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Antibody Production in Mice

VI. EFFECT OF ANTI-CARRIER ANTIBODY ON CELLULAR CO-OPERATION IN THE PRIMARY ANTI-HAPTEN ANTIBODY RESPONSE

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Summary. We studied the effect of passively administered anti-carrier and anti-hapten antibodies on the primary anti-hapten antibody response to hapten–carrier conjugates in mice. Bacterial α-amylase (BαA), Taka-amylase A (TAA) and key-hole limpet haemocyanin (KLH) were used as carrier molecules, and 2,4-dinitrophenyl (DNP) group was used as a haptenic determinant.

Three groups of mice were injected intravenously with anti-carrier antiserum, anti-hapten antiserum and normal serum as the control, respectively, immediately after the immunization with the hapten–carrier conjugate.

The primary anti-carrier antibody response was markedly suppressed by the passively administered anti-carrier antibody but not anti-hapten antibody. However, the primary anti-hapten antibody response was suppressed not only by passively administered anti-hapten antibody but also by the injection of anti-carrier antibody in an early period after the immunization.

When anti-carrier antibody was given twice 0 and 7 days after immunization, the primary anti-hapten antibody response was markedly suppressed and the suppressive effect was observed even at a later period. In contrast, anti-hapten antibody given by the same schedule as above suppressed only the primary anti-hapten antibody response, but not the anti-carrier antibody response.

Passively administered anti-carrier antibody did not suppress carrier-specific helper cell development, but still suppressed the development of B memory cells and antibody formation against carrier determinants. The antigen dose required for the development of B-cell memory was much higher than that necessary for the stimulation of T cells.

Passively administered anti-carrier antibody clearly inhibited the cellular cooperation between carrier-committed helper cells and hapten-specific B cells and the augmented primary anti-hapten antibody response induced by carrier-primed T cells was clearly abolished. Furthermore, mice preimmunized with carrier showed significantly lower anti-hapten antibody response following the hapten–carrier challenge. Moreover, development of hapten-specific memory cells was also suppressed.

Thus, even in the non-specific antibody-induced suppression by anti-carrier antibody, negative feedback effect of a B-cell product with anti-carrier specificity exclusively regulates the B-cell line development and differentiation.

INTRODUCTION

Numerous observations made during the past decade have given us some understanding of the mechanism by which circulating antibody regulates the subsequent antibody response (Möller and Wigzell, 1965; Uhr and Baumann, 1961; Graf and Uhr, 1969). Some evidence suggested that antibody-induced suppression is mediated by covering the antigenic determinants by specific antibody and preventing them from reacting with the immunocompetent cells (Uhr and Möller, 1968). As reported previously using an adoptive cell transfer system (Hamaoka, Takatsu, Masaki, Matsuoka and Kitagawa, 1971), a marked suppression of the secondary antibody response against carrier determinants could be induced by anti-carrier antibody as a result of blocking access of the effective antigenic information from antigen-bearing macrophages to memory cells. The antibody-induced suppression was found to be specific for the haptenic determinants when analysed with a carrier molecule conjugated with two different haptens (Hamaoka, Takatsu and Kitagawa, 1971).

On the other hand, it was also found in the same experimental system that passively administered 7S anti-carrier antibody suppressed not only the secondary antibody response directed to the carrier determinants but also that directed to the haptenic determinants (Hamaoka, Takatsu and Kitagawa, 1973). These observations were interpreted as indicating that binding of 7S anti-carrier antibody with carrier determinants prevents interactions of carrier-specific T cells with the carrier molecule, and inhibits effective cooperation between the helper T cells and hapten-specific B cells (cooperation blocking effect).

In the present paper, we extended the above observations and studied the effect of anti-carrier antibody on the development of carrier-specific helper cells and T–B cell interaction in the primary anti-hapten antibody response. We found that anti-carrier antibody does not suppress the development of carrier-specific T cells, but still suppresses the development of B memory cells specific for the carrier and hapten and subsequently the primary antibody response to both carrier and haptenic determinants. However, under the same experimental conditions, anti-hapten antibody specifically suppresses the development of hapten-specific B memory cells and therefore the primary antibody response only to the haptenic determinant. Anti-carrier antibody suppresses nonspecifically the subsequent antibody response by virtue of blocking the interaction between T cells and carrier molecules, although its effect does not prevent the development of the carrier-specific helper T cell itself. Thus, the negative feedback effect of a B-cell product with carrier specificity exclusively regulates the development and differentiation of the B-cell line.

MATERIALS AND METHODS

Animals

The dd0 albino mice, supplied by the Central Breeding Laboratory of Experimental Animals of Osaka University, were used throughout the experiments.

Proteins

Crystalline bacterial α-amylase (BαA) derived from *Bacillus subtilis* was obtained from Nagase Sangyo Co. Ltd, and crystalline Taka-amylase A (TAA) prepared from 'Taka-diastase Sankyo', was kindly supplied by Dr T. Ikenaka, Niigata University Medical

School. Keyhole limpet haemocyanin (KLH) was the gift of Dr D. H. Campbell, California Institute of Technology, and bovine serum albumin (BSA) was purchased from Sigma Chemical Co. Ltd. These four proteins are not immunologically cross-reacting.

Preparation of hapten–protein conjugates

Dinitrophenyl protein conjugates were prepared according to the method of Eisen, Kern, Newton and Helmreich (1959) using sodium 2,4-dinitrobenzene sulphonate. The following conjugates were prepared: DNP_2–BαA, DNP_{13}–TAA, DNP_{133}–KLH and DNP_{15}–BSA. Subscripts refer to the average number of DNP groups per mole of protein, which was calculated from the absorption reading at 360 mμ and 290 mμ in an alkaline solution.

Assay system of the primary immune response

Mice were immunized by the subcutaneous and intraperitoneal injection of 100 μg of DNP–BαA conjugates in Freund's complete adjuvant. They were bled 7–24 days after the primary antigenic stimulation and the antibodies against DNP and BαA were estimated. In order to estimate the development of immunological memory (priming), the spleens and lymph node cells were taken from those immunized mice 21 days after primary antigenic stimulation and transferred into 600 rad X-irradiated recipients. The size of the B cell memory developed in the primed animals was estimated by the magnitude of the secondary response in recipients after stimulation with DNP–BαA.

Assay system of helper cell activity of BαA-primed cells

The helper cell activity of BαA-primed cells was assayed by an *in vivo culture* system (Hamaoka *et al.*, 1971, 1973). BαA-primed cells were prepared from spleens and mesenteric and cervical lymph nodes of BαA-primed donor mice and mixed with DNP–TAA-primed cells. Then, they were secondarily stimulated by mixing them with DNP–BαA and then transferred into 600 rad X-irradiated recipient mice. The helper cell activity was estimated by the assay of the anti-DNP antibody titre in sera of recipients ten days after the cell transfer.

Determination of antibody titres

Anti-hapten antibody. The anti-DNP antibody titre was estimated by the coprecipitation technique using radio-iodinated [^{131}I]DNP–BSA as the test antigen as described previously (Hamaoka *et al.*, 1971, 1973). Antigen solution (0·2 ml) containing 0·15 μg of [^{131}I]DNP-BSA was added to 0·2 ml of test samples serially diluted in a two-fold manner with 1 : 10 diluted normal mouse serum. After incubation at 37° for 60 minutes, each tube received 0·6 ml of rabbit anti-mouse IgG antiserum, further incubated at 37° for 60 minutes and kept overnight at 4°. After three washes with cold saline, the radioactivity in the precipitates was measured in a Packard well-type scintillation counter. The amount of antigen bound to the precipitate was corrected for non-specific incorporation of the radioactivity in the precipitate. The percentage of antigen bound was plotted against the logarithm of the reciprocal of the dilution factor for each test sample. The curves were approximately linear within the range of 30–70 per cent. The serum dilution at which 50 per cent of added antigen was specifically bound to antibody, was used as the measure of relative binding capacity. The titre of the antibody (ABC/ml) were expressed as the micrograms of DNP–BSA bound by 1 ml of test sample.

Anti-BαA antibody. Anti-BαA antibody titres (units/ml) were estimated by measuring amylase-neutralizing activities according to the method described previously (Hamaoka, Kitagawa, Matsuoka and Yamamura, 1969; Hamaoka and Kitagawa, 1971). A mixture of 0·25 ml of BαA solution (0·3 μg/ml) and 0·25 ml of anti-BαA antiserum (heat-inactivated) was incubated at 37° for 40 minutes. For measurement of activity of BαA added and amylase activity still present in serum after inactivation, saline was substituted for the antiserum and BαA solution, respectively. To the mixture, 0·5 ml of 0·4 M acetate buffer (pH 6·0) and 1 ml of 0·2 per cent soluble starch (substrate) were added and incubated at 37° for 15 minutes. The residual amylase activity was measured by determining the amount of reducing sugar.

The amylase activity was neutralized linearly in proportion to the amount of antibody added, within the range of 0–90 per cent neutralization. The measurement for neutralizing activity of antibody in the serum specimen was done within this range. The enzyme activity which produces reducing substance equivalent to 1 nmol of glucose/minute, was tentatively defined as one amylase unit. The antibody activity which neutralizes eighty amylase units, was defined as one antibody unit.

Antisera

Anti-BαA antisera used for suppression were obtained 10 days after the secondary stimulation with BαA, from recipient mice which had been immunized with BαA 3 months previously. Anti-DNP antisera were prepared by a procedure similar to that for anti-BαA antiserum, using DNP–KLH as antigen.

RESULTS

EFFECT OF PASSIVELY ADMINISTERED ANTI-HAPTEN AND ANTI-CARRIER ANTIBODY ON THE PRIMARY ANTI-HAPTEN ANTIBODY RESPONSE

Three groups of mice were immunized with 200 μg of DNP–BαA in Freund's complete adjuvants (FCA). Two of them were injected intravenously with 1 ml of anti-DNP antiserum (15 ABC) and 1 ml of anti-BαA antibody (600 units), respectively, and the third group received comparable amounts of normal mouse serum immediately after the primary immunization. After the primary immunization, anti-BαA and anti-DNP antibody titres were estimated on days 10, 17 and 24. As can be seen in Fig. 1a, the primary anti-BαA antibody response was markedly suppressed by the previously administered anti-BαA antibody but not by anti-DNP antibody. In contrast, as shown in Fig. 1b, the primary anti-DNP antibody response was suppressed not only by passively administered anti-DNP antibody but also markedly suppressed by the injection of anti-BαA antibody and the suppression was more prominent at an early period after immunization. Thus, as compared with the high anti-DNP antibody response in the control group, anti-DNP antibody in the group receiving anti-BαA antibody was not detectable 10 days after immunization. However, a higher anti-DNP antibody response was obtained 24 days after immunization as compared with the control group, though at this period anti-BαA antibody response was still suppressed. These results clearly indicate that anti-hapten antibody-induced suppression observed in this system is essentially hapten-specific. However, in the case of anti-carrier antibody-induced suppression, the specificity of suppression was somewhat complicated and seemingly depends on the period of primary immunization; namely, in relatively early period of priming (10 days), anti-carrier antibody suppressed both anti-hapten and anti-carrier

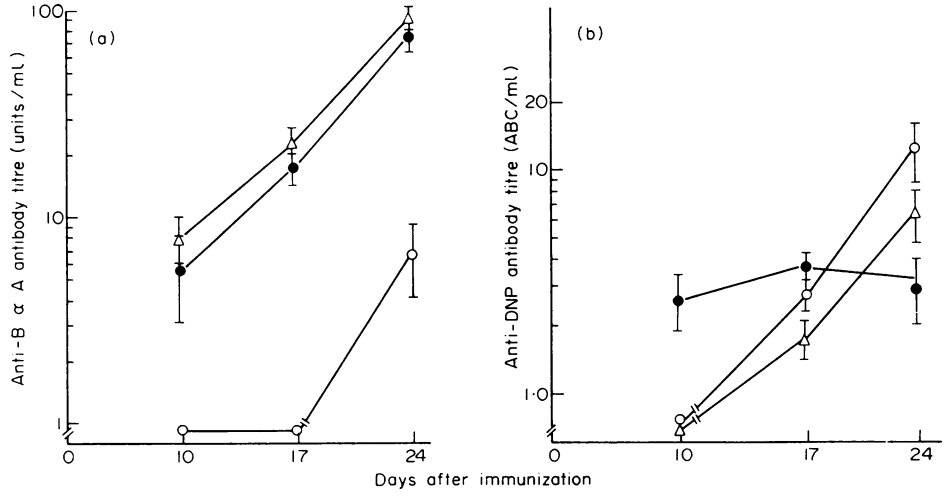

FIG. 1. Effect of passively administered anti-carrier and anti-hapten antibodies on the primary anti-carrier antibody response (a) and the primary anti-hapten antibody response (b). Anti-DNP antiserum (15 ABC), anti-BαA antiserum (600 units) or normal mouse serum was passively administered intravenously into mice immediately after immunization with 200 μg of DNP–BαA. Each symbol represents the mean value of five to six mice. Bars represent the standard errors. (○) Anti-BαA antiserum; (●) normal mouse serum; (△) anti-DNP antiserum.

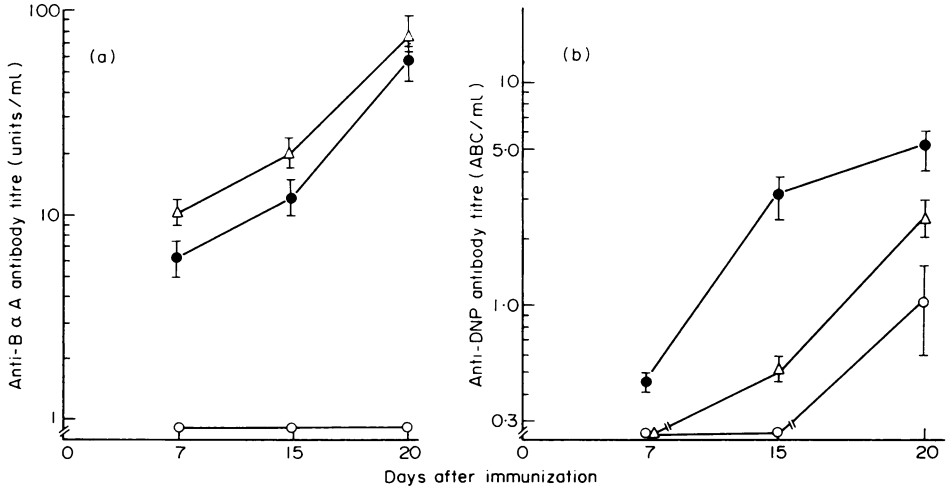

FIG. 2. Effect of passively administered anti-carrier and anti-hapten antibodies on the primary anti-carrier antibody response (a) and the primary anti-hapten antibody response (b). Anti-DNP antiserum (7·5 ABC), anti-BαA antiserum (300 units) or normal mouse serum was passively administered intravenously into mice 0 and 7 days after immunization with DNP–BαA. Each symbol represents the mean value of five to six mice. Each bar represents the standard errors. (○) Anti-BαA antiserum; (●) normal mouse serum; (△) anti-DNP antiserum.

antibody responses. In contrast, at later periods after priming (24 days), the anti-DNP antibody level becomes higher than the control and a suppressive effect of anti-carrier antibody can be demonstrated only on the anti-carrier antibody response. The non-specific suppressive effect of passively administered anti-carrier antibody on the subsequent anti-

hapten primary response was more clearly demonstrated when anti-carrier antibody was given twice after the primary immunization. Thus, the mice given the same amounts of anti-carrier antibody (0·5 ml of anti-BαA serum) 0 and 7 days after the primary immunization showed suppressed anti-DNP antibody response as compared with the control, and a suppressive effect was observed even at later periods after priming (20 days). In this group, the anti-BαA antibody response was completely suppressed (Fig. 2a). In contrast, anti-DNP antibody given at the same timing and the same doses as above again suppressed only the anti-DNP antibody response, but not the anti-BαA antibody response (Fig. 2b).

Thus, the passively administered anti-hapten antibody specifically suppresses only the anti-hapten antibody response. However, the passive administration of anti-carrier antibody clearly suppressed both anti-carrier and anti-hapten antibody responses and this sharply contrasted with what was the case anti-hapten with antibody administration.

Passively administered anti-carrier antibody also clearly suppressed the development of hapten-specific B cell memory during primary immunization. The spleen cells (6×10^7) were taken from the anti-BαA antibody-treated and the control group in the above experiment (Fig. 1) 10 days after primary immunization with DNP–BαA and transferred into another X-irradiated recipient together with an excess number of carrier (TAA) primed cells (6×10^7) taken from other donor mice. Under these experimental conditions, the size of hapten-specific B memory cells developed in the DNP–BαA-immunized mice can be estimated by the anti-DNP antibody response induced by DNP–TAA in the presence of carrier (TAA) primed cells. As shown in Table 1, the development of hapten-specific B cell memory was strikingly suppressed by the passively administered anti-carrier antibody.

TABLE 1

SUPPRESSIVE EFFECT OF PASSIVELY ADMINISTERED ANTI-CARRIER ANTIBODY ON HAPTEN-SPECIFIC MEMORY CELL DEVELOPMENT

Group	Passively administered serum	Secondary anti-DNP antibody response (ABC/ml)
1	Normal serum	3·28
2	Anti-BαA antiserum	0·81

Anti-BαA antiserum (600 units) or normal serum was passively administered intravenously into mice immediately after the immunization with DNP–BαA. Ten days after the immunization, DNP–BαA-primed cells from each group of mice was prepared, mixed with TAA-primed cells and DNP–TAA and transferred into X-irradiated recipient mice. Antibody titres were assayed 10 days after the cell transfer.

The possible mechanism of non-specific suppression of anti-carrier antibody on subsequent anti-hapten antibody response can be considered in at least the following ways. Firstly, anti-carrier antibody suppressed the development of carrier-specific helper cells and consequently, anti-hapten B cells cannot be effectively stimulated by hapten–carrier conjugates. Secondly, anti-carrier antibody does not suppress helper cell development but inhibits the cellular interaction between carrier-specific T cells and hapten-specific B cells by blocking the effective carrier site(s) of hapten–carrier conjugates, so that hapten-specific B memory cells and antibody-forming cells cannot be generated, as observed previously in the induction of the secondary antibody response (Hamaoka *et al.*, 1973).

The following experiments were performed in order to evaluate these possibilities more directly.

EFFECT OF PASSIVELY ADMINISTERED ANTI-CARRIER ANTIBODY ON CARRIER-SPECIFIC HELPER CELL DEVELOPMENT

In order to ascertain whether or not anti-carrier antibody suppresses helper cell development after the immunization with a carrier, fifteen mice were immunized with 50 μg of BαA in Freund's incomplete adjuvant (FICA). The experimental group was given intravenously anti-BαA antibody (320 units) immediately after the immunization, and the control group received normal mouse serum. Seven and 14 days later, five animals from each group were killed and helper cell activity in the spleen cells was estimated in a cell cooperation system with a hapten–carrier conjugate. The cells from each group were mixed with the same pool of DNP–TAA-primed cells which were obtained from mice immunized 40 days before, and transferred into 600 rad X-irradiated recipients and then stimulated intravenously with 100 μg of DNP–BαA. Anti-DNP antibody titres in the recipient mice were estimated 10 days after the cell transfer.

As can be seen in the anti-DNP antibody response of the recipients in Table 2, helper activity in mice immunized with BαA 7 days before and treated with anti-BαA antibody

TABLE 2

EFFECT OF PASSIVELY ADMINISTERED ANTI-CARRIER ANTIBODY ON HELPER CELL DEVELOPMENT

Days after carrier immunization	Passively administered serum	Secondary antibody response	
		Anti-DNP titre (ABC/ml)	Anti-BαA titre (units/ml)
0	None	0·13	0
7	Normal serum	2·36	192
	Anti-BαA antiserum	2·61	13
14	Normal serum	2·81	196
	Anti-BαA antiserum	25·71	5

Normal serum or anti-BαA antiserum (320 units) was injected intraveneously into mice 3 hours before immunization with 50 μg of BαA. After 7 or 14 days, single cell suspensions of lymphoid cells from each donor mice was mixed with DNP–TAA-primed cells and 100 μg of DNP-BαA, and transferred to X-irradiated recipient mice. Antibody titres in recipient mice were estimated 10 days after the cell transfer.

was not significantly different from that in the control mice, and were rather higher 14 days after BαA-immunization. In the anti-BαA antibody-treated group, however, anti-BαA memory cell development was markedly suppressed as compared to the control group, as judged by the responsiveness using our adoptive cell transfer system. These results clearly indicate that passively-administered anti-carrier antibody did not suppress carrier-specific helper cell development, even though development of anti-carrier B cell memory was clearly suppressed.

THE EFFECT OF PRIMING DOSE OF BαA UPON THE DEVELOPMENT OF B MEMORY CELL AND HELPER ACTIVITY

One possibility for the selective suppression of anti-carrier antibody on the development of carrier-specific B-cell memory is that anti-carrier antibody blocks carrier determinant(s) and reduce the number of effective sites for development of B-cell memory, but the reduction is not sufficient to prevent the development of T-cell memory by virtue of the dif-

ference in threshold concentration of antigen for activation of these cells. The dose necessary for a maximum stimulation of T cells may be lower than that of B cells. In order to test this possibility, the priming dose of carrier (BαA) was varied from 0·1 μg to 1000 μg, and the effects of varying doses of carrier on the developments of carrier-specific B cell memory and helper cells were estimated. For the estimation of helper cell activity, the BαA-primed cells (5×10^7) taken from donor mice immunized 21 days previously with various doses of BαA (0·1, 1·0, 50, 200 and 1000 μg) were mixed with the same pool of DNP-TAA primed cells (5×10^7) and transferred into X-irradiated recipient mice (experiment 1). The DNP–TAA-primed cells were obtained from donor mice immunized 45 days previously. The helper activity of various BαA-primed cells populations was estimated by the magnitude of anti-DNP antibody response and the population size of BαA-specific B memory cells was estimated by the anti-BαA antibody production 10 days after the 100 μg of DNP–BαA stimulation. As shown in Table 3, significant helper activity could be detected in BαA-primed cells immunized with either lower (0·1–1·0 μg) or higher (50–1000 μg) doses of BαA, but the highest helper activity was demonstrated with the dose of 1·0 μg of carrier immunization. On the other hand, higher carrier-specific B-cell activity as

TABLE 3

THE EFFECT OF PRIMING DOSE OF BαA UPON HELPER AND AFCP ACTIVITIES OF BαA-PRIMED CELLS

Experiment	Priming dose of BαA (μg)	Secondary antibody response	
		Anti-DNP titre (ABC/ml)	Anti-BαA titre (units/ml)
1 *	0	0·27	0
	0·1	0·47	3·9
	1·0	26·38	6·0
	50	11·22	163·2
	200	7·39	148·0
	1000	3·70	64·8
2 †	0	1·08	0
	0·1	12·8	23
	1·0	36·4	169
	200	4·10	1218

* BαA-primed cells taken from donor mice immunized 21 days previously with various doses of BαA were mixed with DNP–TAA-primed cells and DNP–BαA and transferred to recipient mice.

† BαA-primed cells taken from mice immunized 48 days previously with various doses of BαA were mixed with DNP–KLH-primed cells and DNP–BαA and transferred to recipient mice.

Antibody titres in recipient mice were estimated 10 days after the cell transfer.

reflected by the secondary anti-BαA antibody response could be obtained in the higher range of BαA (50–200 μg). However, 1000 μg of BαA was rather inhibiting for the development of both helper and B cell memory. The dose difference in developmental kinetics between helper T-cell function and B-cell memory was also observed in experiment 2. BαA-primed cells were used 48 days after primary immunization and helper activity was estimated by mixing them with DNP–KLH-primed cells immunized 35 days previously. In agreement with experiment 1, maximum helper cell activity was again observed in cells immunized with 1·0 μg of BαA and maximum B-cell activity was observed in cells immunized with 200 μg of BαA. These observations are consistent with Kettman's finding (Kettman

and Dutton, 1972) that the co-operative function of spleen cells in the response to SRBC reaches a maximum when immunized with lower dose (5×10^6) of antigen and their capacity to produce secondary anti-SRBC antibody was obtained at the higher dose (1×10^9) of antigen. These results show that the antigen dose required for the development of B-cell memory is much higher than that necessary for the stimulation of T cells. These findings suggest that inability of anti-carrier antibody to suppress T-cell helper activity may be due to the threshold difference of antigen-dose for effective cell activation between T and B cells.

SUPPRESSIVE EFFECT OF ANTI-CARRIER ANTIBODY ON CELLULAR COOPERATION BETWEEN HAPTEN-SPECIFIC B CELLS AND CARRIER-SPECIFIC T CELLS ON THE INDUCTION OF PRIMARY ANTI-HAPTEN ANTIBODY RESPONSE

It is well established that, in the primary anti-hapten antibody response to hapten-carrier conjugates, the presence of primed T cells with specificity for the carrier moiety of the conjugates facilitates the stimulation of hapten-specific B cells (Katz, Paul, Goidl and Benacerraf, 1970).

In order to study the effect of anti-carrier antibody on the interaction between hapten-specific B cells and carrier-specific T cells in the induction of a primary anti-hapten antibody response, an augmented primary antibody response was induced by transferring carrier-primed T lymphocytes and the effect of passively administered anti-carrier antibody on the subsequent primary anti-hapten antibody response was studied.

The first group of mice was injected intravenously with 5×10^7 BαA-primed spleen cells taken from donor mice immunized 7 days before with 50 μg of BαA in FICA. The second group received BαA-primed cells and 320 units of anti-BαA antibody mixed together *in vitro*. The third group as control received normal spleen cells. These groups of mice were then immunized intraperitoneally with 200 μg of DNP–BαA in FCA.

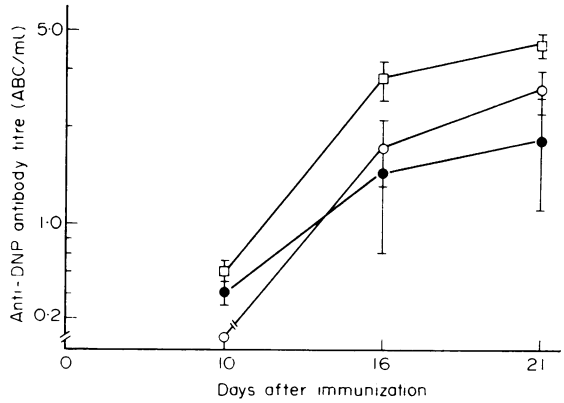

FIG. 3. Suppressive effect of anti-carrier antibody on the cooperative induction of primary anti-hapten antibody response. (□) BαA-primed cells, (○) BαA-primed cells + anti-BαA antiserum or (●) normal spleen cells were injected intravenously into mice 3 hours before immunization with DNP–BαA. Each symbol represents the mean value of five mice. Bars represent the standard errors.

As shown in Fig. 3, the primary anti-DNP antibody response was significantly augmented by transferring BαA-primed cells as compared with the control which received normal cells. In contrast, the augmenting effect of BαA-primed cells was significantly inhibited by the passively administered anti-BαA antibody. These results clearly indicate that anti-

carrier antibody suppresses the effective interaction of carrier-primed T cells with the carrier moiety of hapten-carrier conjugates and inhibits virgin hapten-specific B cells differentiating into antibody-forming cells during the primary anti-hapten immune response.

SUPPRESSIVE EFFECT OF PREEXISTING ANTI-CARRIER ANTIBODY IN THE PRIMARY ANTI-HAPTEN ANTIBODY RESPONSE IN ACTIVELY IMMUNIZED MICE

As shown in previous section, passively administered anti-carrier antibody suppressed anti-hapten primary antibody response through the inhibition of effective cellular interaction between carrier-specific T cells and hapten-specific B cells, but the development of carrier-specific T cells was not suppressed by the presence of anti-carrier antibody.

In order to study further the suppressive effect of anti-carrier antibody in actively immunized animals, carrier-immunization was carried out before primary immunization with DNP–BαA, and the anti-hapten antibody response in carrier-preimmunized mice was compared with non-preimmunized animals. Indeed, by carrier-preimmunization both carrier-specific helper cells and circulating anti-carrier antibody are generated in the same animals, and helper cells and anti-carrier antibody could affect the subsequent primary anti-hapten antibody response in more complicated ways such as augmentation and suppression, respectively. Four groups of mice were preimmunized with various doses of BαA (10, 50, 100 and 200 μg) in FICA and another control group received only saline emulsified in FICA.

Immunization with varying doses of carrier was performed to obtain various levels of carrier-specific helper cells and circulating anti-carrier antibody. Four weeks later, the treated animals received primary immunization with 200 μg DNP–BαA in FCA.

TABLE 4

SUPPRESSIVE EFFECT OF PREEXISTING ANTI-CARRIER ANTIBODY ON THE PRIMARY ANTI-HAPTEN ANTIBODY RESPONSE IN ACTIVELY IMMUNIZED MICE

Preimmunizing dose of BαA	Primary antibody response on day 19		Secondary antibody response on day 10	
	Anti-DNP titre (ABC/ml)	Anti-BαA titre (units/ml)	Anti-DNP titre (ABC/ml)	Anti-BαA titre (units/ml)
0 μg	3·19±0·17	52±27	7·14	221
10	0·14±0·15	1218±101	1·90	394
50	<0·1	1840±186	1·22	864
100	0·29±0·15	1050±600	2·73	1228
200	0·13±0·01	1460±223	1·35	736

Each group of mice was preimmunized with various doses of BαA. Four weeks later, they received primary challenge with DNP–BαA. Nineteen days thereafter, DNP–BαA-primed cells from each donor mice were transferred with DNP–BαA to recipient mice. Antibody titres in donor mice were estimated 19 days after the primary immunization with DNP–BαA and antibody titres in recipient mice were estimated 10 days after the cell transfer.

As shown in Table 4, the group preimmunized with varying doses of BαA showed varying levels of anti-BαA antibody response before the challenge with DNP–BαA, and also showed secondary anti-BαA antibody response to the primary challenge with DNP–BαA. Control groups receiving only FCA showed far smaller anti-BαA antibody responses against the

challenge of DNP–BαA. In contrast, mice preimmunized with BαA showed a significantly lower anti-DNP antibody response following the DNP–BαA challenge than the control on day 19. The suppressive effect of carrier preimmunization was observed over the whole dose range of BαA employed here.

Moreover, the level of development of DNP-specific memory cells was significantly lower than the control as detected by transferring spleen cells from those animals into recipients and stimulating with DNP–BαA. Indeed, as expected, the development of carrier-specific B-cell memory was much more augmented by the carrier-preimmunization than the control.

These results indicate that the anti-carrier antibody generated by carrier-preimmunization suppresses the subsequent anti-hapten antibody response in actively immunized animals, even though carrier-specific helper cells were generated by the same carrier-preimmunization. Thus, the suppressive effect of anti-carrier antibody on the anti-hapten primary immume response is prominent in actively immunized animals, and this abolishes T- to B-cell cooperation.

DISCUSSION

There have been three mechanisms proposed to explain the regulatory influences of antibody on the antibody-producing system (Pincus, Lamm and Nussenzweig, 1971). The first mechanism explains the suppressive effect of antibody by its causing the rapid elimination of antigen from the immune system. Some results in a number of experimental systems can be explained on this basis (Kappler, Hoffman and Dutton, 1971). The second mechanism depends on the regulation of antibody-producing cells by their own products, which has been proposed by Rowley and Fitch (1964) and recently extended by Feldmann and Diener (1970). In their scheme, antibody or an antigen–antibody complex is capable of delivering a suppressive signal to antigen-specific B cells which can be distinguished from the signal from antigen alone.

The third mechanism involves the covering of antigenic determinants with the antibody, thus isolating them from the B-cell receptors. This type of mechanism has been proposed by a number of workers (Uhr and Möller, 1968), and the validity of this reasoning was based on the ability of antibody directed against certain determinants on a molecule to specifically suppress the response to those determinants without affecting the response to other determinants on the same molecule.

Our previous reports (Hamaoka *et al.*, 1971; Hamaoka, Takatsu and Kitagawa, 1971) which analysed the suppressive effect of antibody on the secondary immune response using a technique of memory cell transfer into X-irradiated recipients demonstrated that antibody-induced suppression on induction of secondary response was determinant-specific when using a double hapten conjugate.

Moreover, the capacity of memory cells to respond to antigenic stimulation was not directly impaired by the suppressive antibody. These results clearly indicate that antibody-induced suppression appears to be caused by blocking the effective antigenic determinants which bind to B-cell receptor.

However, when the anti-carrier antibody was applied in this system, passively administered anti-carrier antibody completely suppressed both the anti-carrier and anti-hapten antibody response. From the analysis of T–B cell cooperation in this system, the suppression of anti-hapten antibody response by anti-carrier antibody was the result of failure of

cooperation between carrier-primed T cells and hapten-primed B cells (Hamaoka *et al.*, 1973). The anti-carrier antibody seemed to block the effective carrier site(s) of hapten–carrier conjugate and block the interaction of T cells with carrier molecules.

In the present study, we extended these observations to the primary immune response and demonstrated the non-specific suppression of passively administered anti-carrier antibody on the subsequent anti-hapten primary antibody response. Indeed, in this system, passively administered anti-hapten antibody suppressed only the anti-hapten primary antibody response, but not the anti-carrier antibody response (Figs 1 and 2). These results clearly argue against the notion that the passively administered antibody changes the localization of antigen in the lymphoid system in the primary immune response.

Passively administered anti-carrier antibody suppressed not only anti-carrier antibody response but also the anti-hapten antibody response (Figs 1 and 2). The suppressive effect of anti-carrier antibody on the anti-hapten primary antibody response is non-specific in the sense that antibodies specific for one portion of a molecule can suppress the production of antibodies directed toward another portion.

The mechanism of non-specific suppression of the anti-hapten primary antibody response by administration of anti-carrier antibody could be envisaged in at least two ways. Firstly, anti-carrier antibody suppresses the development of carrier-specific helper cells and consequently, hapten-specific B cells may not be effectively stimulated by hapten-carrier conjugates.

Secondly, as observed in the induction of secondary response, anti-carrier antibody inhibits the cellular interaction between carrier-specific T cells and hapten-specific B cells by blocking of the effective carrier-site(s) of hapten-carrier conjugate, even after the T cells committed to the carrier are generated.

The results presented here strongly support the latter alternative. This derived from the following observations.

Firstly, as shown in Table 2, passively administered anti-BαA antibody did not suppress the helper cell development even though development of anti-BαA B-cell memory was completely inhibited. Kappler *et al.* (1971) also reported that a dose of antiserum which severely suppresses the development of anti-SRBC PFC response did not prevent the increase of SRBC-specific T cells. In general, it is well known that immunological memory is more difficult to suppress by the passive administration of antibody than is the primary humoral antibody response and cell development for cellular immunity such as delayed hypersensitivity, is also relatively insensitive to suppression by antibody. This was probably due to the fact that the antigen dose required for the development of T cells was lower than that necessary for the development of B-cell memory (Table 3).

Secondly, in order to study the effect of anti-carrier antibody on the interaction between hapten-specific B cells and carrier-specific T cells in the induction of primary anti-hapten antibody response, the augmented primary antibody response was induced by transferring carrier-primed T cells and the effect of passively administered anti-carrier antibody on the subsequent primary anti-hapten antibody response was analysed. As shown in Fig. 3, passively administered anti-BαA antibody clearly inhibits the cellular co-operation between carrier-primed helper cells and hapten-specific B cells, and the augmented primary anti-hapten antibody induced by carrier-primed cells was clearly abolished.

Thus, in the primary anti-hapten antibody response, we can demonstrate the same non-specific suppressive effect of anti-carrier antibody on the subsequent anti-hapten antibody response as was observed in the secondary immune response. This is that the

anti-carrier antibody blocks the effective carrier-site(s) of hapten-carrier conjugates and inhibits the interaction of carrier-committed T cells with carrier site(s), and this results in the blocking the co-operation between carrier-specific T cells and hapten-specific B cells (co-operation blocking effect). The co-operation blocking effect of anti-carrier antibody operates on T cells in both pathways of B-cell differentiation, i.e. the differentiation of virgin B cells to memory cells and differentiation of B memory cells to antibody-forming cells, since when the primary anti-hapten antibody response was suppressed by anti-carrier antibody, the development of hapten-specific memory cells was also suppressed at the same time.

This non-specific suppression of anti-hapten antibody formation by anti-carrier antibody was also clearly demonstrated in carrier-preimmunized animals. In this case, carrier-specific helper cells were generated at the same time by the carrier immunization (as can be seen in Table 3), but the suppressive effect of anti-carrier antibody on anti-hapten immune response was so strong that the anti-hapten antibody response to be induced by hapten-carrier immunization was completely suppressed by the carrier preimmunization (Table 4).

There have been some experimental results showing that antibodies corresponding to one antigenic determinant non-specifically suppress the immune response against different antigenic determinants present on the same molecule.

In guinea-pigs, antibodies specific to the Fab and Fc portions of human γ-globulin were equally effective in suppressing production of antibodies to all antigenic determinants of human γ-globulin (Henny and Ishizaka, 1970). Uhr and Bauman (1961) showed that three anti-toxin molecules could effectively neutralize the immunogenicity of one toxin molecule. Similarly, the response to sheep erythrocytes seems to be suppressed under the conditions where only a minor fraction of the determinants of the antigen is covered by antigen. On the other hand, Pincus *et al.* (1971) demonstrated that passively administered antiserum specific for Fab or Fc portion of HGG, can simultaneously suppress or enhance the primary immune response against different determinants of the same antigen molecule. A similar phenomenon was also reported by McBride and Shierman (1971) using chicken erythrocyte antigen. In our results, as shown in Fig. 1, a single administration of anti-carrier antibody suppressed the primary anti-DNP antibody response at an early period (10 days after immunization) but enhanced at a later period (24 days after immunization). However, when the anti-carrier antibody was adminstered twice after primary immunization, anti-carrier antibody suppressed the primary anti-hapten antibody at a later period after immunization.

If the antibody binds to the antigenic determinants located near to the carrier-site(s) with which the carrier-committed T cells interact as shown in the induction of secondary response (Hamaoka *et al.*, 1973), the antibody would abolish the cooperation of carrier-committed T cells. Thus, in this example, suppression would be non-specific at the level of antigenic determinants.

However, in a long course of immunization such as during the primary immune response, the presence of anti-carrier antibody does not suppress T-cell development, and as soon as the effect of passively administered antibody disappears, the augmenting effect of carrier-primed T cells starts to operate and the subsequent anti-hapten antibody response would be augmented. Thus, by this mechanism, the seemingly conflicting observations above can be explained very clearly.

Finally, the mechanism of antibody-induced suppression in T–B-cell interaction

suggests to us a very interesting feature of negative feedback regulation by B-cell products. This is that anti-hapten antibody blocks the hapten determinants and prevents them from interacting with B cells and only suppresses the hapten-specific B-cell response. On the other hand, anti-carrier antibody blocks the carrier-determinants and inhibits the carrier-specific B cells from interacting with carrier determinants as well as T-cell interaction with carrier. Thus, the anti-carrier antibody produces a non-specific effect on the subsequent B-cell responses to both hapten and carrier determinants through prevention of the B-cell triggering mechanism induced by T cells, but development of helper T-cell activity is not suppressed by this process. In this sense, the effect of antibody-induced immune regulation, which is a negative feedback mechanism by B-cell products, is restricted to control of the B-cell line.

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