REGULATION OF THE IMMUNE RESPONSE

II. Qualitative and Quantitative Differences Between Thymus- and Bone Marrow-Derived Lymphocytes in the Recognition of Antigen*

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One of the primary characteristics of an immune response is the specificity of antigen recognition. This specificity is manifest in the activities of both thymus-derived lymphocytes (T cells)[1] and bone marrow-derived lymphocytes (B cells) and in the antibody produced by the B cells (1–11). We have studied the specificity of the T cells that mediate the helper function in the response of mice to heterologous erythrocyte antigens and have observed that these cells display a less restricted specificity than the antierythrocyte antibody produced by B cells (12).

Similar observations have recently been reported by others working with erythrocyte (13–15) and protein[2] (16–18) antigens; however, Rajewsky et al., using heterologous serum albumin antigens, (19, 20) failed to see any qualitative differences between the specificities of helper T cells and of humoral antibody.

A shortcoming in several of the relevant studies is that T cells were not established as limiting in the assay for their activity and that therefore exaggerated cross-reactivities with other antigens might have been measured. In the present study we measure T cell helper activity in the in vitro response of mouse spleen cells to the hapten trinitrophenol (TNP), coupled to an erythrocyte carrier, using a titration technique that assures that T cell activity is the limiting component in the system. We confirm our previous observation of the less restricted specificity of T cells compared with antibody and discuss the implica-

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[1] *Abbreviations used in this paper:* B cells, bone marrow-derived lymphocytes; BRBC, burro erythrocytes; BSS, balanced salt solution; CRBC, chicken erythrocytes; GRBC, goat erythrocytes; HGG, human gamma globulin; HRBC, horse erythrocytes; NIP, 4-hydroxy-5-iodo-3-nitrophenacetate; PFC, plaque-forming cells; SRBC, sheep erythrocytes; T cells, thymus-derived lymphocytes; (T, G)-A--L, poly-L(Tyr, Glu)-poly-D, L-Ala-poly-L-Lys; TNP, trinitrophenol; TRBC, toad erythrocytes.

[2] Ruben, T., and J. Chiller. Cross-tolerance: localization at the thymus. Manuscript in preparation.

tions of this finding with respect to the sensitivity and regulation of the cellular and humoral immune responses.

Materials and Methods

Mice.—8–12-wk-old hybrid mice (BDF_1), raised in our own colony from C57BL/6 female × DBA/2 male, were used in all experiments.

Antigens.—Sheep erythrocytes (SRBC) from a single animal (no. 446) and horse, goat, and chicken erythrocytes (HRBC, GRBC, and CRBC, respectively), each pooled from a number of animals, were obtained from the Colorado Serum Company (Denver, Colo.). Burro erythrocytes (BRBC) from a single animal (Maggie) were obtained from Davis Laboratories, Inc. (Davis, Calif.). Toads (*Bufo marinus*) from the Miami Pet Farm (Miami, Fla.) were the generous gift of Dr. Stanley Mendoza. Erythrocytes (TRBC) were pooled from a number of toads. TNP-coupled erythrocytes were prepared by the method of Rittenberg and Pratt (21) as modified by Kettman and Dutton (22). In vivo immunizations were given intravenously in 0.2 ml balanced salt solution (BSS). In vitro immunizations were 0.05 ml of a 0.2% suspension of erythrocytes ($\sim 2 \times 10^6$) per culture.

Cultures.—Spleen cell suspensions were cultured by the method of Mishell and Dutton (23), as modified by Kettman and Dutton (22), for the TNP-erythrocyte antigens. The cell concentration was 10^7 spleen cells per culture in all experiments.

Antisera.—Primary anti-SRBC sera were pooled from nine mice 5 days after a primary immunization with 2×10^8 SRBC. For secondary anti-SRBC serum, 50 mice were given a primary immunization with 2×10^7 SRBC, followed 3–4 mo later with a secondary immunization with 2×10^8 SRBC. The animals were bled 5 days later, and the sera were pooled.

Hemagglutination Titers.—Microhemagglutination titers were obtained in disposable titration plates (Cooke Engineering Co., Alexandria, Va.), 25-μl transfer loops being used. The diluant was BSS, and 25 μl of a 1% suspension of erythrocytes were added to each well.

Assay of Antibody-Producing Cells.—Cells producing antibody specific for erythrocyte determinants were enumerated by the Jerne hemolytic plaque assay (24), as modified by Mishell and Dutton (23). IgM or "direct" plaques were developed with guinea pig complement alone. IgG or "indirect" plaques were developed with guinea pig complement containing a 1/200 dilution of rabbit antimouse IgG.

TNP-specific antibody-producing cells were assayed by the method of Rittenberg and Pratt (21) as modified by Kettman and Dutton (22), using TNP coupled to HRBC. In each assay a parallel determination was made by assaying with TNP-free HRBC. This HRBC "background" was subtracted from the value obtained with TNP-HRBC to calculate the number of TNP-specific plaque-forming cells (PFC). The correction seldom amounted to more than 10%.

Assay of Thymus-Derived Helper Cells.—The helper activity of T cells was assayed by a modification of the method of Kettman and Dutton (6, 7, 22). Helper activity was equated with the ability of spleen cells from mice primed with a carrier erythrocyte to enhance the in vitro anti-TNP response of normal spleen cells when presented with TNP coupled to the same or another carrier erythrocyte. The method is represented schematically in Fig. 1. It is reasonable to assume that the immunization of mice with a carrier erythrocyte to increase the helper T cell activity in the spleen has little or no effect on the concentration of TNP-specific precursors. Therefore, as one cultures normal spleen cells diluted with increasing numbers of carrier-primed spleen cells, keeping the total cell number constant (represented on the abscissa), the concentration of TNP-specific precursors remains constant in each culture. However, since there is more T cell activity in the primed cells than in the normal cells, the concentration of helper T cell activity in the cultures increases linearly with the dilution. At the beginning of the dilution, T cell activity would be limiting; but it is con-

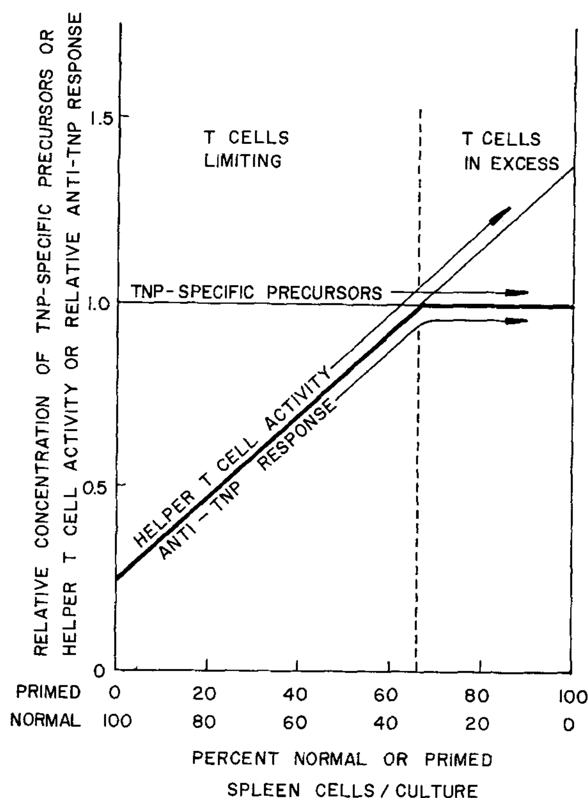

FIG. 1. Schematic representation of the technique for assaying helper T cell activity. See text for explanation.

ceivable that as the dilution proceeds, a point is reached at which the system becomes saturated with T cell activity. Past this point T cells would be in excess; and TNP-specific precursors would become limiting. If the anti-TNP response of the cultures is linearly related to the limiting component, then one would predict that the response would follow the heavy line through the dilution. The relative increase in helper T cell activity that accompanies priming could be estimated from the initial slope of this titration curve.

Fig. 2 shows an example of this dilution titration in which normal spleen cells and SRBC-primed spleen cells and TNP-SRBC as antigen are used. The anti-TNP response is plotted vs. the number of carrier-primed spleen cells per culture. The anti-TNP response of the normal cells was 337 PFC/10^6 recovered cells on day 4 of culture. As these cells were diluted with primed cells, the anti-TNP response rose linearly, eventually reaching a peak at about 1,050 PFC/10^6. At this point the cultures contained 15%, or 1.5×10^6, primed spleen cells. As the titration continued, the response plateaued as predicted. The initial slope (first four points) of the titration curve was taken as a measure of T cell activity, the best straight line was fit to the data, and a slope of 461 anti-TNP PFC/10^6 recovered cells per 10^6 primed cells was calculated.

At priming doses of below approximately 10^6 SRBC, titration curves similar to those in Fig. 2 are obtained. At higher priming doses, one still obtains a linear initial slope; but rather

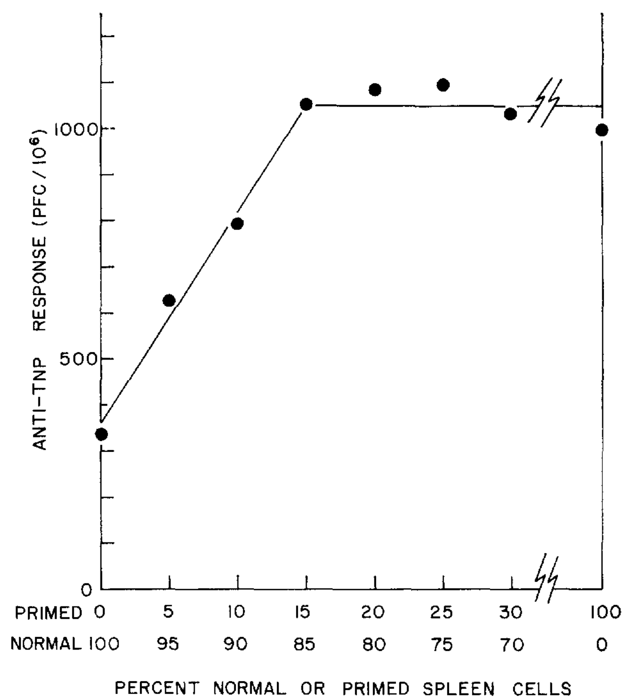

FIG. 2. Assay of helper T cell activity in spleen cells from SRBC-primed mice. Three mice were injected with 2×10^5 SRBC each. After 4 days, a pooled spleen cell suspension was prepared from these and from normal mice. Cultures were prepared from various proportions of primed and normal cells, keeping the total cell concentration at 10×10^6 per culture. The cultures were immunized with TNP-SRBC and, after 4 days, were assayed for TNP-specific PFC, pooling four cultures for each determination. Results are reported as anti-TNP PFC/10^6 recovered spleen cells.

than a sustained plateau, one observes that the anti-TNP response begins to fall off at high concentrations of carrier-primed spleen cells in the cultures. The explanation for this effect is unclear. It may be due to some inhibitory component (introduced into the cultures with the primed spleen cells) such as (*a*) anti-SRBC antibody, (*b*) too many helper T cells or "suppressor" T cells, or (*c*) an expanded population of SRBC-specific B cell precursors, which compete with anti-TNP precursors for the available T cells. In any event, initial titration slopes having proven to be linear at all priming doses, routinely complete titrations are not performed, but simply four to six concentrations of primed cells to determine the initial slope. And in the graphic presentation of data, for ease of comparison of several titrations plotted on the same axes, the ordinate scale is adjusted so that for each titration the ordinate intercept is zero. Thus the ordinate axis records the enhancement of the anti-TNP response above that of normal cells alone.

An example is given in Fig. 3, which shows a series of titrations for mouse spleen cells that had been primed with different doses of SRBC, titrated against a common pool of normal cells. In this example, as the priming dose increased, the concentration of T cell activity increased, until a maximum was reached at a dose of 1.6×10^6 SRBC.

The technique can be used to assay helper T cell activity with any of a number of eryth-

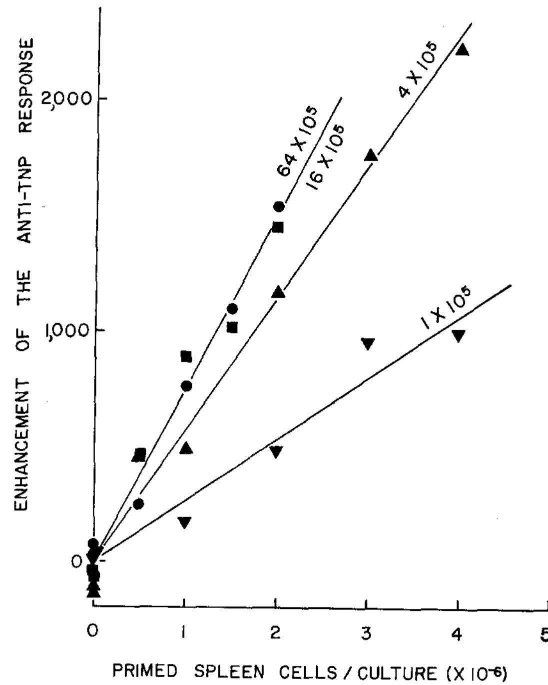

FIG. 3. Helper T cell activity vs. priming dose of SRBC. Groups of three mice were primed with either 64×10^5 (●), 16×10^5 (■), 4×10^5 (▲), or 1×10^5 (▼) SRBC. After 4 days, spleen cell suspensions were prepared from each group and were titrated against a common pool of normal cells, as described in Fig. 2. The least squares method was used to fit the best straight line to the data, and the ordinate intercept was adjusted to zero for each titration. The results are reported as the enhancement of the anti-TNP response in anti-TNP PFC/10^6 recovered cells vs. the number of primed spleen cells per culture. The slopes calculated from these data were 748, 744, 578, and 268, respectively, for priming doses of 64, 16, 4, and 1×10^5 SRBC.

rocyte carriers. The data from five experiments are plotted in Fig. 4. Spleen cells from mice that had been immunized with a particular carrier erythrocyte were titrated against normal spleen cells, TNP coupled to the same erythrocyte being used as antigen. In each case the anti-TNP response of the normal cultures was enhanced by the addition of carrier-primed cells, showing that helper activity is limiting for each erythrocyte in the spleens of normal animals, but rises rapidly after priming.

RESULTS

The specificity of anti-SRBC antibody for SRBC and other heterologous erythrocytes was determined by means of a number of functional assays: the ability of the antibody to agglutinate the erythrocytes in a microhemagglutination assay, the ability of the antibody to lyse the erythrocytes in a hemolytic plaque assay, and the ability of the antibody to suppress a primary in vitro immune response to the erythrocyte antigens or to the hapten TNP coupled to the erythrocytes as carriers.

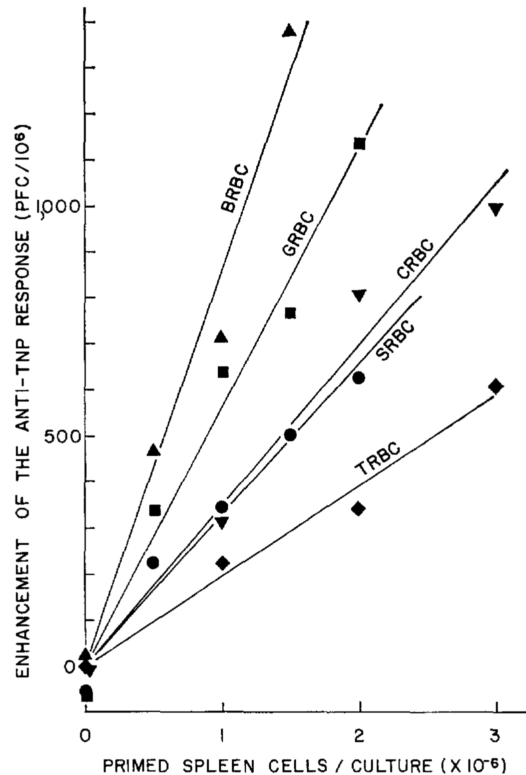

FIG. 4. Helper T cell activity for various carrier erythrocytes. The results are from five experiments. In each experiment a group of three mice was immunized with an optimal dose of a particular erythrocyte carrier: BRBC (▲), GRBC (■), CRBC (▼), SRBC (●) and TRBC (◆). After 4 days, a spleen cell suspension was prepared and titrated against normal cells, as described in Fig. 2, immunizing the cultures with TNP coupled to the same carrier erythrocyte. Three to four cultures were pooled for each determination. The data are presented as in Fig. 3. Immunizing doses: 2×10^6 BRBC, 2×10^6 GRBC, 4×10^7 CRBC, 2×10^7 SRBC, 2×10^6 TRBC. Calculation of the slopes of these titration lines yields values of 861, 564, 350, 329, and 195 anti-TNP PFC/10^6 recovered cells per 10^6 primed cells, respectively, for the carriers BRBC, GRBC, CRBC, SRBC, and TRBC.

The results of the hemagglutination assays are shown in Table I. Both primary and secondary anti-SRBC sera show a high degree of specificity for SRBC. Cross-reactivity with GRBC was detected in the secondary serum, but no cross-reactivity with the other erythrocytes was seen in either serum.

Comparable results were obtained with the hemolytic plaque assay (Table II). After a primary in vivo immunization with SRBC, IgM or direct PFC were detected in the spleen with both SRBC and GRBC, but not with BRBC or CRBC. Similarly, after a secondary immunization with SRBC, IgG or indirect PFC were observed with SRBC and GRBC, but not with BRBC or CRBC.

TABLE I

Specificity of Hemagglutination by Primary and Secondary Anti-SRBC Serum

Antigen	Hemagglutination titer (log_2)	
	Primary*	Secondary*
SRBC	6.2 ± 0.6‡	9.9 ± 1.9‡
GRBC	<1	3.8 ± 0.6
BRBC	<1	<1
CRBC	<1	<1
TRBC	<1	<1

* For preparation of sera, see Materials and Methods.

‡ Average of three determinations; 95% confidence limits.

TABLE II

Specificity of In Vivo Anti-SRBC PFC

	Exp. no.	Mouse no.	Antigen used in assay							
			SRBC		GRBC		BRBC		CRBC	
			PFC/10⁶	%	*PFC/10⁶*	%	*PFC/10⁶*	%	*PFC/10⁶*	%
Primary direct PFC	1	1	391	100	72	18	0.1	0.03	1.8	0.46
		2	438	"	93	21	1.9	0.43	0.7	0.16
		3	834	"	164	20	0.6	0.07	0.3	0.04
		4	492	"	80	16	0.2	0.04	0.9	0.18
		5	318	"	73	23	<0.1	<0.03	1.2	0.38
		6	227	"	38	17	0.1	0.04	0.4	0.18
		Av		100		19		0.11		0.23
Secondary indirect PFC	2	1	5950	100	1440	24	1.1	0.02	<0.4	<0.01
		2	6470	"	1210	19	0.4	0.01	3.8	0.06
		3	4460	"	1480	33	0.9	0.02	<0.4	<0.01
		Av		100		25		0.02		0.03
	3	1	3400	100	1820	54	9.0	0.26	<0.4	<0.01
		2	3430	"	1210	35	7.9	0.23	0.4	0.01
		3	3580	"	1650	46	4.3	0.12	0.4	0.01
		Av		100		45		0.20		0.01

Exp. 1. Six mice were immunized with 2×10^8 SRBC. 4 days later, spleen cell suspensions were prepared and assayed for direct hemolytic PFC, various erythrocytes being used in the assay. Exps. 2 and 3. Three mice were immunized with 2×10^7 SRBC. 4 mo later, a secondary immunization with 2×10^8 SRBC was given. 5 days later, spleen cell suspensions were prepared and assayed for indirect hemolytic PFC, various erythrocytes being used in the assay. Results are reported as PFC per 10^6 spleen cells and as the percent of the results obtained with SRBC in the assay.

TABLE III

Specificity of In Vitro Anti-SRBC PFC

	Exp. no.	Antigen used in assay															
		SRBC				GRBC				BRBC				CRBC			
		+Ag	−Ag	Difference	%	+Ag	−Ag	Difference	%	+Ag	−Ag	Difference	%	+Ag	−Ag	Difference	%
Normal	1	428	49	379	100	36	26	10	2.6	1	1	0	0	35	29	6	1.6
	2	433	39	394	"	54	16	38	9.6	1	1	0	0	26	35	−11	−2.8
	3	2,955	57	2,898	"	190	26	164	5.7	2	5	−3	−0.10	60	80	−20	−0.7
	4	592	30	562	"	61	22	39	6.9	3	4	−1	−0.18	33	16	17	3.0
	Av				100				6.2				−0.07				0.28
SRBC primed	1	21,281	528	20,753	100	3,140	394	2,746	13	15	8	7	0.03	42	61	−19	−0.09
	2	15,716	447	15,269	"	3,289	344	2,945	19	2	4	−2	−0.01	21	22	−1	−0.01
	3	18,229	443	17,786	"	3,281	228	3,053	17	3	1	2	0.01	115	74	41	0.23
	Av				100				16				0.01				0.04

Spleen cells from normal mice or from mice immunized 4 days previously with 2×10^7 SRBC were cultured with (+ Ag) or without (−Ag) the addition of SRBC to the cultures. 4 days later, the cultured cells were assayed for direct hemolytic PFC, various erythrocytes being used in the assay. The results are reported as PFC per 10^6 recovered spleen cells. The SRBC-stimulated portion of the response was calculated (Difference), and is also reported as the percent of the results obtained with SRBC in the assay.

To assure that the specificity of the response to SRBC was not different in vitro, the cross-reactivity of anti-SRBC PFC in cultures of normal and SRBC-primed spleen cells immunized with SRBC was determined (Table III). Since there is a spontaneous appearance of PFC specific for most erythrocytes in this culture system, in each case a parallel determination was made in cultures in which the SRBC antigen was omitted and the number of antigen-induced PFC was calculated. The results are for IgM or direct PFC only, since in our hands the IgG response is poor in this in vitro system. Again, considerable cross-reactivity is seen with GRBC, but not with BRBC or CRBC.

Finally, the ability of anti-SRBC serum to suppress a primary in vitro response was determined, using the various erythrocytes as antigens themselves or as carriers for the hapten TNP. The results are presented in Table IV. Anti-SRBC serum suppressed both the antierythrocyte and antihapten response when SRBC or GRBC were used as antigen or carrier, but not when BRBC or CRBC were used.

With this pattern of cross-reactivity established at the antibody level, we determined the specificity at the level of SRBC-primed helper T cells. Mice were primed with SRBC, and the ability of the SRBC-primed helper T cells to help anti-TNP responses involving other carriers was determined. A typical experiment is shown in Fig. 5. It can be seen that the SRBC-primed helper cells also are capable of enhancing the anti-TNP response when GRBC and, to a smaller

TABLE IV

Specificity of the Suppression of the In Vitro Immune Response by Anti-SRBC Serum

Culture immunogen	Anti-SRBC serum added	In vitro immune response (PFC/10^6 recovered spleen cells)				
		Anti-SRBC	Anti-GRBC	Anti-BRBC	Anti-CRBC	Anti-TNP
TNP-SRBC	−	3,881	—	—	—	193
	+	43				53
TNP-GRBC	−	—	1,046	—	—	447
	+		11			64
TNP-BRBC	−	—	—	881	—	394
	+			1,031		363
TNP-CRBC	−	—	—	—	954	702
	+				753	713
None	−	150	25	11	49	21
	+	67	6	1	51	53

Cultures of spleen cells from normal mice were immunized with either TNP-SRBC, TNP-GRBC, TNP-BRBC, TNP-CRBC, or no immunogen in the presence or absence of 3 μl of secondary anti-SRBC serum per culture. 4 days later, the cultures were harvested and assayed for both the antierythrocyte and anti-TNP response. Results are reported as PFC per 10^6 recovered spleen cells.

extent, when BRBC are used as carrier. No significant enhancement is seen with CRBC or TRBC as carrier. An estimation of the extent of cross-reactivity is calculated by comparing the slopes of the titration lines. In the experiment in Fig. 5, assuming 100% for the slope when SRBC is used as carrier, the slopes are 95, 18, 2, and −5 when GRBC, BRBC, CRBC, and TRBC, respectively, are used as carrier. The summarized results of a number of similar experiments are shown in Table V. The antigenic similarities between SRBC and GRBC detected at the antibody level were also seen at the helper-cell level, where they appear to be more extensive. The similarities between SRBC and BRBC were detected only at the helper-cell level.

To see if this type of cross-reactivity is a property peculiar to SRBC-primed T cells, reciprocal experiments were performed with BRBC and TRBC as the priming antigens. The results are presented in Figs. 6 and 7. TRBC-primed T cells cross-react significantly with none of the tested carriers, whereas, as expected, BRBC-primed T cells cross-react with SRBC (~15%), but not with CRBC or TRBC.

DISCUSSION

We have examined the specificity of both the helper T cells and the antibody produced in the response of mice to a series of heterologous erythrocytes. In determining the antigen specificity of the antibody produced in response to SRBC, we selected as additional test antigens erythrocytes from the closely related species goat and from the progressively more distant species burro, chicken, and toad. The specificity was determined by a number of functional

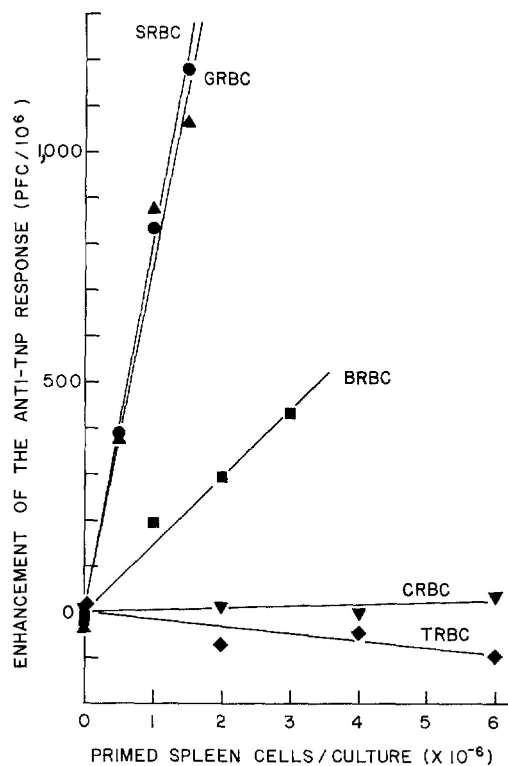

FIG. 5. Specificity of SRBC-primed helper T cell activity. Spleen cells pooled from three mice that had been primed 4 days previously with 2×10^6 SRBC were added in increasing numbers to cultures of normal spleen cells, keeping the total cell concentration at 10^7 per culture. Cultures were then immunized with either TNP-SRBC, TNP-GRBC, TNP-BRBC, TNP-CRBC, or TNP-TRBC. After 4 days the anti-TNP responses of the cultures were assayed by the hemolytic plaque assay. The influence of the SRBC-primed spleen cells on the anti-TNP response of the normal cells is plotted as the enhancement of the anti-TNP response above the normal response (PFC/10^6 recovered cells) vs. the number of SRBC-primed spleen cells per culture. The TNP erythrocyte carrier is indicated for each titration, and the best straight line is fit to the data. The anti-TNP responses (PFC per 10^6 recovered cells) of normal spleen cells alone with the respective carriers were: SRBC, 118; GRBC, 122; BRBC, 132; CRBC, 68; TRBC, 221. The helper activities with each of the carriers calculated from the slope of the titrations (PFC per 10^6 recovered cells per 10^6 primed cells) were: SRBC, 799; GRBC, 759; BRBC, 148; CRBC, 4; TRBC, −16.

TABLE V

Summary of the Specificity of SRBC-Primed Helper T Cell Activity

	In vitro TNP carrier			
	GRBC	BRBC	CRBC	TRBC
Average percent cross-reactivity with SRBC	113	29	2.4	0.0
No. of determinations	6	13	6	5
Range	82–159	2–67	−2–5	−2–2
Standard deviation	±26	±18	±3.7	±1.6

The protocol for each experiment was as in Fig. 5. The SRBC-priming dose varied from 10^6 to 10^7. The slopes of titration curves were calculated, and cross-reactivity with SRBC was estimated as described in the text.

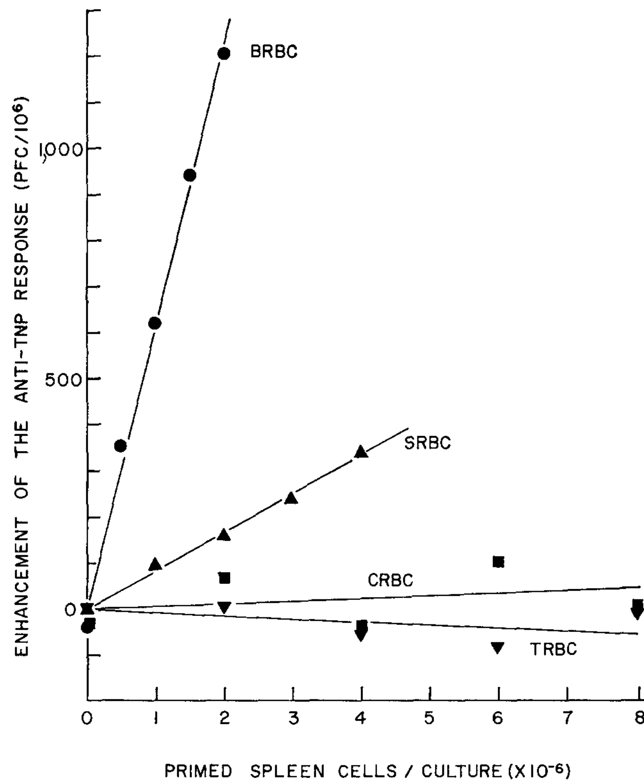

FIG. 6. Specificity of BRBC-primed helper T cell activity. The experimental protocol is the same as in Fig. 5, except that the priming immunogen was 2×10^6 BRBC and the culture immunogens were TNP-BRBC, TNP-SRBC, TNP-CRBC, and TNP-TRBC. The anti-TNP responses (PFC per 10^6 recovered cells) of normal spleen cells alone with the respective carriers were: BRBC, 360; SRBC, 216; CRBC, 357; TRBC, 274. The helper activities with each of the carriers calculated from the slope of the titration lines (PFC per 10^6 recovered cell per 10^6 primed cells) were: BRBC, 620; SRBC, 83; CRBC, 6; TRBC, −7.

assays: the ability to agglutinate antigen, the ability to cause hemolysis, and the ability to suppress the immune response. Whereas anti-SRBC antibody was demonstrated to cross-react with GRBC at all the levels tested, no cross-reactivity was seen with BRBC, CRBC, or TRBC. These findings are consistent with previous reports in the literature (1, 2, 23).

To determine the specificity of the helper T cells that appear in the spleen after immunization with SRBC, the ability of these SRBC-primed helper T cells to enhance an in vitro anti-TNP response was measured, using the various test erythrocytes as carrier for the TNP. In each case titrations were performed to assure that T cell activity was assayed when limiting.

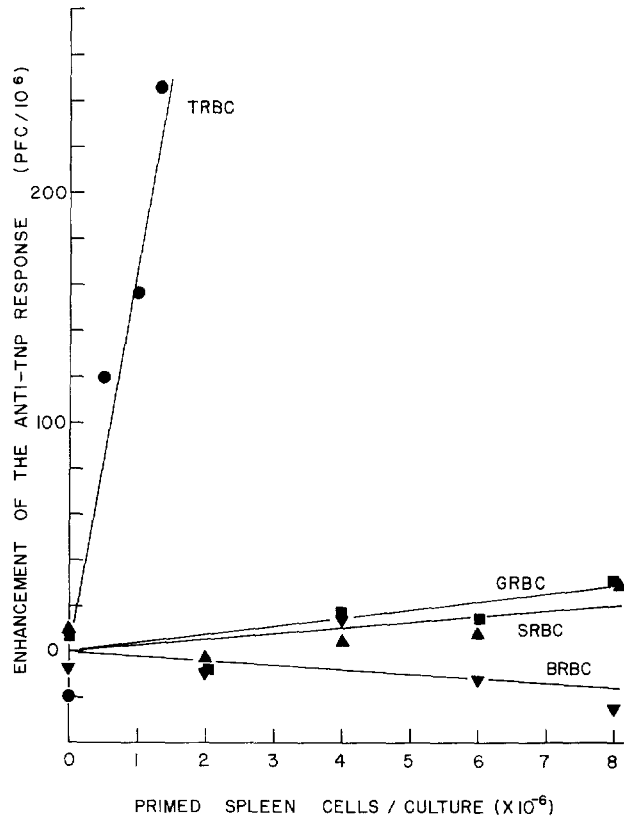

FIG. 7. Specificity of TRBC-primed helper T cell activity. The experimental protocol is the same in Fig. 5, except that the priming immunogen was 2×10^6 TRBC and the culture immunogens were TNP-TRBC, TNP-SRBC, TNP-BRBC, and TNP-GRBC. The anti-TNP responses (PFC per 10^6 recovered cells) of the normal spleen cells alone with the respective carriers were: TRBC, 114; SRBC, 25; BRBC, 72; GRBC, 72. The helper activity with each of the carriers calculated from the slope of the titrations (PFC per 10^6 recovered cells per 10^6 primed cells) were: TRBC, 167; SRBC, 3; BRBC, −2; GRBC, 3.

The results (Fig. 5, Table V) show that SRBC-primed T cells cross-react with GRBC; however, the degree of cross-reaction (> 100%) is higher than that seen at the antibody level (6–50%). A significant cross-reactivity is also observed with BRBC (~30%), although no significant cross-reactivity is seen with this erythrocyte at the antibody level (<0.20%). That this enhancement of the anti-TNP response is not due to some general nonspecific stimulus produced by the SRBC-primed cells is shown by the lack of significant enhancement when either CRBC or TRBC are used as the TNP carrier.

The antigenic similarity between SRBC and BRBC was also demonstrated in a reciprocal experiment in which spleen cells from mice that had been im-

munized with BRBC were titrated against normal cells using BRBC, SRBC, CRBC, or TRBC as TNP carriers (Fig. 6). As predicted on the basis of the preceding experiment, these helper T cells cross-reacted with SRBC ($\sim$15%), but not with CRBC or TRBC. In a control experiment, the specificity of TRBC-primed T cells was tested with TRBC, GRBC, SRBC, and BRBC (Fig. 7). There was no cross-reactivity with the other erythrocytes.

These data confirm and quantitate our preliminary findings on the cross-reactivity of SRBC-primed helper cells with GRBC and BRBC (12). Recently others working with erythrocyte antigens have reported similar results. Cunningham and Sercarz (13), working in a transfer system in mice, have found that helper T cells primed by cow erythrocytes can also help in the response to SRBC and HRBC and that the extent of this cross-reaction is greater than that seen at the PFC level. Playfair (14) has recently reported that radio-resistant helper T cells induced by priming with a number of mammalian erythrocytes (rabbit, pig, human, horse) could help the response of irradiated, bone marrow-restored recipients to SRBC. Haritou and Argyris (15), using a rosette assay, have shown considerable cross-reactivity between SRBC and HRBC at the T cell level, whereas these antigens do not cross-react at the PFC level.

Rajewsky et al. (19, 20) have studied the specificity of the helper cells involved in the response of mice to the hapten 4-hydroxy-5-iodo-3-nitrophenacetate (NIP) coupled to either sheep or bovine serum albumin as carrier. Although they conclude that the extent of cross-reactivity of the helper T cells for these two antigens closely follows the extent of cross-reactivity seen at the serum antibody level, several other workers have recently reported differences between T cells and B cells in the recognition of protein antigens. Thompson et al. (16) have demonstrated that antibodies produced in guinea pigs to the antigens, lysozyme, and reduced, carboxymethylated lysozyme do not cross-react, whereas extensive cross-reactivity is seen at the level of T cell-mediated, delayed hypersensitivity. Similar experiments have been performed by Parish (17), using bacterial flagellin and acetylated flagellin in rats, and by Cooper (18), using, in mice, flagella from two strains of *Salmonella* that show no serological cross-reactivity. Ruben and Chiller[2] have shown that human gamma globulin (HGG) is capable of inducing unresponsiveness at the T cell level, but not at the B cell level, to both bovine and horse gamma globulin.

Several of these studies and our own previous findings suffer from the shortcoming that the assay of T cell activity involves a method in which this activity may not always be limiting and that therefore exaggerated cross-reactivities may be measured. In the experiments reported here, the performance of titrations, which allowed greater confidence in the quantitation of the assay, established that the helper T cell activity was limiting.

The basis of the apparently broader specificity range of helper cells compared with antibody is as yet unexplained. Several possibilities exist.

Some T cells may be nonspecific or may each carry a number of specificities. This possibility seems unlikely in view of the lack of cross-reactivity of SRBC-primed T cells with CRBC or TRBC.

It may be that the receptors on T cells are different from those on B cells, which would result in the two populations' "seeing" different determinants on the antigen. The T cell receptor may be a special class of Ig that does not appear in the humoral antibody, or it may be another type of molecule altogether. In the case of SRBC and BRBC, the cross-reaction of the T cell could then be mediated by a determinant, or determinants, that does not participate in the production of humoral antibody. Although this is a troublesome position to defend on theoretical grounds, there is some evidence to support it.

There is a considerable body of evidence documenting the existence of immunoglobulin receptors on B cells; however, there is still controversy about the nature of the receptor on T cells. There are reports of failure to directly demonstrate Ig on the surface of T cells with anti-Ig reagents (25–28), although others have been successful (29, 30). There are conflicting reports on the ability to detect surface Ig with a radioiodine labeling technique (31–34). On the other hand, several indirect methods have indicated the presence of functional Ig receptors on T cells. Lesley et al. (35) have shown that helper T cells can be killed by treatment with antikappa serum and complement. Also, Greaves and Hogg (36, 37) have demonstrated the inhibition of rosette formation by T cells with anti-Ig reagents. Mason and Warner (38) have inhibited cells that mediate transplantation immunity and delayed hypersensitivity with anti-Ig reagents.

Other evidence suggesting the T cell receptors may differ from B cell receptors is the reported existence of antigenic determinants that are specific for either T cells or B cells, for example, those of glucagon (39) and encephalomyelitic protein (40). The genetically controlled lesions in the response of some mice to the synthetic polypeptide poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] could be due to the absence of T cells with specificity for the antigen since a perfectly normal anti-(T,G)-A--L antibody response can be elicited in these mice if the (T,G)-A--L is coupled to immunogenic carriers (41, 42).

The above evidence suggests that our observation of the less-restricted specificity of helper T cells compared with antibody may be explained by a basic difference in the receptors of T cells and B cells. However, our results are also compatible with the assumption that the receptors on T cells and B cells are the same, the differences being explained on the basis of sensitivity rather than specificity.

This difference in sensitivity may be at the level of antigen stimulation. For instance, the stimulation of B cells may be restricted to cells with relatively high affinity for the antigen, whereas T cells may respond over a much

broader range of affinity and therefore cross-react with other antigens in the low-affinity range where B cells are not stimulated.

The difference in sensitivity of T cells and B cells to antigenic stimulus has been shown by a number of workers. Parish has demonstrated an inverse relationship between humoral antibody production and cellular immunity when a flagellin antigen is chemically modified by acetylation. He suggests that the effect is explained by a lower affinity requirement for the stimulation of T cells than for B cells (17).

Falkoff and Kettman (43) have shown in this TNP-erythrocyte system that helper T cells are stimulated by very low doses of antigen, which fail to stimulate B cells. This selective stimulation of T cells by low doses of antigen has been demonstrated by others (44–46). In addition, studies have shown that tolerance is induced in T cells by much lower doses of antigen than are required for B cells (47, 48).

Finally, we must consider the possibility that the differences in sensitivity of T cells and B cells might be at the level of the mediation of function rather than at the level of stimulation. The affinity of the antibody required for the agglutination or lysis of antigen or the suppression of the immune response may be considerably higher than that required by the receptors that mediate T cell functions. Therefore, the different phenomena may show different patterns of cross-reactivity, even though they are mediated by identical molecules.

Although we cannot distinguish among the possible explanations for our findings, discussed above, we have established that the T cells that mediate the helper function for erythrocyte antigens display a broader range of specificity than does the antibody produced in a humoral response. The biological significance of our findings is substantiated by the observation by Kettman (49) that the cells that mediate delayed hypersensitivity to these erythrocyte antigens in vivo show the same broad range of specificity as the helper cells. This differential activity of SRBC-primed T cells and anti-SRBC antibody with BRBC provides a useful system for separating the cellular and humoral immune responses.

Our findings and those of others discussed above suggest that the response of T cells is extremely sensitive, but of rather broad specificity. From a teleological point of view this allows the animal to become easily sensitized or "primed" to a wide range of environmental antigens. The B cell response, on the other hand, may be reserved for a more substantial contact with antigen, e.g. during a bacterial infection, showing a narrower specificity in order to deal with a specific antigen during a crisis. These findings also suggest that, by proper manipulation or selection of antigen, one can selectively stimulate a cellular immune response in the absence of a humoral response, thus providing a tool that may prove useful in situations in which the production of

humoral "blocking" antibody interferes with the successful rejection of a tumor by cytotoxic T cells (50).

SUMMARY

The specificity of antigen recognition by thymus-derived helper cells (T cells) and antibody was examined in mice, heterologous erythrocyte antigens from sheep (SRBC), goat (GRBC), burro (BRBC), chicken (CRBC), and toad (TRBC) being used. Antibody specificity was tested by a number of functional assays: hemagglutination, hemolysis, and immune suppression. The specificity of T cells was determined by titrating their ability to help the in vitro antitrinitrophenol (TNP) responses of mouse spleen cultures immunized with the hapten coupled to the various test erythrocytes as carrier. Anti-SRBC antibody cross-reacted with GRBC, but not with BRBC, CRBC, or TRBC. In contrast, SRBC-primed helper T cells cross-reacted with both GRBC and BRBC, but not with CRBC or TRBC, indicating a difference in the specificity of antigen recognition between the cellular and the humoral immune responses.

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