HAPTEN-SPECIFIC IgE ANTIBODY RESPONSES IN MICE

II. Cooperative Interactions Between Adoptively Transferred T and B Lymphocytes in the Development of IgE Response*

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The induction of humoral immune responses to a variety of antigens requires the cooperative participation of both thymus-derived (T)[1] and bone marrow-derived (B) lymphocytes. The former cells exert very definite regulatory influences on the responses of IgM and IgG B lymphocyte precursors of antibody-forming cells with respect to triggering the differentiative events following exposure to antigen (reviewed in ref. 1). Evidence obtained in recent studies by other investigators (2–8) has strongly indicated the participation of T cell functions in hapten-specific IgE antibody responses in rats and rabbits. However, the precise nature of T cell regulation of IgE antibody production is largely unknown. Specifically, a crucial question concerns whether or not the effect of activated helper T cells on IgE production follows the same general pattern, both qualitatively and quantitatively, observed in the elicitation of IgG antibody responses and, by extension, whether the same or different T cells or T cell products operate on the two antibody class precursor lymphocytes, respectively.

In order to evaluate these questions, we have recently developed and described a system for the adoptive transfer of IgE antibody responses in inbred mice (9). In those initial studies, specific IgE antibody responses to the hapten 2,4-dinitrophenyl (DNP) conjugated to either keyhole limpet hemocyanin (KLH) or protein extracts of *Ascaris suum* (ASC) were transferred to irradiated recipients using spleen cells from syngeneic donors sensitized to produce IgE antibodies (9). In the present report, we describe the utilization of this adoptive transfer system to demonstrate cooperative interactions between distinct determinant-specific (i.e., hapten and carrier, respectively) mouse spleen cell populations in the production of IgE as well as IgG antibodies.

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[1] *Abbreviations used in this paper:* ABC, antigen-binding capacity; ASC, *Ascaris suum*; B lymphocytes, bone marrow-derived lymphocytes; BGG, bovine gamma globulin; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; KLH, keyhole limpet hemocyanin; MEM, minimum essential medium; OVA, hen ovalbumin; PCA, passive cutaneous anaphylaxis; T lymphocytes, thymus-derived lymphocytes.

Moreover, these studies demonstrate that T lymphocytes bearing the θ-isoantigen function as the carrier-specific helper cells for IgE antibody production, analogous, in this sense, to the situation for IgG antibody responses. Finally, evidence bearing on similarities and differences between T cell regulation of IgG and IgE antibody responses, respectively, has been obtained in certain of the experiments reported herein.

Materials and Methods

Proteins and Hapten-Protein Conjugates.—KLH was purchased from Pacific Bio-Marine Supply Co., Venice, Calif. ASC, extracted from *Ascaris suum* as described by others (10), were kindly supplied by Dr. Kurt J. Bloch. Bovine gamma globulin (BGG) and hen ovalbumin (OVA), five times recrystallized were obtained from Pentex Biochemicals, Kankakee, Ill. The following DNP conjugates were prepared as previously described (11, 12): DNP_{14}-KLH, $DNP_{2.1}$-ASC, DNP_{32}-BGG, and DNP_8-OVA. Subscripts refer to the number of moles of DNP per 100,000 molecular weight units of KLH; per mole of carrier for BGG and OVA and moles of DNP $\times 10^{-7}$ per milligram of ASC protein.

Animals.—Mice of the inbred lines A/J and BALB/c and SJL were obtained from the Jackson Laboratories, Bar Harbor, Maine. Random-bred white female CFW mice were obtained from Carworth Div. Becton, Dickinson, and Co., New City, N. Y. All mice were immunized or used as recipients at 8–12 wk of age.

Immunizations and Adoptive Cell Transfers.—Mice used as donors of DNP-primed spleen cells were immunized by intraperitoneal injection of either DNP-KLH (2 μg) or DNP-ASC (10 μg) mixed with 2–10 mg of $Al(OH)_3$ gel in a total volume of 0.5 ml of saline. $Al(OH)_3$ gel (alum) was prepared by mixing equal volumes of 2N $Al_2(SO_4)_3$ and 2N NaOH as described by Levine and Vaz (13). Mixtures of hapten-protein conjugates with alum were prepared immediately before use.

Donors of carrier-primed cells were immunized with 10 μg of either KLH or ASC administered intraperitoneally either mixed with 2–4 mg of alum or emulsified in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich., containing 0.5 mg/ml *Mycobacterium butyricum*). At varying times after priming (see Results), the primed donor mice were killed and their spleens removed. Single cell suspensions in Eagle's minimum essential medium (MEM) were prepared, washed, and transferred intravenously to syngeneic, irradiated (500 r) recipients. Usually 50×10^6 cells of a given type were administered to each recipient. Immediately after cell transfer, secondary challenge was performed intraperitoneally with a DNP-protein conjugate or carrier protein alone mixed with alum. All recipient mice were bled from the retroorbital plexus 7 days after cell transfer and challenge and serum anti-DNP antibody levels were determined as described below.

Depletion of T Lymphocytes.—The preparation of anti-θ serum, determination of anti-θ serum cytotoxicity, and treatment of spleen cells with anti-θ serum plus complement has been detailed in previous studies from this laboratory (14).

Measurement of Anti-DNP Antibodies.—

IgE antibodies: The level of reaginic (IgE) anti-DNP antibodies in pools of sera from groups of five mice were determined by passive cutaneous anaphylaxis (PCA) reactions using shaven CFW or SJL test mice as described by Levine and Vaz (13). Briefly, 25 μl aliquots of serum from each mouse within a given group were pooled and serially diluted (twofold) in saline. 50 μl of each serum dilution were injected intradermally into the dorsal skin surface of the test mice. 48 h after intradermal sensitization, DNP-specific PCA reactions were elicited by intravenous injection of 500 μg of DNP-BGG in 0.3 ml of 0.5% Evans blue dye dissolved in 5% dextrose-water solution. Reactions were read and recorded as the reciprocal of the highest dilution of serum evoking threshold PCA reactivity (5 mm diameter). Sera which failed to elicit detectable PCA reactions were arbitrarily assigned a value of <5.

It is well established that homocytotropic antibodies detected by this procedure are of the IgE class (15). Details of their DNP specificity have been previously described (9).

IgG serum antibodies: Serum IgG anti-DNP antibody levels were determined in individual sera by a modified Farr technique (16, 17) using [^{3}H]DNP-ε-amino-*N*-caproic acid (11). Using standard curves constructed from specific anti-DNP antibody of corresponding strains of mice, percentage of binding was converted into amount of specific anti-DNP antibody in microgram per milliliter of serum. For statistical analysis, antibody levels of individual animals were logarithmically transformed and geometric means and standard errors were calculated. In those mice in which no significant antibody could be detected in the serum, a value of 0.10 μg/ml was arbitrarily assigned to allow logarithmic transformation of the data. Group comparisons were made employing Student's *t* test.

Measurement of Anti-KLH Antibodies.—

IgE antibodies: These were determined by PCA reactions performed as described above for anti-DNP. Test antigen was 200 μg of dissociated KLH in 0.3 ml of 0.5% Evans blue dye.

IgG antibodies: Antibodies to KLH were titrated by the antigen-binding capacity (ABC) test previously described (18). By using ^{125}I-labeled dissociated KLH, the binding antibodies were coprecipitated by rabbit antimouse immunoglobulin antiserum. The ABC value was calculated from the dilution of antiserum that bound 33% of the antigen. Titers are expressed as micrograms of antigen *N* precipitated by 1.0 ml of antiserum.

RESULTS

Cooperation between Hapten-Primed and Carrier-Primed Lymphocytes in the Elicitation of Adoptive Secondary Hapten-Specific IgE Antibody Responses.— The successful adoptive transfer of DNP-specific IgE antibody responses with spleen cells from suitably primed donor mice (9) made it possible to investigate the nature of cell interactions participating in the production of IgE antibodies in vivo. To this end, we have utilized the classical Mitchison (19) model for adoptive transfer of mixtures of spleen cells primed to different determinants as detailed in the following series of experiments carried out in A/J mice.

The protocol and the anti-DNP antibody responses obtained in the first experiment are summarized in Fig. 1. Anti-KLH antibody responses of selected groups are given in the top of Table I. Recipients of only DNP-KLH-primed cells developed very good adoptive secondary anti-DNP responses of both IgE and IgG antibody classes upon challenge with DNP-KLH (group I), whereas DNP-ASC failed to elicit any detectable response in such mice (group II). The same was true for anti-KLH antibody responses of the IgE class in these two groups of mice (Table I). On the other hand, recipients of DNP-KLH-primed cells which received, in addition, a second transfer of ASC-primed cells manifested good secondary anti-DNP responses to DNP-ASC (groups III and IV). However, the magnitude of the responses obtained of the IgE and IgG antibody classes, respectively, varied in such mice depending on the nature of adjuvant employed for carrier immunization. Thus, recipients of cells from donors primed with ASC mixed with alum (group III) had clearly better IgE responses than recipients of cells from donors primed with ASC in CFA (group IV), whereas the converse was true for IgG anti-DNP antibody

responses. These differences will be explored in greater detail in subsequent experiments described below.

Although recipients of only DNP-KLH-primed cells, without additional helper spleen cells, failed to respond to a secondary challenge made with DNP-ASC alone (group II), such recipients developed appreciable levels of

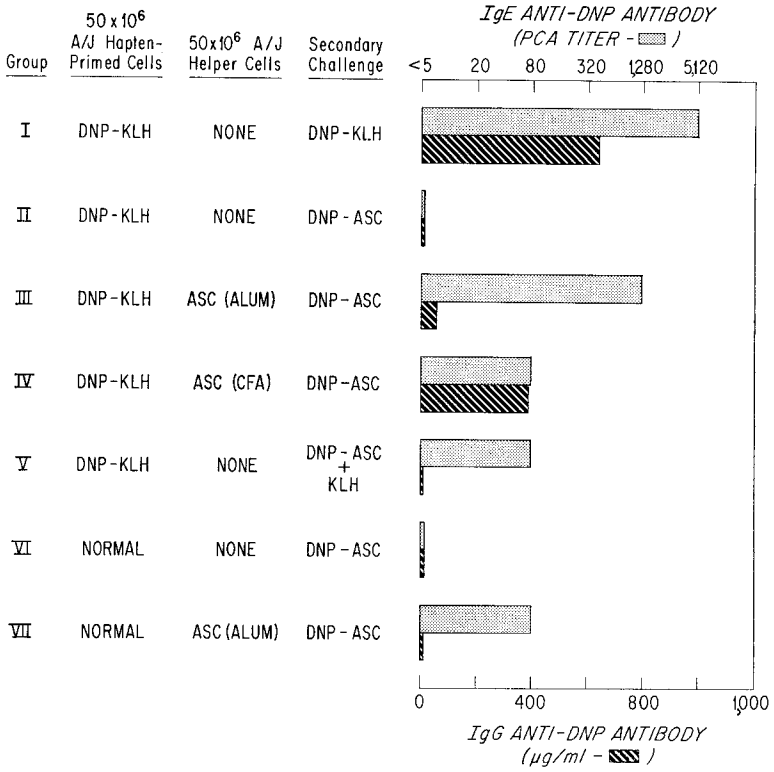

FIG. 1. Irradiated (500 r) A/J mice received one or more syngeneic donor cell types intravenously as indicated. DNP-KLH-primed cells came from 7 wk-primed donors; ASC-primed cells came from 3 wk-primed donors. Recipients were secondarily challenged intraperitoneally with alum mixtures of 2 μg DNP-KLH, 10 μg DNP-ASC, or a mixture of 10 μg DNP-ASC plus 2 μg unconjugated KLH as shown. The IgE and IgG serum anti-DNP antibody levels of groups of five mice on day 7 after secondary challenge are illustrated.

IgE anti-DNP antibodies when challenged with a mixture of DNP-ASC plus unconjugated KLH (group V). Note that under the present conditions a selective IgE response was elicited; no appreciable increase in IgG anti-DNP antibodies was observed. This phenomenon also is further explored in a later experiment in this report (see below). Not unexpectedly, recipients in group V also developed good IgE anti-KLH antibody responses (Table I).

Finally, whereas mice that received normal donor spleen cells alone failed to

produce detectable IgE or IgG antibody after challenge with DNP-ASC (group VI), the additional transfer of ASC (alum)-primed spleen cells permitted the development of IgE (but no detectable IgG) anti-DNP antibodies after such challenge. This form of "augmented" primary antihapten response has been previously demonstrated to occur, under appropriate circumstances of carrier preimmunization, in IgG responses of intact guinea pigs and rabbits (11).

The protocol and anti-DNP responses obtained in a reciprocal experiment are illustrated in Fig. 2. Anti-KLH antibody responses of certain groups are summarized in the bottom of Table I. As shown in Fig. 2, recipients of DNP-ASC-primed cells developed substantial IgE and IgG anti-DNP antibody responses upon challenge with DNP-ASC (group I). Secondary challenge with DNP-KLH failed to elicit detectable anti-DNP antibody responses (group II) unless recipients were given an additional inoculum of spleen cells from KLH-primed donors (groups III and IV). As in the preceding experiment, somewhat better IgE anti-DNP responses were obtained with KLH-primed cells from donors primed with an alum mixture (group III) than from donors given a CFA emulsion of KLH (group IV). This difference was even more striking in terms of the IgE anti-KLH antibody responses developed in these same groups of recipients (compare groups III and IV, Table I). However, the difference observed be-

TABLE I

Adoptive Secondary Anti-KLH Antibody Responses

Experiment	Group	Protocol		Secondary challenge	Serum Anti-KLH antibody	
		Hapten-primed cells	Helper cells		IgE	IgG
					*PCA**	*μg/ml‡*
	I	DNP-KLH	None	DNP-KLH	1,280	ND
1	II	DNP-KLH	None	DNP-ASC	<5	ND
(Fig. 1)	V	DNP-KLH	None	DNP-ASC + KLH	1,280	ND
	II	DNP-ASC	None	DNP-KLH	<5	0
	III	DNP-ASC	KLH (Alum)	DNP-KLH	5,120	11.6
2	IV	DNP-ASC	KLH (CFA)	DNP-KLH	320	2.9
(Fig. 2)	VI	Normal	None	DNP-KLH	<5	0
	VII	Normal	KLH (Alum)	DNP-KLH	5,120	10.8
	VIII	Normal	KLH (CFA)	DNP-KLH	320	5.0

See legend to Figs. 1 and 2 for protocol.

ND, Not done.

* The data are expressed as the reciprocal of the highest PCA titer elicited by pooled sera from five mice of each recipient group bled 7 days after secondary challenge.

‡ The data are expressed as geometric mean anti KLH antibody levels of sera from individual mice in each group on day 7 after secondary challenge.

tween these two adjuvants with respect to IgG anti-DNP antibody production in the preceding experiment (Fig. 1) was not apparent in the IgG anti-DNP responses obtained in the present experiment. In contrast, recipients of KLH (alum)-primed cells developed significantly higher IgG anti-KLH antibody responses than did recipients of KLH (CFA)-primed cells (Table I). The specificity of the helper effect of KLH-primed spleen cells in this experiment is

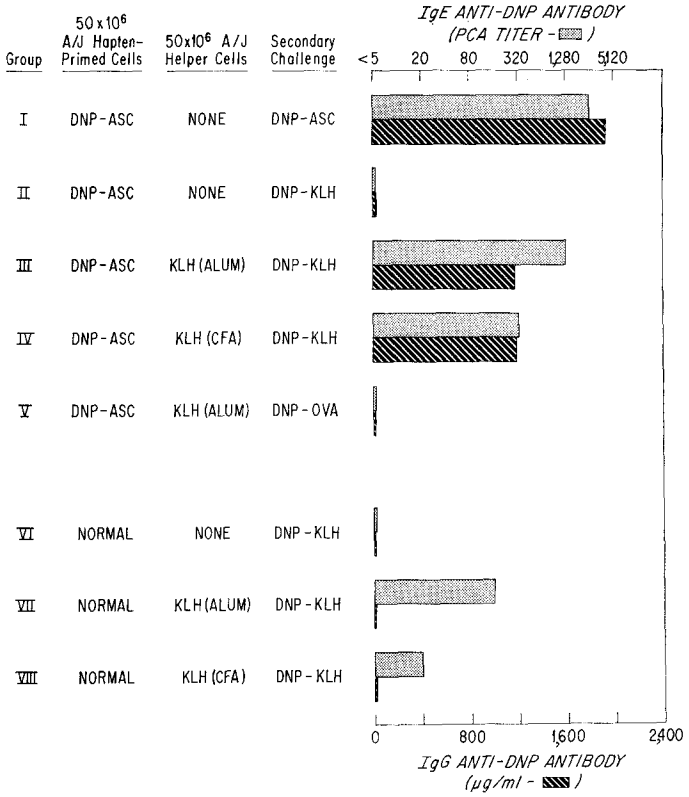

FIG. 2. Irradiated A/J mice received one or more donor cell types intravenously as indicated. DNP-ASC-primed cells came from 5 wk-primed donors; KLH-primed cells came from 6 wk-primed donors. Recipients were secondarily challenged intraperitoneally with alum mixtures of either 10 μg DNP-ASC, 2 μg DNP-KLH, or 10 μg DNP-OVA as shown. The IgE and IgG serum anti-DNP antibody levels of groups of five mice on day 7 after secondary challenge are illustrated.

clearly illustrated by the failure of a completely unrelated DNP-protein, DNP-OVA, to stimulate secondary IgE or IgG responses in recipients of both DNP-ASC and KLH (alum)-primed cells (group V).

The capacity to obtain an augmented adoptive primary IgE anti-DNP response is again illustrated in the present experiment by groups VI–VIII

(Fig. 2). It is interesting that the best augmented primary IgE anti-DNP responses were manifested by recipients of KLH-primed cells from donors immunized with alum (group VII). As shown in Table I, recipients of KLH-primed cells in groups VII and VIII developed good adoptive secondary anti-KLH antibody responses of both IgE and IgG classes. Again, significantly higher anti-KLH responses of both classes were manifested by recipients of cells from donors primed with an alum mixture (group VII).

Identification of Carrier-Specific Helper Cells as θ-Bearing T Lymphocytes in Cooperative IgE Antibody Responses.—The establishment of a cooperative IgE

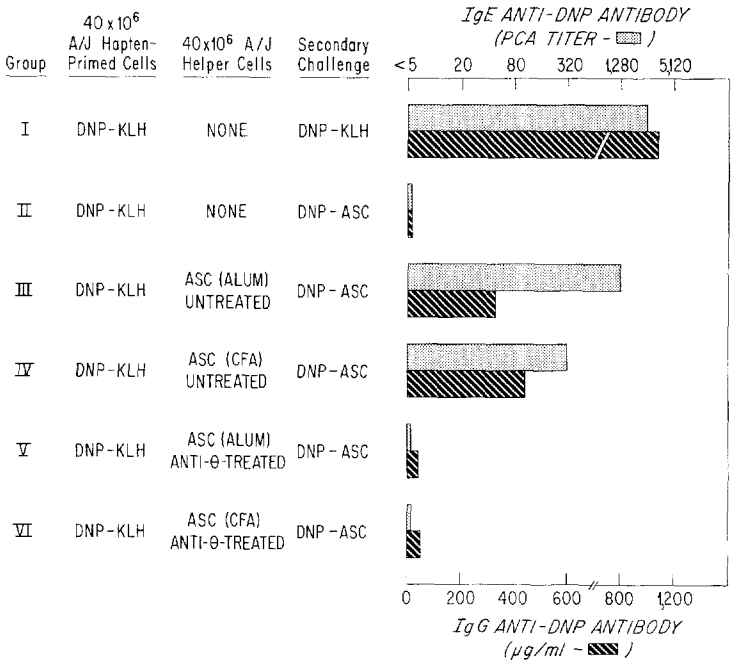

FIG. 3. Same basic protocol as described in Figs. 1 and 2. Donor cell types were obtained 6 wk after priming with the antigen indicated. The doses of antigens employed for secondary challenge were the same as in Figs. 1 and 2. Each group consisted of five mice and the serum IgE and IgG anti-DNP antibody levels on day 7 after challenge are illustrated.

response system in the present studies afforded us the opportunity to demonstrate directly T cell participation in such responses using classical techniques for depletion of T lymphocytes by anti-θ serum cytolysis.

The protocol and data from this experiment are depicted in Fig. 3. Control recipients in groups I and II developed and failed to develop, respectively, secondary anti-DNP responses of IgE and IgG antibody classes depending on whether DNP-KLH (group I) or DNP-ASC (group II) was employed for

secondary challenge. As before, additional transfer of ASC-primed spleen cells conferred the capacity of DNP-KLH-primed cells to develop adoptive secondary responses to DNP-ASC (groups III and IV), the magnitude of IgE and IgG antibody class responses being again slightly inversely related to one another depending upon the adjuvant (alum or CFA) used for carrier-priming. Treatment of such ASC-primed spleen cells with anti-θ serum plus complement to deplete T lymphocytes from the helper cell donor population completely abolished IgE and markedly diminished IgG anti-DNP antibody production (groups V and VI).

Comparisons of the Effects of Freund's Adjuvant vs. Alum in Priming Carrier-Specific Helper Cells Participating in IgE and IgG Antibody Responses.—In the preceding experiments, differences were noted in the magnitude of responses obtained in the IgE and IgG antibody classes which appear to be related to the type of adjuvant employed in priming donors of carrier-specific helper cells. These findings could reflect appreciable quantitative and/or qualitative differences in the helper T cell populations stimulated under different conditions of adjuvant administration and prompted us to explore this possibility by varying the number of helper T cells participating in the adoptive secondary anti-DNP response. The predictive reasoning behind this type of experiment is that if differences in the predominant antibody class response elicited with the help of a given adjuvant-primed T cell population results from the participation of qualitatively distinct T cells, then the magnitudes of the IgE and IgG responses, respectively, should vary with helper cell dose and perhaps independently of one another. Parallel variance of the IgE and IgG responses in relation to the number of helper T cells argues against, but does not rule out, the existence of two distinct T cell participants.

A/J irradiated recipients were injected with 30×10^6 syngeneic spleen cells from 8 wk-primed DNP-ASC donor mice. Two groups of such recipients received no additional cell transfer while nine groups received a second inoculum consisting of varying numbers of KLH-primed cells from donor mice primed 2 wk earlier with 10 μg of KLH administered either as an alum mixture or a CFA emulsion. With the exception of one group (challenged with 10 μg of DNP-ASC plus alum), all recipients were secondarily challenged with 2 μg of DNP-KLH plus alum and then bled 7 days later.

The results are summarized in Table II. Note that the magnitudes of both IgE and IgG anti-DNP antibody responses were appreciably higher when KLH-primed cells were developed with alum rather than CFA. However, the responses in both antibody classes tended to vary in parallel with one another in relation to the number of cells used and irrespective of the adjuvant employed for carrier-priming. Hence, this experiment argues against the involvement of different KLH helper T cells for both immunoglobulin classes which may be favored more or less by the type of adjuvant used for priming.

Elicitation of Adoptive Secondary Cooperative IgE Anti-DNP Antibody Responses by Administering Hapten and Carrier Determinants on Separate Molecules.—The capacity to elicit DNP-specific IgE antibody production in the first experiment (Fig. 1) by administering the appropriate haptenic and carrier determinants simultaneously but on separate molecules was investigated more extensively in the following series of experiments.

A summary of the protocol and results of one such experiment is illustrated in Fig. 4. In groups of mice that did not receive helper cells very good adoptive secondary anti-DNP responses of both IgE and IgG antibody classes were

TABLE II

Helper Activity of Varying Numbers of Carrier Specific Cells on IgE and IgG anti-DNP Antibody Responses

KLH(alum)-primed cells		Anti-DNP antibody		KLH(CFA)-primed cells		Anti-DNP antibody	
Group	No. of cells transferred	IgE	IgG	Group	No. of cells transferred	IgE	IgG
		*PCA**	*μg/ml*‡			*PCA**	*μg/ml*‡
I	None	<5	2.0	VI	1×10^6	20	44.3
II	6×10^6	160	683.3	VII	6×10^6	40	495.3
III	12×10^6	160	998.3	VIII	12×10^6	40	754.1
IV	25×10^6	320	2,283.5	IX	25×10^6	80	1,151.0
V	50×10^6	640	2,573.7	X	50×10^6	160	1,005.6

A/J irradiated recipients of 30×10^6 DNP-ASC primed cells received an additional transfer (except group I) of varying numbers of KLH primed cells from donors primed with KLH plus alum (groups II–V) or KLH in CFA (groups VI–X). All of the groups shown above were secondarily challenged with DNP-KLH. A control group of recipients of DNP-ASC cells alone that was challenged with DNP-ASC (not shown) developed IgE responses of 2,560 PCA titer and IgG responses of 2,488 μg/ml.

* The data are expressed as the reciprocal of the highest PCA titer elicited by pooled sera from five mice of each recipient group bled 7 days after secondary challenge.

‡ The data are expressed as geometric mean anti-DNP antibody levels of sera from individual mice in each group on day 7 after secondary challenge.

elicited by challenge with the homologous conjugate, DNP-ASC (group I), whereas no detectable responses were elicited by DNP-KLH alone (group II). On the other hand, simultaneous challenge with DNP-KLH together with unconjugated ASC incued a significant adoptive secondary anti-DNP response restricted as before (Fig. 2), to the IgE class (group IV). That this latter effect requires the administration of both of the relevant haptenic (DNP) and carrier (ASC) determinants is demonstrated by the failure to elicit IgE antibody production by challenging with unconjugated ASC alone (group III).

The capacity to elicit an adoptive secondary IgE response in this system by administering DNP-KLH together with ASC is clearly improved by the additional transfer of ASC-primed cells (groups V and VI). However, in contrast

to the observations in the preceding experiments in which carrier-primed cells from alum-primed donors were somewhat better helper cells for IgE antibody production than CFA-primed cells, the reverse appears to be the case in the conditions of the present experiment. Moreover, recipients of ASC (CFA)-primed helper cells manifested clear stimulation of IgG as well as IgE antibody production (group VI) which was statistically significant as compared to appropriate control (groups II–V). This latter observation was indeed surprising in view of the previous failure of others (20–22) including ourselves (23)[2] to demonstrate such a phenomenon for IgG antibody responses in vivo.

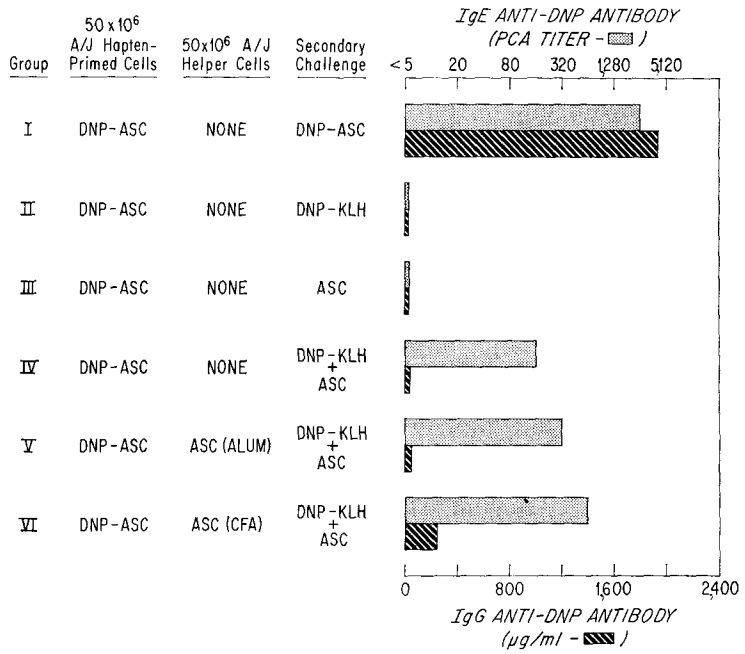

FIG. 4. Protocol, timing, etc. as in previous figures. DNP-ASC primed cells came from 5 wk-primed donors; ASC primed cells came from 6 wk-primed donors. Secondary challenge was made as indicated with either 10 μg DNP-ASC, 2 μg DNP-KLH, 10 μg unconjugated ASC, or a mixture of the latter two antigens. The IgG anti-DNP antibody responses in recipients in group VI were significantly higher ($0.02 > P > 0.01$) than those of recipients in either group IV or group V.

A second experiment of this basic design carried out in BALB/c mice is depicted in Fig. 5. The following points are noteworthy about the data: (*a*) There was a small but appreciable secondary IgE response to the heterologous conjugate (DNP-ASC) in the absence of transferred helper cells (groups II and VII) in BALB/c mice; this has not been routinely observed by us in A/J

[2] Katz, D. H., W. E. Paul, and B. Benacerraf. 1970. Unpublished observations.

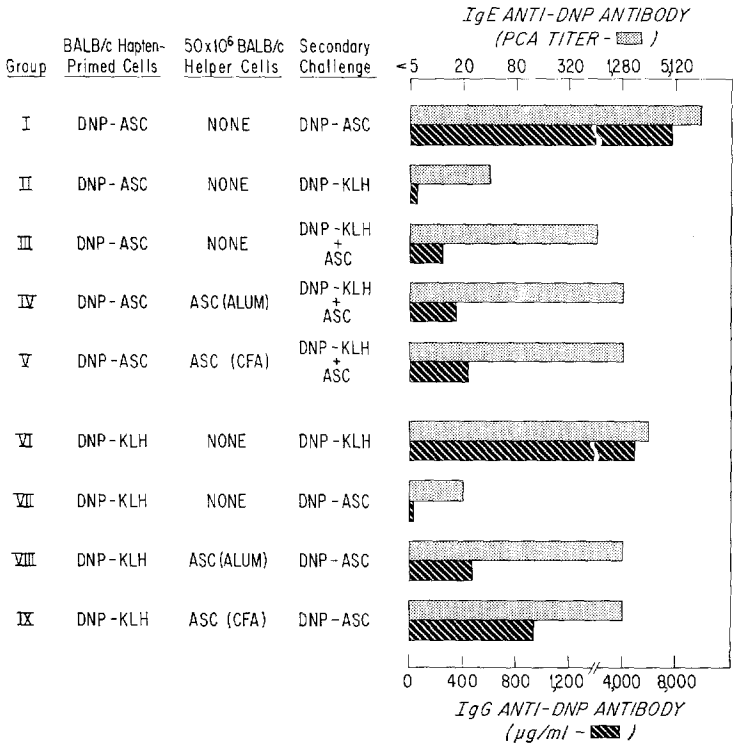

FIG. 5. Irradiated BALB/c mice received one or more donor cells intravenously as indicated. DNP-KLH- and DNP-ASC-primed cells came from 13 wk-primed donors; ASC (alum)- and ASC (CFA)-primed cells came from 2 wk-primed donors. 35×10^6 DNP-ASC and 20×10^6 DNP-KLH-primed donor cells were employed, respectively. Mice were challenged with the antigen or mixture of antigens shown (same doses as in Fig. 4) and then bled 7 days later. Serum anti-DNP IgE and IgG antibodies of groups of five mice are illustrated. Comparisons between IgG responses in various groups yielded the following P values: (*a*) group II vs. group III, $0.10 > P > 0.05$; (*b*) group II vs. group IV or group V, $0.005 > P > 0.001$ in both cases; (*c*) group VII vs. group VIII or IX, $0.005 > P > 0.001$ and $0.001 > P$, respectively; (*d*) group VIII vs. group IX, $0.30 > P > 0.20$.

strain mice. (*b*) The simultaneous challenge with DNP-KLH plus ASC induced secondary anti-DNP responses in both IgE and IgG antibody classes without additional helper cells (but relatively higher IgE than IgG response, group III). (*c*) The additional transfer of ASC-primed cells clearly improved both IgE and IgG anti-DNP responses whether or not the priming adjuvant employed had been alum or CFA (group IV and V). (*d*) The cooperative response to DNP-ASC obtained with mixtures of DNP-KLH and ASC-primed cells (groups VIII and IX) appeared to be less affected, in terms of IgE antibody production, by the type of adjuvant (alum or CFA) used for priming helper cells than was observed in A/J mice (cf. Fig. 1 and 3); the IgG anti-DNP

response, however, was about twofold higher in recipients of ASC (CFA) cells (group IX) than in recipients of ASC (alum) cells (group VIII).

A third experiment in this series, also performed in BALB/c mice, was designed to determine to what extent the duration of carrier-priming may play a role in the effects of T cells derived from CFA or alum-primed donors on IgE and IgG antibody production. The protocol and data are illustrated in Fig. 6. Cells from donors primed 2 or 10 wk earlier with KLH either with alum

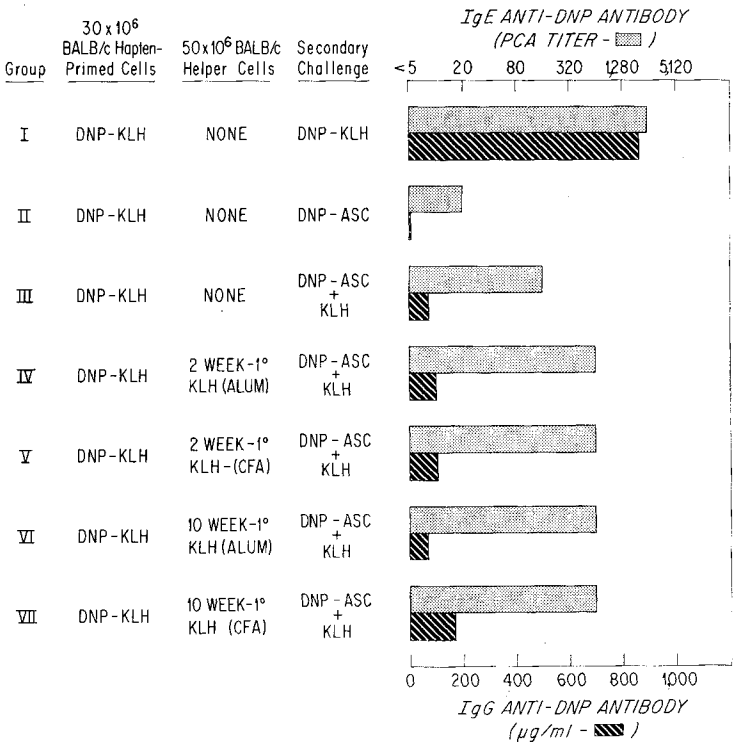

FIG. 6. Protocol similar to that of Fig. 5. Statistical comparisons of IgG anti-DNP antibody responses between group II and groups III–VII yielded P values of either $0.005 > P > 0.001$ or $0.001 > P$ in all cases. Each group consisted of five mice.

or in CFA were evaluated for their capacity to affect the secondary responses of DNP-KLH-primed cells elicited by the concomitant administration of DNP-ASC plus KLH. Again note the detectable IgE response to the heterologous conjugate alone, DNP-ASC (group II) and the clear increase in both IgE and IgG anti-DNP antibodies after challenge with DNP-ASC plus KLH without additional helper cells (group III). The addition of KLH-primed helper cells resulted in further increases in IgE responses to such challenge (groups IV–VII) but did not markedly improve the magnitude of IgG antibody pro-

duction (with the possible exception of group VII). The main point of this experiment is, however, that the transferred KLH-primed cells exerted comparable effects, which were at all times enhancing, regardless of either the duration of priming or the nature of adjuvant employed for such priming.

DISCUSSION

The present studies have established conditions for the demonstration of cooperative interactions between specific T and B lymphocyte populations in the development of IgE antibody responses in vivo in mice. This has been accomplished by utilizing a system which permits the successful adoptive transfer to irradiated recipients of DNP-specific secondary IgE responses with spleen cells from suitably primed syngeneic donor mice (9). Thus, adoptively transferred DNP-KLH or DNP-ASC-primed spleen cells produced high levels of anti-DNP antibodies of both IgE and IgG antibody classes in response to challenge with the appropriate homologous priming conjugate but failed to develop more than meager responses to the reciprocal heterologous conjugate. However, when spleen cells from donors primed to the second carrier were concomitantly transferred with hapten-primed lymphocytes, secondary IgE anti-DNP responses were consistently obtained upon challenge with the heterologous conjugate. Moreover, we have been able to elicit augmented primary IgE anti-DNP antibody responses to either DNP-ASC or DNP-KLH after adoptive transfer of spleen cells from donors primed only to the carrier, ASC or KLH, respectively.

This adoptive transfer system has enabled us to provide direct proof for the participation of T lymphocytes in antibody responses of the IgE class. Thus, the capacity of ASC-primed spleen cells to effectively cooperate with the DNP-KLH-primed lymphocytes in the adoptive secondary response to DNP-ASC could be abolished by in vitro treatment of such cells with anti-θ serum plus complement. This was true not only for the anti-DNP response of the IgG antibody class, as initially demonstrated by Raff in a similar system (24), but for the IgE antibody class as well. Although T cell participation in IgE antibody responses has been circumstantially implied by previous observations of others (2–8), it is perhaps relevant that we could demonstrate a comparable degree of sensitivity to anti-θ serum cytolysis of T cells operative in both the IgE and IgG class responses.

Certain implications arise from the results presented here concerning regulatory mechanisms involved in IgE and IgG antibody production, respectively. Principal among these is the very clear impression that IgE antibody responses, and, accordingly, the IgE B lymphocyte precursors, are inherently more sensitive to, at least, the enhancing effects of T cell functions. This tendency is exemplified by (*a*) the more readily demonstrable enhanced adoptive primary in the IgE vs. IgG class by carrier-primed cells, and (*b*) by the greater facility with which IgE anti-DNP antibody production could be elicited by con-

comitant administration of the DNP and relevant carrier determinants on separate molecules (Figs. 1 and 4–6). Thus, DNP-ASC-primed cells readily developed significant IgE anti-DNP responses upon challenge with DNP-KLH plus ASC. The elicitation of IgG antibody production under these conditions was variable at best, but could be appreciably improved (as was the IgE response) by the transfer of additional carrier (ASC)-primed cells. The latter observation clearly demonstrates, moreover, the importance of the relative frequency of the carrier-specific T cell in the development of such responses. It should be reiterated that previous attempts to elicit IgG anti-hapten responses in vivo by challenging with hapten and carrier determinants on separate molecules have been usually unsuccessful (20–23).[2] However, such responses have been demonstrated to occur for IgM and IgG production in vitro (25) and recently Kishimoto and Ishizaka have demonstrated such responses to occur in IgE as well as IgG and IgM anti-DNP antibody responses in vitro (8). Our ability to demonstrate IgG as well as IgE anti-DNP responses in vivo to carrier and hapten determinants on separate molecules in the present studies probably reflects the inherently strong immunogenicity of the carrier proteins employed (ASC and KLH).

If we interpret the easily demonstrable IgE responses to challenge with distinct determinants on separate molecules as indicative of greater sensitivity of IgE B cells to enhancing T cell effects, can we assume that such B cells are comparably more sensitive to suppressive T cell functions? Indeed, recent studies of Tada and his associates have demonstrated a striking suppressive effect of carrier-specific helper cells on IgE anti-DNP antibody production in rats (26, 27). The data obtained in the present studies do not permit any conclusions on this question since, as such, we failed to observe clear examples of suppression of the IgE anti-DNP response. What we did find were substantial differences in magnitude of responses which were, under certain conditions, related to the nature of the adjuvant employed for carrier-priming. Thus, CFA immunization resulted in a slight dissociation in the helper effect of ASC-primed T cells for IgE and IgG antibody production in the adoptive secondary response of DNP-KLH primed B cells to DNP-ASC (Figs. 1 and 3). Whereas such cells tended to promote higher IgG anti-DNP responses than ASC-primed cells from alum-immunized animals, the latter cells clearly favored higher IgE antibody responses. This was not the case with cells from KLH-primed donors, where alum immunization resulted in a high level of helper function for both IgE and IgG antibody production. In contrast, CFA immunization resulted in a lower level of KLH-specific helper cell function for both IgE and IgG precursor B cells (Fig. 2 and Table I).

Moreover, the above mentioned dissociation in helper function of ASC-primed cells for IgE and IgG responses to challenge with a single molecule containing both hapten and carrier determinants was no longer demonstrable in the experiments in which responses were elicited by concomitant administra-

tion of the two determinants on separate molecules. Thus, additional transfer of ASC-primed cells from donors immunized with CFA was at least as effective as transfer of ASC (alum)-primed cells in improving the IgE secondary response of DNP-ASC-primed cells to challenge with DNP-KLH plus ASC (Figs. 4 and 5). This was also true for KLH-specific helper T cells where it was further shown that, beyond 2 wk, duration of carrier-priming exerted no substantial difference in effect on the ultimate magnitude of IgE antibody production (Fig. 6). Perhaps of greatest significance in these latter experiments was the obvious lack of suppressive effects of such carrier-primed cells on IgE anti-DNP antibody production under the conditions employed.

Taken collectively, these results may be interpreted to indicate a lack of evidence for the existence of different T cells responsible for regulating IgE and IgG B cells, respectively. This interpretation could be based on the data cited above concerning effects of adjuvant immunization on helper cell function for either IgE or IgG anti-DNP responses. In terms of relative magnitude of IgE vs. IgG responses, the role of the adjuvant employed for carrier-priming appears to matter considerably for a given antigen (ASC) under one set of circumstances but not under others (i.e., challenge with two separate determinant moieties), whereas for another antigen, KLH, the role of adjuvant is consistent for the magnitude of both antibody classes. (In this respect, it is interesting to note that, incidental to what we've done in these studies, it became obvious that KLH is such an inherently strong antigen that its incorporation in CFA tends to diminish resulting helper cell function for both IgE and IgG antibody responses as compared to alum). Furthermore, the finding that the magnitudes of IgE and IgG anti-DNP antibody responses, varied in parallel with one another and independently of the mode of adjuvant immunization used for carrier priming as the ratio of helper T cells to DNP-specific B cells was shifted (Table II) argues against, but does not rule out the existence of distinct helper T cell populations for IgE and IgG B cells, respectively. Our interpretation of more sensitive IgE B cells must take into account the failure to see some dissociation between IgE and IgG responses at the lower doses of helper T cells transferred in the latter experiment. However, this could be explained by the fact that we employed high numbers (probably a great excess) of DNP-primed B cells. Experiments currently underway using limiting numbers of DNP-primed lymphocytes should resolve this question.

These interpretations appear at first glance to be somewhat at variance with the recent findings of Ishizaka and Okudaira from in vivo studies of anti-DNP IgE antibody responses in mice (7) and those of Kishimoto and Ishizaka in in vitro studies in rabbits (6, 8). In their in vivo mouse system, which did not involve adoptive cell transfers, they investigated the effects of supplemental or preimmunization with carrier protein on the subsequent IgE anti-DNP response of such mice to DNP coupled to the same carrier. Under certain dose conditions for carrier preimmunization, the IgE anti-DNP response

was suppressed whereas IgG anti-DNP and both IgE and IgG anticarrier antibody responses were normal or even accelerated (7). However, as the authors themselves pointed out, the role of circulating anticarrier antibodies could be very great in contributing to their observations and requires further study (7, 28). In the adoptive transfer system employed in the present studies, circulating anticarrier or antihapten antibodies are not present in the adoptive recipient at the time of antigen challenge and, therefore, cannot influence the ultimate result. This major difference in experimental design may well account for any apparent discrepancies in these and other data on IgE antibody production.

Finally, it is quite clear that despite the participation of T cell function in both IgE and IgG antibody responses, there are sufficiently overt inherent differences in the operation of regulatory influences on the respective B cell populations to warrant great interest and further intensive analysis. The principle issue, as we see it at present, is whether such differences in control mechanisms involved in these two antibody class responses reflect the existence of distinct classes of regulatory T cells and/or their products, or, rather, a wide difference of inherent sensitivity of the respective B cell populations to the same T cells and their product. This issue is not yet resolved, although we are currently inclined toward favoring an explanation based at least partially on demonstrated inherent differences in B cell sensitivity to the same T cell influences. Being cognizant of differences of IgE vs. IgG responses with different antigens, however, it will also be of critical importance to study the helper T cell generated by immunization with these molecules in order to grapple more specifically with these questions. Indeed, the experiments reported here were performed with antigens and modes of immunizations deliberately to promote high IgE responses, since our primary purpose was to demonstrate the positive role of helper T cells in IgE synthesis. Studies designed to explore the relative control of IgE vs. IgG responses and the possible existence of two types of T cells or cell products for these respective responses requires a different approach. To this end, an evaluation must be made of the respective properties of helper T cells generated by antigens and immunization known to favor responses in one or the other antibody classes and their effects on adoptive secondary IgE responses such as those employed in these studies.

SUMMARY

The present studies have established conditions for the demonstration of cooperative interactions between specific T and B lymphocyte populations in the development of IgE antibody responses in vivo in mice. This has been accomplished by utilizing a system which permits the successful adoptive transfer to irradiated recipients of DNP-specific secondary IgE responses with spleen cells from suitably primed syngeneic donor mice. Thus, adoptively transferred DNP-KLH or DNP-ASC-primed spleen cells produced high levels

of anti-DNP antibodies of both IgE and IgG antibody classes in response to challenge with the appropriate homologous priming conjugate but failed to develop more than meager responses to the reciprocal heterologous conjugate. However, when spleen cells from donors primed to the second carrier were concomitantly transferred with hapten-primed lymphocytes, secondary IgE ant-DNP responses were consistently obtained upon challenge with the heterologous conjugate. Moreover, we have been able to elicit augmented primary IgE anti-DNP antibody responses to either DNP-ASC or DNP-KLH after adoptive transfer of spleen cells from donors primed only to the carrier, ASC or KLH, respectively.

This adoptive transfer system has enabled us to provide direct proof for the participation of θ-bearing T lymphocytes in antibody responses of the IgE class. Thus, the capacity of ASC-primed spleen cells to effectively cooperate with the DNP-KLH-primed lymphocytes in the adoptive secondary response to DNP-ASC could be abolished by in vitro treatment of such cells with anti-θ serum plus complement. This was true not only for the anti-DNP response of the IgG antibody class, but for the IgE antibody class as well.

These studies have, furthermore, demonstrated the capacity to stimulate secondary anti-DNP antibody production in vivo by the concomitant administration of the DNP and relevant carrier determinants on separate molecules. This was more readily seen in the IgE than in the IgG antibody class. Thus, DNP-ASC-primed cells developed significant IgE, but more variable IgG, anti-DNP responses upon challenge with DNP-KLH plus unconjugated ASC. Antibody responses of both classes elicited in this manner were appreciably improved by the transfer of additional carrier (ASC)-primed cells. These and other results presented herein suggest that IgE B lymphocyte precursors may be inherently more sensitive than IgG B cells to at least certain of the functions of T lymphocytes concerned with regulatory mechanisms involved in antibody production.

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REFERENCES

1. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15:**1.
2. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J. Immunol.* **106:**1019.
3. Tada, T., and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. V. Cell Cooperation in the anti-hapten homocytotropic antibody response. *J. Immunol.* **107:**1137.

4. Tada, T., K. Okumura, and M. Taniguchi. 1972. Regulation of homocytotropic antibody formation in the rat. VII. Carrier functions in the anti-hapten homocytotropic antibody response. *J. Immunol.* **108:**1535.
5. Kishimoto, T., and K. Ishizaka. 1972. Regulation of antibody response *in vitro*. III. Role of hapten-specific memory cells and carrier specific helper cells on the distribution of anti-hapten antibodies in IgG, IgM and IgE classes. *J. Immunol.* **109:**612.
6. Ishizaka, K., and T. Kishimoto. 1973. Cellular basis of reaginic antibody formation *in vitro*. *In* Control Mechanisms in Reagin-mediated Hypersensitivity. L. Goodfriend, editor. Marcel Dekker, Inc., New York.
7. Ishizaka, K., and H. Okudaira. 1973. Reaginic antibody formation in the mouse. II. Enhancement and suppression of anti-hapten antibody formation by priming with carrier. *J. Immunol.* **110:**1067.
8. Kishimoto, T., and K. Ishizaka. 1973. Regulation of antibody response *in vitro*. VI. Carrier specific helper cells for IgG and IgE antibody response. *J. Immunol.* In press.
9. Hamaoka, T., D. H. Katz, K. J. Bloch, and B. Benacerraf. 1973. Hapten-specific IgE antibody responses in mice. I. Secondary IgE responses in irradiated recipients of syngeneic primed spleen cells. *J. Exp. Med.* **138:**306.
10. Strejan, G., and D. H. Campbell. 1967. Hypersensitivity to Ascaris antigens. I. Skin-sensitizing activity of serum fractions from guinea pigs sensitized to crude extracts. *J. Immunol.* **98:**893.
11. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. Exp. Med.* **132:**261.
12. Benacerraf, B., and B. B. Levine. 1962. Immunological specificity of delayed and immediate hypersensitivity reactions. *J. Exp. Med.* **115:**1023.
13. Levine, B. B., and N. M. Vaz. 1970. Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagen production in the mouse. *Int. Arch. Allergy.* **39:**156.
14. Katz, D. H., and D. P. Osborne, Jr. 1972. The allogeneic effect in inbred mice. II. Establishment of the cellular interactions required for enhancement of antibody production by the graft-vs.-host reaction. *J. Exp. Med.* **136:**455.
15. Bloch, K. J. 1967. The anaphylactic antibodies of mammals including man. *Progr. Allergy.* **10:**84.
16. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. *J. Infect. Dis.* **103:**329.
17. Green, I., B. Benacerraf, and S. H. Stone. 1969. The effect of the amount of mycobacterial adjuvants on the immune response of strain 2, strain 13, and Hartley strain guinea pigs to DNP-PLL and DNP-GL. *J. Immunol.* **103:**403.
18. Unanue, E. R., and J. C. Cerottini. 1970. The immunogenicity of antigen bound to the plasma membrane of macrophages. *J. Exp. Med.* **131:**711.
19. Mitchison, N. A. 1969. Cell populations involved in the immune response. *In* Immunological Tolerance. M. Landy and W. Braun, editors. Academic Press. Inc., New York. 149.

20. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* **1:**18.

20a. Rajewsky, K., V. Schirrmacher, S. Nase, and N. K. Jerne. 1969. The requirement of more than one antigenic determinant for immunogenicity. *J. Exp. Med.* **129:**1131.

21. Hamaoka, T., K. Takatsu, and M. Kitagawa. 1971. Antibody production in mice. IV. The suppressive effect of anti-hapten and anti-carrier antibodies on the recognition of hapten-carrier conjugate in the secondary response. *Immunology.* **21:**259.

22. Kettman, J., and R. W. Dutton. 1971. Radioresistance of the enhancing effect of cells from carrier-immunized mice in an *in vitro* primary immune response. *Proc. Natl. Acad. Sci. U.S.A.* **68:**699.

23. Unanue, E. R., and D. H. Katz. 1973. Immunogenicity of macrophage-bound antigens: The requirement for hapten and carrier determinants to be on the same molecule for T and B lymphocyte collaboration. *Eur. J. Immunol.* In press.

24. Raff, M. C. 1970. Role of thymus-derived lymphocytes in the secondary humoral immune response in mice. *Nature* (*Lond.*). **226:**1257.

25. Sulica, A., R. Tarrab, and M. Sela. 1971. Stimulation *in vitro* with protein carrier of antibodies against a hapten. *Science* (*Wash. D.C.*). **170:**1108.

26. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. *J. Immunol.* **107:**1682.

27. Tada, T., K. Okumura, M. Taniguchi. 1973. Regulation of homocytotropic antibody formation in the rat. VIII. An antigen-specific T cell factor that regulates anti-hapten homocytotropic antibody response. *J. Immunol.* In press.

28. Hamaoka, T., K. Takatsu, and M. Kitagawa. 1973. Antibody production in mice. V. The suppressive effect of anti-carrier antibodies on cellular cooperation in the induction of secondary anti-hapten antibody responses. *Immunology.* **24:**409.