-derived (T cell)[1] and bone marrow–derived (B cell) lymphocytes. Synergism between T cell and B cell components has been demonstrated for (*a*) humoral responses against heterologous erythrocytes and serum proteins, (*b*) in "carrier-specificity" phenomena, and (*c*) in graft-vs.-host cell-mediated immunity (1). In addition, the demonstration of specific immunological memory and tolerance in T cell–mediated cellular responses, and in B cell–mediated humoral responses, suggests that both populations contain cells which are capable of displaying antigenic specificity (1). With respect to humoral immunity, although both classes of cells can respond to an antigenic stimulus, only the B cell line can synthesize exportable immunoglobulin (3–5). T cells do not secrete antibody, but appear to be necessary to facilitate antibody production by B cells. But the mechanism of interaction between these two populations in the immune response is not known.

Recently, we reported that augmented responses similar to those after a second exposure to the same antigen need not be specific (6). Our data indicated that the addition of specific antigen to a population of primed cells results in the enhancement of the response to another unrelated antigen present in the environment. This is consistent with the idea, suggested by Davies et al., that a mediator, released by specifically stimulated T cells, acts on the antibody-producing B cells to increase their response.

The experiments described here were designed to determine whether specific stimulation of cells from the thymus of primed mice results in augmentation of

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[1] *Abbreviations used in this paper:* B cell, bone marrow–derived lymphocyte; PFC, plaque-forming cell; SRBC, sheep erythrocytes; T cell, thymus-derived lymphocyte; TT, fluid tetanus toxoid.

an immune response by normal spleen cells to an unrelated antigen. We added graded numbers of syngeneic thymocytes from mice primed with tetanus toxoid to suspension-cultures of normal spleen cells which were stimulated with sheep erythrocytes (SRBC). The addition of the priming antigen to such cultures resulted in significant enhancement of the anti-SRBC plaque-forming cell (PFC) response compared to aliquots cultured without the priming antigen. Similar cultures of normal thymocytes and normal spleen cells did not show this effect.

6-wk-old DBA/2 female mice were immunized with 10 Lf of fluid tetanus toxoid (TT). Mice were shown to be primed, by finding antitoxin in their serum, as previously described (6). Cultures were prepared from the spleens of immunized and normal mice 30–60 days after injection by a modification of the method of Mishell and Dutton (6). Suspensions of primed and normal thymocytes were prepared by excising the upper two-thirds of the thymic lobes, care being taken to avoid fascial tags which might contain lymph node tissue. Thymus lobes were teased apart and treated similarly to the spleen cell suspensions. Cells were resuspended in complete medium (6) to a final concentration of 20 million cells/ml. Experimental cultures contained 20 million normal spleen cells and 10 million or 1 million primed or normal thymocytes. Uniform cell density was maintained in all groups of cultures. Cultures were stimulated with approximately 3 million SRBC on day 0 and with 1 ng/ml of TT on day 2. Cells releasing anti-SRBC hemolysins were enumerated on day 5 by a modification of the direct technique of Jerne and Nordin (6).

The results of a representative group of experiments are shown in Table I. Addition of 1 ng/ml of TT on day 2 to SRBC-stimulated, normal spleen cell cultures containing 10 million thymocytes primed with TT, resulted in a significant enhancement of the anti-SRBC PFC response compared to similar cultures without the priming antigen (groups 1 and 2). In contrast, the addition of TT to cultures with similar numbers of normal spleen cells and normal thymocytes resulted in a slight depression in the anti-SRBC response (groups 3 and 4). Addition of TT to positive control (groups 5 and 6) and negative control cultures (groups 7 and 8) resulted in significant augmentation or slight depression, respectively, of the PFC response, as previously reported (6). We obtained no anti-SRBC plaques in SRBC-stimulated cultures of primed or normal thymocytes cultured in the presence of priming antigen nor in its absence (groups 9 and 10).

To control for the possibility that the enhanced response was due to either lymph node cells or peripheral blood lymphocytes contaminating the primed thymocyte population, we performed a similar experiment substituting 10 million primed spleen cells for the 10 million primed thymocytes (Table II, groups 9 and 10). Addition of TT to such cultures did not produce an elevated anti-SRBC PFC response (Table II, experiments 4 and 5). All controls responded as shown in Table I.

In another set of experiments, we measured the effect of adding TT on day 2 to mixed cultures of 1 million primed or normal thymocytes and 20 million normal spleen cells that received SRBC on day 0. It can be seen that addition of priming antigen to these cultures failed to elicit an augmented anti-SRBC response

(Table II, experiments 1–3). Enhancement was observed in positive controls, while slight depression was seen in the negative controls.

The results indicate that antigenic stimulation affects a relatively small number of cells in the thymus in such a way that they respond specifically to it

TABLE I

Effect on the Anti-SRBC PFC Response of Adding 1 ng of TT on Day 2 to Mixed Cultures of 10 Million TT-Primed Thymocytes and 20 Million Normal Spleen Cells Stimulated with SRBC on Day 0

Group	Cultured cells (in millions)	TT	Anti-SRBC plaques per 10^6 recovered cells					Average enhancement of anti-SRBC PFC response
			Experiment					
			1	2	3	4	5	
1	10 Primed Thy	+	1390	1720	2363	3647	3160	
	20 Normal Spl*		(39)‡	(70)§	(38)‡	(38)§	(94)§	56%
2	10 Primed Thy	−	1000	1010	1710	2640	1627	
	20 Normal Spl*							
3	10 Normal Thy	+	983	1187	1680	2846	2352	
	20 Normal Spl*		(−11)	(−16)	(−18)	(−22)	(−5)	−14%
4	10 Normal Thy	−	1103	1420	2047	3640	2467	
	20 Normal Spl*							
5	20 Primed Spl	+	1637	2220	2010	5130	3367	
			(34)‖	(50)§	(36)§	(48)‖	(66)§	47%
6	20 Primed Spl	−	1223	1483	1483	3460	2032	
7	20 Normal Spl	+	1070	1750	1960	3660	2800	
			(−24)	(−2)	(−13)	(−11)	(−3)	−11%
8	20 Normal Spl	−	1413	1783	2247	4120	2892	
9	20 Primed Thy	+ or −	0	0	0	0	0	0
10	20 Normal Thy	+ or −	0	0	0	0	0	

Thy, thymocytes; Spl, spleen cells; TT, tetanus toxoid; Nos. in parentheses indicate degree of enhancement.

* Similar results were obtained with 10 million spleen cells.

‡ Symbols represent P values calculated by Student's t test. $P < 0.005$

§ $P < 0.001$.

‖ $P < 0.010$.

again on restimulation. This is apparent from the data which show that the addition of TT to 10 million, but not 1 million TT-primed thymocytes, cultured with 20 million normal spleen cells, enhances the anti-SRBC PFC response.

The data support the concept that at least some components of antigen-

specific immunological memory reside in the T cell population. These observations are consistent with the supposition that the capacity of thymus cells to collaborate with bone marrow cells in humoral responses can be specifically enhanced by prior exposure of the thymocytes to the test antigen (7–10). Similar

TABLE II

Effect on the Anti-SRBC PFC Response of Adding 1 ng of TT on Day 2 to Mixed Cultures of 1 Million TT-Primed Thymocytes (or 10 Million TT-Primed Spleen Cells) and 20 Million Normal Spleen Cells Stimulated with SRBC on Day 0

Group	Cultured cells (in millions)	TT	Anti-SRBC plaques per 10^6 recovered cells					Average enhancement of anti-SRBC PFC response
			Experiment					
			1	2	3	4	5	
1	1 Primed Thy	+	3167	3440	3267			
	20 Normal Spl		(6)	(4)	(−14)			−1%
2	1 Primed Thy	−	2987	3427	3787			
	20 Normal Spl							
3	1 Normal Thy	+	2107	3233	3330			
	20 Normal Spl		(−35)	(−15)	(−2)			−17%
4	1 Normal Thy	−	3240	3807	3400			
	20 Normal Spl							
5	20 Primed Spl	+	2273	4060	3372	3453	3553	
			(58)‡	(33)§	(44)‡	(62)‡	(67)‡	53%
6	20 Primed Spl	−	1443	3060	2347	2127	2127	
7	20 Normal Spl	+	2467	2640	3440	2067	2400	
			(−20)	(−17)	(−19)	(−3)	(0)	−12%
8	20 Normal Spl	−	3073	3200	4247	2127	2407	
9	10 Primed Spl	+				1620	2413	
	20 Normal Spl					(−6)	(4)	−1%
10	10 Primed Spl	−				1727	2320	
	20 Normal Spl							

See Table I for explanatory footnotes.

findings of antigenic specificity and immunological memory of thymocytes have been reported in a cell-mediated graft-vs.-host system (11). Furthermore, the fact that the adoptive secondary response to hapten-protein conjugates can be abrogated by treatment of carrier-primed cells (T cells) with anti-θ serum is additional evidence that at least a portion of immunological memory resides in the T cell population (12).

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Note Added in Proof.—Using the carbon-injection technique of Leckband and Boyse (1971. *Science (Washington).* **172**:1258), we were unable to detect parathymic lymph nodes adherent to, or within, the thymic capsule of DBA/2 mice; hence, it is unlikely that contaminating lymph node cells were responsible for the enhancing effect observed in mixtures of primed thymocytes and spleen cells.