ANTIBODY-SPECIFIC IMMUNOREGULATION*

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In recent years much evidence has accumulated which demonstrates that an animal's immune system has the capacity to recognize its own antibody idiotypes. These findings suggest a potential role for self-idiotypic recognition in controlling B-cell antibody responses (1–3). In this report we describe experiments designed to test the postulate that subsequent to primary immunization and concomitant with an initial antibody response to an antigen an animal develops the ability to specifically regulate the synthesis of antibodies specific for the stimulating antigen. This regulation may be envisaged as a means by which the immune system specifically limits the expression of antigen-specific B cells once an adequate humoral immune response has ensued.

The kinetics of appearance of antigen-specific antibody in the serum of immunized animals after primary immunization has been well documented (4–7). Although the antibody synthesized is specific for the stimulating antigen, for most antigens the antibody population is extremely heterogeneous. This heterogeneity is a reflection of the heterogeneity of the B-cell repertoire in that each B cell synthesizes antibody of a single specificity (4, 8–12). On this basis one would predict that, if an immunoregulatory mechanism does indeed develop after a primary antibody response, it would be highly specific for the B cells synthesizing the particular antibodies induced during the primary immune response. Thus, antibody responses to unrelated antigens would be unaffected in the immunized animal. Considering the heterogeneity of the initial antibody response, this regulation would presumably involve the recognition of individual antibody-producing B cells.

A further consideration which must be made is that secondary antibody responses, by definition, occur in previously immunized animals. This would imply that the population of secondary B cells which is generated as a consequence of primary immunization (4, 13, 14) is not susceptible to immunoregulatory mechanisms elicited in an individual during a primary antibody response. If a regulatory mechanism does involve the specific recognition of B cells synthesizing antibody elicited during primary immunization one might predict that secondary B cells would be susceptible to this regulation as evidence exists which suggest that secondary B cells may, in part, express the same antibody idiotypes as the B cells from nonimmune animals (15, 16). However, secondary B cells differ from primary B cells in many characteristics of their stimulation and tolerance induction (4, 14, 16) and these differences may explain their ability to be stimulated in an environment which suppresses primary B-cell antibody synthesis.

We have employed the in vitro splenic focus fragment culture system to investigate whether a specific immunoregulation develops after immunization (4, 17). The results indicated that only 25–35% of dinitrophenyl (DNP)[1]-specific

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CH, constant region of the immunoglobulin heavy chain; DNP, 2,4-dinitrophenyl; FL, fluorescein; Hy, hemocyanin; NIP, 4-hydroxy-3-iodo-5 nitrophenyl; NP, 4-hydroxy-3-nitrophenyl; TNP, 2,4,6-trinitrophenyl; VH, variable region of the immunoglobulin heavy chain.

primary B cells which responded to DNP-hemocyanin (Hy) in recipients immunized with Hy alone could be stimulated by DNP-Hy in recipients previously immunized with DNP-Hy. This inhibition appeared to affect exclusively DNP-specific primary B cells expressing the recipients allotype-linked locus immunoglobulin genes and did not affect secondary DNP-specific B-cell responses.

Materials and Methods

Proteins and Haptenated Proteins. *Limulus polyphemus* Hy was purchased from Worthington Biochemical Corp., Freehold, N. J. The preparation of 2,4-dinitrophenylated-hemocyanin (DNP_{10}-Hy, 10 mol of DNP per 100,000 g of Hy) and 2, 4, 6-trinitrophenylated-hemocyanin (TNP_{10}-Hy) have been previously described (4). Fluoresceinated hemocyanin (FL_{10}-Hy) was prepared as previously described (18). The preparation of 4-hydroxy-3-nitrophenylated-Hy (NP_{10}-Hy) and 4-hydroxy-3-iodo-5-nitrophenylated-bovine serum albumin (NIP_{10}-BSA) has been described elsewhere (19). DNP_{10}-BSA, TNP_{10}-BSA, and FL_{10}-BSA were prepared as described elsewhere (4, 18).

Animals. 6- to 8-wk-old BALB/cAnN mice were acquired through the Institute for Cancer Research, Philadelphia, Pa. B10.D2 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. CB20 mice were supplied by Dr. Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md., through the Litton Bionetics, Kensington, Md. breeding colony operating under National Cancer Institute contract no. NO1-CB-92142.

Immunizations. BALB/c and B10.D2 mice used as carrier-primed recipients of cell transfers received an intraperitoneal injection of 0.1 mg Hy in complete Freund's adjuvant (CFA) 5–8 wk before use. Animals used as hapten-Hy-primed recipients were immunized in one of two ways. Mice received either a single intraperitoneal injection of a mixture of 0.1 mg Hy and 0.1 mg hapten-Hy in CFA 5–8 wk before use or 0.1 mg Hy in CFA in a single intraperitoneal injection 5–8 wk before use followed by a separate injection of 0.1 mg hapten-Hy in CFA 2 wk before use. Groups of animals immunized in these two ways were used interchangeably in the studies presented since results of initial studies demonstrated no difference in the immunoregulatory capacity of mice immunized by either regimen. Animals used as donors of secondary B cells were injected with 0.1 mg DNP_{10}-Hy in CFA intraperitoneally 5–8 wk before use.

Cell Transfers and Fragment Cultures. The methodology for obtaining monoclonal antibody responses in splenic fragment cultures has been previously described (4, 17). Briefly, spleens from immunized or unimmunized mice were prepared in a single cell suspension by perfusion of the spleen with Dulbecco's modified Eagles medium. Appropriate numbers of cells from these suspensions were injected intravenously into carrier-primed or hapten carrier-primed recipients which had been irradiated at 1,300 rad 6 h earlier. Fragment cultures of recipient spleens were prepared 16 h after cell transfer and fragments were individually stimulated with the appropriate hapten-Hy conjugate at a hapten concentration of 10^{-6} M. Anti-DNP, anti-TNP, anti-FL, and anti-NP antibodies were detected in culture fluids collected 9 and 12 days after in vitro stimulation by using a modification (16) of a solid phase radioimmunoassay described by Catt and Tregar (1, 20). DNP_{10}-BSA, TNP_{10}-BSA, FL_{10}-BSA, or NIP_{10}-BSA were used to coat wells of disposable polyvinyl chloride microtiter plates, Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va. Antibodies to DNP, TNP, and FL in culture fluids were detected in this assay in microtiter plates coated with the homologous hapten-BSA conjugates. Anti-NP antibodies were detected by assaying culture fluids in microtiter plates coated with NIP_{10}-BSA. Culture fluids were quantitatively assayed for anti-hapten antibody by using ^{125}I-labeled anti-mouse Fab, $IgG_1(\gamma_1)$, or IgM (μ) antibodies as detecting reagents (4, 21).

Results

The Effect of Hapten-Carrier Immunization on Primary B-Cell Responses. Previous reports have indicated that antibody specific for DNP can be detected in the serum of mice approximately 3–5 days after immunization with DNP-Hy (6, 7). The anti-DNP antibody concentration in the serum then increases dramatically from the 2nd to 3rd wk postimmunization and reaches maximum levels of 0.1–0.2 mg/ml during this period. The concentration of serum

TABLE I

Primary Anti-DNP-Specific Donor B-Cell Responses in Hy and DNP-Hy Immunized Recipient Mice at Various Times after Recipient Immunization

No. of weeks postimmunization with DNP-Hy before cell transfer*	Total no. donor cells analyzed × 10^{-6}‡	No. positive foci per 10^6 spleen cells transferred§	Response detected in recipients immunized with Hy alone‖
			%
Unimmunized	48	1.83	–
2	32	0.44	24.0
4	16	0.25	13.7
8	32	0.75	41.0
18	16	0.50	27.3
22	8	0.50	27.3

* All donor and recipient mice were of the BALB/c strain. All donor mice were immunized with 0.1 mg Hy in CFA 8–22 wk before cell transfer.

‡ Each recipient received 4×10^6 donor BALB/c spleen cells.

§ All cultures were stimulated in vitro with DNP-Hy at a DNP concentration of 10^{-6} M. Positive foci were detected by assaying culture fluids for anti-DNP antibody.

‖ This number represents the percent of positive foci detected in Hy-primed recipient mice which were detected in DNP-Hy + Hy immunized recipient mice.

anti-DNP antibody decreases gradually thereafter and anti-DNP antibody is barely detectable 8 wk after immunization (6). The experiments to be presented in this report were initiated to determine whether during the course of such a primary immune response there is a progressive change in the environment of the immune host which specifically affects the responses of primary B cells.

The in vitro fragment culture system was employed to compare primary donor B-cell responses to DNP-Hy in DNP-Hy immunized recipients at various times after immunization to those of the same donor cell population transferred to recipients immunized with Hy alone. In the splenic fragment culture system, primary B-cell responses to hapten-carrier conjugates are absolutely dependent on the carrier-priming of the recipient mouse (4). Therefore, all DNP-Hy immunized mice were also immunized with nonhaptenated Hy to insure carrier recognition for other hapten-Hy conjugates in the recipients spleens. The results shown in Table I indicate that the number of DNP-specific primary B cells responding in fragment cultures derived from recipients immunized with DNP-Hy was significantly decreased as compared to the number of DNP-specific B cells responding in spleen fragments of recipients immunized with Hy alone. This decrease was observed as early as 2 wk after immunization when serum anti-DNP antibody concentrations in recipients were at a maximum, to as late as 8–22 wk after immunization when anti-DNP antibody was barely detectable in the serum of recipient mice.

A summary of the results obtained by using a variety of haptens coupled to Hy for both in vivo immunization and in vitro stimulation is presented in Table II. As shown, only 29.5% of all DNP-specific primary B cells produced detectable DNP-specific antibody in DNP-Hy immunized BALB/c mice as compared to the same donor cells in Hy immunized recipient mice. Similar results were obtained by using hapten-Hy conjugates other than DNP-Hy (such as TNP-Hy and FL-Hy) for recipient immunization and in vitro stimulation.

TABLE II

Primary Anti-Hapten-Specific Donor B-Cell Responses in Recipient Mice Immunized with Either Carrier or Hapten Carrier

Recipient immunization*	In vitro stimulating hapten-Hy conjugate‡	No. donor cells analyzed × 10^{-6}	No. positive foci per 10^6 spleen cells transferred§	Response in Hy-primed recipients‖
				%
Hy	DNP –Hy	48	1.83	–
DNP –Hy +Hy	DNP –Hy	52	0.54	29.5
Hy	TNP –Hy	20	1.85	–
TNP –Hy +Hy	TNP –Hy	20	0.55	29.7
Hy	FL –Hy	16	1.40	–
FL –Hy +Hy	FL –Hy	24	0.50	35.7

* Donor and recipient mice were of the BALB/c strain. Recipient mice received 4 × 10^6 donor spleen cells. Recipients were primed as described in Materials and Methods section.

‡ All in vitro cultures stimulated with hapten-Hy conjugates at 10^{-6} M hapten.

§ Positive foci were detected by assaying culture fluids for antibody specific for the in vitro stimulating hapten.

‖ This number represents the percent of positive foci detected in Hy immunized recipient mice which were detected in hapten-Hy + Hy immunized recipients.

The responses of primary B cells to other haptens coupled to Hy in DNP-Hy and TNP-Hy immunized recipients were tested and the results are presented in Table III. As shown, the frequencies of anti-FL primary B-cell responses in DNP-Hy immunized recipients and anti-NP primary B-cell responses in TNP-Hy immunized recipients were, if anything, slightly enhanced as compared to responses of the same donor cell population in recipients primed with Hy alone. Preliminary experiments not presented have indicated that the frequency of DNP-specific primary B-cell responses may be nearly equivalent in TNP-Hy and Hy immunized recipient mice. These results indicate that the decrease in the frequency of DNP-specific B-cell responses in DNP-Hy immunized recipients can not be attributed to the lack of adequate Hy-specific T-cell help since responses to heterologous haptens coupled to Hy were not diminished in these recipients. Thus, the inability of primary B cells to respond to hapten-Hy conjugates in recipient mice which have been previously immunized to that hapten coupled to Hy appear to be the result of a specific, irradiation resistant regulation of primary B-cell responses present in the immunized recipient.

The Target of the Immunoregulation. Experiments were carried out to identify the gene products of primary B cells which served as targets of this suppressive phenomenon. Appropriate mouse strain combinations were selected so that donor and recipient strains shared genes in the major histocompatibility complex of the mouse (*H-2*), thus maximizing primary B-cell responsiveness (22–24) but differed, to various degrees, in genes outside that complex. The results of these experiments, summarized in Table IV, demonstrate that if donor and recipient strains shared only genes within the *H-2* complex and differed in all genes outside that complex, as in the BALB/c and B10.D2 combination, the frequency of DNP-specific primary B-cell responses was equivalent in Hy and

TABLE III

The Hapten-Specific Responses of Donor Cells in Recipient Mice Immunized with Carrier or Hapten Carrier Complexes

Recipient priming*	In vitro antigen‡	No. cells analyzed × 10^{-6}	No. positive foci per 10^6 cells transferred§	Response in Hy-primed recipients‖
				%
Hy	DNP −Hy	48	1.80	−
DNP −Hy +Hy	DNP −Hy	54	0.54	29.3
Hy	FL −Hy	32	1.40	−
DNP −Hy + Hy	FL −Hy	32	1.50	107.1
Hy	NP −Hy	60	0.25	−
TNP −Hy +Hy	NP −Hy	60	0.30	125.0

* All donor and recipient mice are of the BALB/c strain. 4 × 10^6 donor cells were transferred to each recipient stimulated with FL-Hy or DNP-Hy in vitro and 10 × 10^6 cells were transferred to each recipient stimulated with NP-Hy in vitro.

‡ All cultures were stimulated in vitro with hapten Hy conjugates at 10^{-6} M hapten.

§ Positive foci were detected by assaying culture fluids for antibody specific for the in vitro stimulating hapten.

‖ This number represents the percent of positive foci detected in Hy immunized recipient mice which were detected in hapten-Hy + Hy immunized recipients.

TABLE IV

The Identification of the Genetic Region Associated with the Suppression of Primary B-Cell Responses

Donor*	Donor allotype‡	Recipient	Recipient allotype‡	Recipient priming	No. cells analyzed × 10^{-6}	No. anti-DNP positive foci per 10^6 cells transferred§	Response in Hy-primed recipients
							%
BALB/c	a^1	BALB/c	a^1	Hy	48	1.83	−
BALB/c	a^1	BALB/c	a^1	DNP −Hy +Hy	52	0.54	29.5
B10.D2	a^2	BALB/c	a^1	Hy	32	1.88	−
B10.D2	a^2	BALB/c	a^1	DNP −Hy +Hy	32	2.00	106.4
CB20	a^2	BALB/c	a^1	Hy	16	1.87	−
CB20	a^2	BALB/c	a^1	DNP −Hy +Hy	16	2.00	106.9

* 4 × 10^6 donor cells were transferred to each recipient. All donor and recipient strains are of the *H-2*^{d} haplotype (26).

‡ Allotype according to Leiberman et al. (25).

§ All cultures were stimulated in vitro with DNP-Hy at 10^{-6} M DNP. Positive foci were detected by assaying culture fluids for DNP-specific antibody.

DNP-Hy immunized recipients. Further, if donor and recipient strains were identical with the exception of genes which code for immunoglobulin heavy chains, as in the BALB/c and CB20 strain combination (25), the frequency of donor anti-DNP specific B-cell responses in DNP-Hy-primed recipients was equivalent to the frequency in Hy-primed recipients. Although not shown in

Table IV, similar results were obtained by using B10.D2 and CB20 mice as recipients of BALB/c donor cells. Thus, it appears that, after immunization with a hapten-carrier complex, a regulatory mechanism is established which affects only hapten-specific B cells which respond to the hapten with heavy chains common to the recipient strain. It should be noted that the unimpaired responsiveness of B10.D2 and CB20 DNP-specific primary B cells in DNP-Hy immunized BALB/c recipients implies that the regulation is unlikely the result of a nonspecific suppression mediated by recipient antibody specific for DNP-Hy.

The Effect of Recipient Hapten-Carrier Immunization on Secondary B-Cell Responses. As a consequence of primary immunization with T-dependent antigens, animals appear better equipped to respond to the same antigen upon reimmunization. After secondary immunization, serum antibody concentrations reach higher levels earlier and persist in the serum longer than after primary immunization (4–7). This change in the kinetics of the humoral response has been related in part to the generation of a population of secondary B cells (4, 13, 14). Since it is clear that these secondary B cells are able to respond in vivo to DNP-Hy in the milieu of the DNP-Hy immunized animal, it was of interest to determine if hapten-specific secondary B cells would be fully responsive in the fragment cultures derived from hapten-carrier-primed recipients. The results shown in Table V demonstrate that spleen cells from BALB/c mice previously immunized to DNP-Hy responded in approximately the same frequency when transferred to Hy-primed or DNP-Hy-primed recipients. This was also true for spleen cells from FL-Hy immunized BALB/c mice transferred to either Hy or FL-Hy immunized BALB/c recipients. It thus appears that secondary B cells are not susceptible to the regulatory mechanism established after a primary response to T-dependent antigens which affects primary B-cell responses.

Discussion

Based on the assumption that the induction and progression of the humoral immune response is a specifically regulated biological process, several investigators have suggested theories of immunoregulation and tested these experimentally. Many models of immunoregulation propose a regulatory role for serum antibody specific for determinants of the stimulating antigen and antigen antibody complexes in both the stimulation and inhibition of B-cell responses to these particular determinants (27, 28). Generally, in these models, the level of serum antibody modulates the immune response by a feedback mechanism which is dependent on the concentration of antigen antibody complexes or the availability of unbound antigenic determinants. More recently, it has been proposed that regulation of the humoral immune response may be mediated directly through the specific recognition of the idiotypic determinants of the surface receptors of the antigen-specific responding B cells (1–3, 15, 29, 30). The rationale for this model is based on the fact that most antigens elicit antibodies bearing many different idiotypic specificities and it is thought that selective regulation of these idiotypes might be mediated better through anti-idiotypic recognition than through antigen.

The experiments presented here were carried out to determine whether immunized animals, after or during a primary humoral immune response,

TABLE V

Secondary Anti-Hapten-Specific B-Cell Responses in Recipients Immunized with Carrier or Hapten-Carrier

Donor immunization*	Recipient immunization	In vitro antigen	No. cells analyzed × 10^{-6}	No. positive foci per 10^6 cells transferred‡	Response in Hy-primed recipients
					%
DNP −Hy	Hy	DNP −Hy	16	6.94	–
DNP −Hy	DNP −Hy +Hy	DNP −Hy	16	6.78	97.7
FL −Hy	Hy	FL −Hy	8	2.62	–
FL −Hy	FL −Hy +Hy	FL −Hy	8	2.25	85.9

* All donor and recipient mice are of the BALB/c strain. 2×10^6 donor cells were transferred to each recipient.

‡ Positive foci detected by assaying culture fluids for antibody specific for the in vitro stimulating hapten.

develop the capacity to specifically regulate the response of primary B cells to the immunizing antigen. To this end, BALB/c mice were immunized with either Hy alone or DNP-Hy in addition to Hy and used as recipients of donor primary B cells in the splenic fragment culture system. By immunizing recipients with DNP-Hy and Hy and comparing results obtained in these recipients to those obtained in recipients immunized with Hy alone, it was possible to assess the effect of DNP-Hy immunization on primary anti-DNP responses in a splenic environment which provides carrier-specific help (4). The results demonstrate that for weeks after immunization with DNP-Hy, immunized mice were not capable of enabling the responses of most transferred DNP-specific primary B cells. This nonpermissive environment for primary anti-DNP B-cell responses was specific for the immunizing hapten since FL- and NP-specific primary B-cell responses were unimpaired in DNP-Hy or TNP-Hy immunized recipients, respectively, as compared to Hy-primed recipient mice. By selecting appropriate donor recipient strain combinations, it was possible to demonstrate that genes or gene products of the immunoglobulin heavy chain locus played an essential role in determining if primary B cells were subject to this suppression.

Thus, it would appear that during an initial humoral immune response to a T-dependent antigen, a regulatory mechanism is induced which specifically limits the stimulation of hapten-specific primary B cells. The studies reported here have characterized several aspects of this immunoregulation. (*a*) The suppression is irradiation resistant. (*b*) The suppression is specific for responses to the inducing hapten and may discriminate between very similar cross-reacting haptens. (*c*) The regulatory process is evident only in immunized individuals and acts only on primary B cells and specifically those which share genes coding for immunoglobulin heavy chains with the immunized recipient mouse. (*d*) The lack of response of primary B cells is not due to the absence of appropriate carrier-specific T-cell help since other haptens on the same carrier induce primary responses. (*e*) Maintenance of the suppression does not appear to be dependent on the presence of anti-DNP antibody in the serum of recipient mice.

It is possible, however, that anti-DNP antibody, as a serum product or as receptors of responding DNP-specific B cells, may have been essential for the induction of this regulatory phenomenon.

In postulating a mechanism by which DNP-Hy immunized recipients are capable of specifically regulating primary B-cell stimulation two points are critical: that the suppression be specific and that it pertain only to B cells expressing the same immunoglobulin genes as the recipient. We have not demonstrated that this regulation is idiotype specific, in that the available genetic data could indicate that either VH or CH genes may provide targets of this immunoregulation. However, knowing that the regulation is antibody specific, the specificity of the regulation for the response to the immunizing hapten might argue that this is a regulation mediated through specific idiotype recognition.

Much evidence has accumulated which attests to the ability of antibody to specific immunoglobulin idiotypes or allotypes to suppress or stimulate B cells and thus regulate responses of B cells bearing the particular idiotype or allotype (29, 31, 32). These findings have led Rodkey, Köhler, and Jerne to propose regulatory networks of antibody idiotypes and anti-idiotypes interacting to modulate or regulate immune responses (1–3). However, the immunoregulatory capacity of anti-idiotypic reactivity has generally been observed experimentally in vivo only after passive transfer of anti-idiotypic antibody to animals which respond to a particular antigen with antibody of that idiotype (29, 33). Attempts to generate anti-idiotypic antibody in animals which respond to antigens with that idiotype have succeeded only after multiple repeated immunization with the antigen or by immunization with chemically modified or polymerized antigens (1, 34, 35). No clear evidence therefore has previously existed which establishes a role for anti-idiotypic reactivity in regulation during the normal course of an immune response after conventional immunization.

The exact mechanisms by which anti-idiotypic recognition regulates B-cell responsiveness is unknown, but any of several identifiable modes of cell receptor-anti-receptor interaction may be responsible for the antibody-specific immunoregulation presented in this report. It is possible, for example, that the homing pattern of transferred idiotype-bearing cells in hapten-carrier immunized recipients is altered by anti-idiotypic recognition. In addition, anti-idiotypic antibody has been shown capable of blocking B-cell receptor binding to antigen thus inducing an acute B-cell suppression (31). Long-term B-cell suppression specific for receptor allotype or idiotype induced by the administration of anti-allotypic or anti-idiotypic antibody has been shown to depend on the generation of allotype or idiotype-specific T cells (29, 32, 36). Understanding which of these various mechanisms pertains to the antibody-specific immunoregulation described here may be of great biological interest since this immunoregulation is induced as the result of a conventional primary humoral immune response rather than the passive transfer of antibody.

A final aspect of the observed phenomenon which warrants discussion is the relative nonsusceptibility of secondary B cells to this suppression. There is evidence to suggest that secondary B cells share receptor idiotypes with the primary B-cell repertoire (15, 16). If this is indeed the case, secondary B cells should also be recognized by this antibody-specific regulation. However, several

laboratories, including this one, have demonstrated that, as a population, secondary B cells are characteristically different from the B cells of nonimmune animals, particularly with respect to the ease with which they can be antigenically stimulated (4, 14, 16). This may explain the stimulation of secondary B cells in an environment which is nonpermissive for primary B-cell stimulation. More importantly, this difference between primary and secondary B cells may have enormous biological relevance in that the existence of secondary B cells may provide the opportunity for the regulation of primary B-cell responses while enabling the animal to respond to the same antigen upon restimulation. In this light one could postulate that, if antigen stimulated only the regulatory limb of the immune response during a primary immunization without generating secondary B cells, animals could be rendered functionally tolerant to the antigen.

These findings have had important practical applications in allowing primary B-cell responses to antigens for which carrier molecules are not readily available. Primary B-cell responses in the fragment culture system are absolutely dependent on carrier priming of the recipient mouse (4, 16, 17). In the past it was not possible to obtain carrier-specific help for primary in vitro responses to viruses and bacteria by immunizing recipients with the particular virus or bacterium. These experiments not only offer a possible explanation for why this was the case since primary B cells specific for the same determinants would have been suppressed but, more importantly, the difficulty can now be bypassed by selecting the appropriate strain combinations for analysis so that donor and recipient strains do not share immunoglobulin heavy chain genes. Employing this method we have been able to generate primary B-cell responses to a variety of complex antigens including varius strains of influenza virus.

Summary

In recent years, much evidence has accumulated which demonstrates that an animal's immune system has the capacity to recognize its own antibody idiotypes. These findings suggest that self-idiotypic recognition may potentially play a role in the regulation of B-cell responses. The experiments presented in this report were carried out to determine if an animal develops the ability to specifically regulate the synthesis of antibodies specific for an antigen, subsequent to primary immunization to the particular antigen and concomitant with an initial antibody response. Employing the splenic fragment culture system we have compared the response of primary donor B cells in irradiated recipients which have been previously immunized to hemocyanin (Hy) alone or dinitrophenyl (DNP)-Hy plus Hy. The results indicated that only 25–30% of DNP-specific B cells stimulated by DNP-Hy in Hy immunized recipients could be stimulated by DNP-Hy in recipients immunized with Hy as well as DNP-Hy. B-cell responses to other haptens, such as fluoresceinated-Hy, and secondary DNP-specific B-cell responses were unaffected in DNP-Hy immunized animals. The nontrivial and specific nature of the observed decrease in primary DNP-specific B-cell responses was verified by the finding that the response of CB20 donor cells, which differ from BALB/c mice only in the immunoglobulin heavy chain allotype-linked locus, was unaffected in BALB/c recipient mice which had been immunized with DNP-Hy. Thus, it appeared that during a primary humoral immune response to a T-dependent antigen, an antibody-specific regula-

tory mechanism is induced which specifically limits the stimulation of hapten-specific primary, but not secondary, B cells. The important implications that these findings have for the understanding of the control of primary B-cell responses and the generation of secondary B cells is discussed.

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