SELECTIVE ROLES OF THYMUS-DERIVED LYMPHOCYTES IN THE ANTIBODY RESPONSE

I. Differential Suppressive Effect of Carrier-Primed T Cells on Hapten-Specific IgM and IgG Antibody Responses*

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It is rapidly becoming clear that in certain antibody responses thymus-derived lymphocytes (T cells) are definitely endowed with a role to suppress responses of bone marrow-derived lymphocytes (B cells) to antigen (1–12). Such inhibitory effect of T cells led to a designation of "suppressor T cell" and is being recognized in large measure as an important regulatory device in various antibody responses. Some forms of immunological tolerance are also explained by the active suppression by T cells rather than the elimination of B-cell clones (12–14).

The suppression by T cells has been shown to be mediated by both antigen-specific and nonspecific mechanisms, while both pathways may be equally important for the regulation of antibody synthesis. Our previous studies (3) demonstrated that T cells primed with a carrier antigen suppressed an ongoing IgE antibody synthesis by B cells against a hapten coupled to the same carrier; thus this suppression appeared to be antigen-specific. The presence of a subcellular component of T cells that specifically suppresses the B-cell response was also reported (15).

Our more recent studies on the specific suppression of IgM antibody response by T cells (11) showed another important fact, namely, that the T cell's suppressive effect on B cells varied depending on the time when the suppressor cells were given to the immunized recipients. The suppressor effect on the IgM antibody response was found to be more pronounced if the primed T cells were given to the recipients at a time when antibody-forming cells were starting to appear in their spleen rather than at the time of immunization. These observations suggest that the susceptibility of B cells to the suppressive effect of T cells is inherently different depending on the maturation stage of B cells and on the immunoglobulin classes which they are destined to produce. The present experiments were undertaken to study the mode and sites of action of suppressor T cells on antibody response in mice with special reference to the differential effect of T cells on B cells which produce IgM and IgG antibodies.

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Materials and Methods

Animals.—BALB/c 6J mice, 8–12 wk old, raised in our laboratory, were used throughout.

Antigen.—Keyhole limpet hemocyanin (KLH)[1] was purchased from Calbiochem, San Diego, Calif. Bovine serum albumin (BSA) and bovine gamma globulin (BGG) were obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio. They were coupled with 2,4-dinitrophenyl (DNP) hapten by the method of Eisen et al. (16). The following DNP conjugates were used in the present studies: DNP_{730}-KLH (assuming average mol wt of KLH as 7,000,000), DNP_{43}-BGG and DNP_{34}-BSA. Subscripts refer to the number of DNP groups per molecule of carrier proteins. *Bordetella pertussis* vaccine containing 3×10^{10} killed organisms was kindly supplied by Dr. S. Hashizume of the Chiba Serum Institute, Chiba, Japan.

Immunization.—Mice were primarily immunized with an intraperitoneal injection of 100 μg of DNP-KLH or DNP-BGG mixed with 10^9 pertussis vaccine in a total volume of 0.2 ml. Such animals served as the recipients of carrier-primed cells (see below). Animals were killed at various intervals after the immunization, and the number of DNP-specific antibody-forming cells in the spleen was assayed by the hemolytic plaque technique as described below.

To induce secondary anti-DNP antibody response, animals were primed with an intraperitoneal injection of 100 μg of DNP-KLH without adjuvant, and 4 wk later were secondarily challenged with 100 μg of DNP-KLH with or without 10^9 pertussis vaccine. The PFC response was determined 3 days after the secondary immunization.

Preparation of Carrier-Primed Cells and Experimental Procedure.—Donors of carrier-primed cells were immunized with two intraperitoneal injections of 100 μg of KLH or BGG without adjuvant at a 2-wk interval. Animals were killed 2 wk after the second injection, and their thymuses and spleens were removed. They were teased and minced with forceps in a small amount of MEM and then gently pressed between two glass slides to release cells from fibrous tissues. After straining through no. 200 stainless steel mesh, they were washed three times with cold MEM and resuspended in MEM at a concentration of 2.5×10^8 cells/ml.

The suspension of carrier-primed thymocytes or spleen cells were passively transferred intravenously into the recipient animals in quantities of 0.2 ml containing 5×10^7 cells. The cell transfer was made at different time points in the primary antibody response of the recipient, i.e., simultaneously with, 2 days or 3 days after the primary immunization with DNP-KLH (see Results). In the secondary antibody response, cells were given simultaneously with the secondary challenge with DNP-KLH.

In some experiments, the carrier-primed spleen cells were treated in vitro with AKR anti-C3H θ antiserum (anti-θ) and guinea pig complement (C) or rabbit antimouse thymocyte serum (ATS) and C at 37°C for 1 h. The treatment usually killed 20–30% of the spleen cells. The treated cells were washed with MEM and immediately transferred into recipient animals.

Assays for Antibody-Forming Cells.—The number of DNP-specific antibody-forming cells in the spleen of immunized animals was estimated by the hemolytic plaque method of Cunningham and Szenberg (17). Sheep erythrocytes (SRBC) were coupled with DNP-BSA by chromic chloride (18). The optimal coupling for hemolysis was confirmed at each time by passive hemolysis with a standard anti-DNP antiserum. Direct plaque-forming cells (PFC) developed without rabbit antimouse IgG (anti-IgG) were considered to be IgM antibody-forming cells. Indirect PFC was developed by adding 1:100 dilution of anti-IgG and was considered as an IgG antibody producer. The number of indirect PFC was determined by subtracting the number of direct PFC from the total PFC developed with anti-IgG.

Statistical Analysis.—The geometric means and standard deviations were calculated from

[1] *Abbreviations used in this paper:* ATS, anti-thymocyte serum; BGG, bovine gamma globulin; BSA, bovine serum albumin; BPV, *Bordetella pertussis* vaccine; C, guinea pig complement; KLH, keyhole limpet hemocyanin; MEM, Eagle's minimal essential medium; PFC, plaque-forming cell.

the logarithmically transformed PFC numbers of at least six similarly treated mice at each time. P values were determined by the student's t test.

RESULTS

Kinetics of DNP-Specific Primary Antibody Response in BALB/c Mice Immunized with DNP-KLH.—Mice immunized with 100 μg of DNP-KLH and 10^9 pertussis vaccine exhibited normal sequential IgM and IgG antibody responses against the DNP group as measured by the numbers of direct and indirect PFC in the spleen. As shown in Fig. 1, the peak IgM antibody response occurred on day 3 amounting to 50,000 direct PFC/spleen. The number of indirect PFC at this time was low and variable. The peak indirect PFC response dropped on day 6 at a time when direct PFC had already been declining since its peak on day 3. The maximum number of indirect PFC was about 10,000/

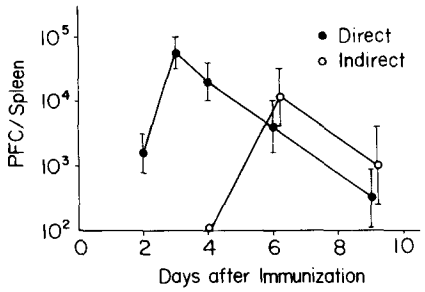

FIG. 1. Kinetics of DNP-specific PFC response in BALB/c mice immunized with 100 μg of DNP-KLH and 10^9 BPV. Each point and bracket are the geometric mean and standard deviation.

spleen on day 6. Both direct and indirect PFC responses were short-lived and barely detectable on day 10 (Fig. 1).

Suppressive Effect of Carrier-Primed T Cells on the Hapten-Specific Antibody Response.—5×10^7 thymocytes or spleen cells obtained from KLH- or BGG-primed donors were passively transferred to the syngeneic recipient that was concomitantly immunized with DNP-KLH plus pertussis vaccine. The kinetics of DNP-specific PFC responses are shown in Fig. 2. Animals given the cells from BGG-primed donors showed no difference in the magnitude and pattern of the PFC responses from those of the control animals, whereas mice given the cells from KLH-primed donors showed markedly altered responses following the same immunization with DNP-KLH. As is evident from Fig. 2 and Table I, animals given KLH-primed thymocytes or spleen cells exhibited a strong suppression of indirect PFC (IgG) response rather than of direct PFC (IgM) response. The number of maximum indirect PFC on day 6 was nearly 2 log scale lower than that of the control groups that had been given BGG-primed cells. In contrast, the peak direct PFC response on day 3 was only slightly depressed,

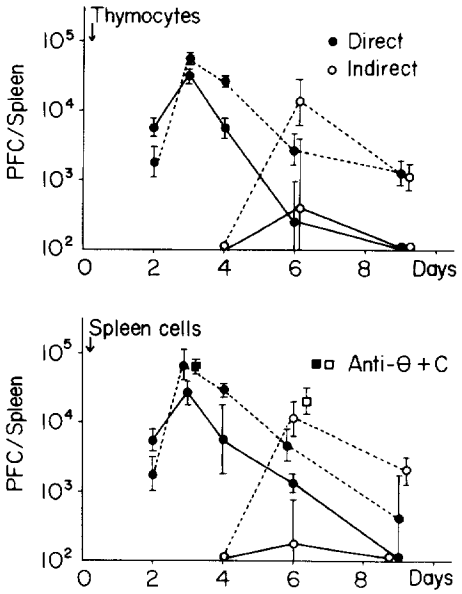

FIG. 2. Kinetics of DNP-specific PFC response in mice given thymocytes (upper panel) and spleen cells (lower panel) from donors immunized with KLH (solid line) or BGG (broken line) on day 0 (arrow). Closed and open squares in the lower panel represent the numbers of direct and indirect PFC in animals given KLH-primed spleen cells treated with anti-θ and C. Each point and bracket are the geometric mean and standard deviation of at least six similarly treated mice.

although this difference was still statistically significant ($P < 0.05$). This difference in the direct PFC numbers between suppressed and unsuppressed groups was, however, much more pronounced on day 6 (Fig. 2). This indicates that the carrier-primed cells were capable of suppressing IgM antibody formation in a later phase of the primary antibody response, resulting in the earlier termination of IgM response.

The suppressive activity of KLH-primed spleen cells was completely abrogated by in vitro treatment of the cells with anti-θ and C or ATS and C before cell transfer, indicating that the spleen cells responsible for the suppression of antibody response are, in fact, θ-bearing T cells.

A reverse experiment was performed to confirm the specificity of the suppression using DNP-BGG as immunizing antigen instead of DNP-KLH. Recipient mice were given the same 5×10^7 KLH-primed cells or BGG-primed cells, and were immunized with 100 μg of DNP-BGG and pertussis vaccine. With this immunizing antigen DNP-specific PFC was meaningfully detected only on day 6. Although the magnitude of the antibody response was low, it was confirmed that BGG-primed cells but not KLH-primed cells were capable of suppressing both direct and indirect PFC responses in the DNP-BGG-immunized mice (Table I).

TABLE I

Suppression of Hapten-Specific Antibody Response by Passively Transferred Carrier-Primed Thymocytes and Spleen Cells in the Primary Antibody Response

Immunizing antigen	Cells* transferred	PFC/spleen on day 3‡		PFC/spleen on day 6‡	
		Direct	Indirect	Direct	Indirect
DNP-KLH + BPV	None	56,100 ×/÷ 1.99	v§	3,950 ×/÷ 2.35	11,000 ×/÷ 2.77
	KLH-primed Th	27,900 ×/÷ 1.34	v	306 ×/÷ 3.53	500 ×/÷ 2.02
	KLH-primed Spl	28,700 ×/÷ 1.66	v	1,340 ×/÷ 1.36	89 ×/÷ 3.98
	" anti-θ treated	65,200 ×/÷ 1.29	v	7,140 ×/÷ 1.67	20,600 ×/÷ 1.74
	" ATS treated	52,400 ×/÷ 1.61	v	3,690 ×/÷ 1.25	11,700 ×/÷ 1.55
	BGG-primed Th	56,800 ×/÷ 1.18	v	2,970 ×/÷ 1.80	14,000 ×/÷ 2.58
	BGG-primed Spl	68,700 ×/÷ 1.71	v	4,720 ×/÷ 1.99	11,600 ×/÷ 1.78
DNP-BGG + BPV	None	v	v	2,080 ×/÷ 1.60	10,200 ×/÷ 1.82
	BGG-primed Th	v	v	873 ×/÷ 1.81	287 ×/÷ 5.77
	KLH-primed Th	v	v	1,900 ×/÷ 1.12	9,840 ×/÷ 1.53

* 5×10^7 thymocytes or spleen cells were passively transferred on day 0.

‡ Geometric means and standard deviations calculated from more than six similarly treated animals; ×/÷ means "multiply and divide".

§ Low and variable.

Specificity of the T-Cell-Mediated Suppression.—Since the above studies indicated that the cells primed with a carrier antigen on which immunizing hapten was coupled can effectively suppress the antibody response against the hapten on the homologous carrier, the next experiment was designed to learn whether the same suppression could be induced by an interaction between unrelated antigen and T cells primed with this second antigen. Animals were given either KLH-primed cells or BGG-primed cells, and then subsequently immunized with the mixture of DNP-BGG and uncoupled KLH, or with the mixture of DNP-KLH and uncoupled BGG. If the interaction between the uncoupled carrier and corresponding T cells elaborate nonspecific factors that suppress anti-DNP antibody response, it should be predictable that the hapten-specific PFC response is suppressed by the concurrent injection of uncoupled heterologous carrier and T cells.

As a control for this experiment, normal mice were immunized with the mixture of DNP-KLH and BGG, or DNP-BGG and KLH. DNP-specific PFC response in these groups immunized with two antigens was slightly lower than that induced by a single hapten-carrier conjugate (Table II). However, even if such animals were concomitantly given the cells primed with the second carrier on which immunizing hapten was not coupled, no significant suppression of anti-DNP PFC response was observed. As shown in Table II, animals given KLH-primed cells produced comparable numbers of direct and indirect PFC to those of the control upon immunization with DNP-BGG and KLH. Similarly, no suppression was detectable in animals given BGG-primed cells upon subsequent immunization with DNP-KLH and uncoupled BGG. Thus, it is suggested that in order to elicit effective suppression the hapten must be present on the same carrier by which suppressor T cells had been primed.

TABLE II

Failure to Suppress the Antibody Response by Simultaneous Injection of Second Antigen and the Cells Primed with this Second Antigen

Immunizing antigen	Cells* transferred	PFC/Spleen on day 6‡	
		Direct	Indirect
DNP-BGG + KLH§	None	1,060 ×/÷ 1.24	7,320 ×/÷ 1.16
	KLH-primed Th	892 ×/÷ 1.20	7,420 ×/÷ 1.29
	KLH-primed Spl	1,560 ×/÷ 1.69	5,960 ×/÷ 1.34
DNP-KLH + BGG§	None	1,840 ×/÷ 1.39	5,190 ×/÷ 1.36
	BGG-primed Th	4,830 ×/÷ 1.86	6,150 ×/÷ 2.06
	BGG-primed Spl	3,890 ×/÷ 1.02	3,230 ×/÷ 1.49

* 5×10^7 thymocytes or spleen cells were passively transferred on day 0.
‡ Geometric means and standard deviations calculated from more than six similarly treated animals.
§ The second antigens.

Effect of the Time of Cell Transfer on IgM and IgG Antibody Response.—Since our previous studies (3, 11) indicated that suppressor T cells were more effective in terminating the pre-established IgE and IgM antibody responses than preventing the induction of these responses, we tested the effect of differing cell transfer time points on the subsequent antihapten IgM and IgG antibody responses in the present system. Groups of recipient mice were immunized by the standard method with DNP-KLH and pertussis vaccine, and KLH-primed thymocytes or spleen cells were transferred into these recipients 2 days or 3 days after instead of simultaneously with the immunization. In both cases control mice were given BGG-primed thymocytes or spleen cells on day 2 or 3, and the kinetics of PFC responses were compared with those of KLH-primed cell recipients.

Fig. 3 shows the results of the experiment in which KLH-primed cells were given on day 2. Both thymocytes and spleen cells from KLH-primed donors produced a significant suppression of anti-DNP antibody response, while here again BGG-primed cells failed to alter the immune responsiveness of the recipients. The overall effect of KLH-primed cells given on day 2 was comparable to that induced by the cell transfer on day 0, except for the more significant suppression of the peak direct PFC response ($P < 0.02$).

Suppression of indirect PFC response observed on day 6 was, however, still more prominent than of direct PFC with a difference of more than one log scale of magnitude ($P < 0.001$). It was also confirmed that the suppressive effect of KLH-primed spleen cells was completely abrogated by the in vitro treatment with anti-θ and C before the cell transfer.

In contrast to the above findings, the cell transfer on day 3 produced a markedly different effect on the recipients' anti-DNP antibody responses. As shown in Fig. 4, the number of direct PFC, which was already at the maximal before the cell transfer, declined very rapidly following the passive transfer of KLH-primed thymocytes or spleen cells, at a much greater rate than observed in the control animals given BGG-primed cells. This suppressive effect on direct PFC

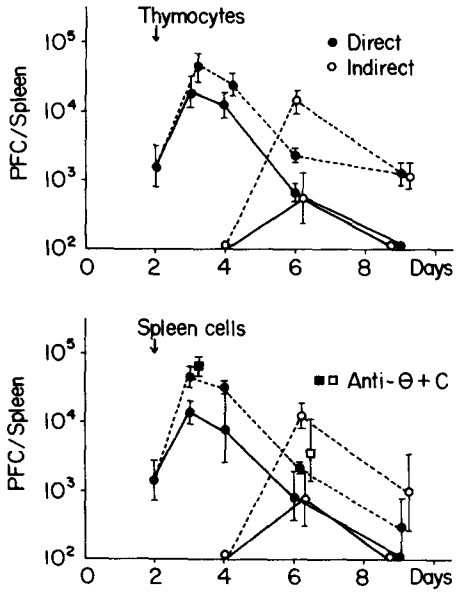

FIG. 3. Kinetics of DNP-specific PFC response in mice given KLH- or BGG-primed thymocytes (upper panel) and spleen cells (lower panel) on day 2 (see legends for Fig. 2).

was already evident on day 4, only 24 h after the transfer of KLH-primed cells, and became more pronounced on day 6. By contrast, the cell transfer on day 3 produced much less suppressive effect on indirect PFC response than that induced by the same KLH-primed cells given on day 0 or 2. It is apparent that KLH-primed cells given on day 3 were effective in suppressing the pre-established IgM antibody response, resulting in an earlier termination of the response, whereas they were no longer very effective in inhibiting the IgG antibody response which was just starting at the time of cell transfer.

Differential Suppressive Effect of KLH-Primed Cells on the Secondary IgM and IgG Antibody Responses against DNP-KLH.—Secondary antihapten antibody response was induced in animals primed 4 wk earlier with DNP-KLH and secondarily challenged with DNP-KLH with or without pertussis vaccine. In both cases of secondary immunization, animals produced good secondary anti-DNP antibody response which was characterized by increases in both maximal direct and indirect PFC in their spleens as observed 3 days after the secondary injection of the antigen (Table III).

5×10^7 KLH-primed thymocytes or spleen cells were passively transferred into such animals simultaneously with the secondary immunization. As summarized in Table III, both KLH-primed thymocytes and spleen cells produced a moderate suppression of the anti-DNP secondary antibody response, but their effect was evidently more profound in the suppression of indirect PFC response ($P < 0.001$) than of direct PFC response ($P < 0.05$). The suppressive effect of spleen cells was also eliminated by the treatment with anti-θ and C. Although

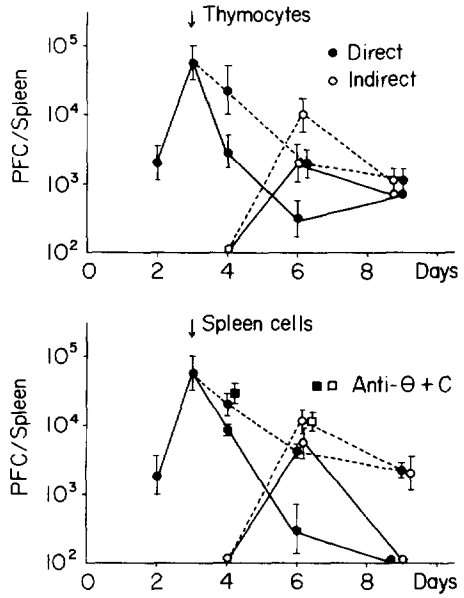

FIG. 4. Kinetics of DNP-specific PFC response in mice given KLH- or BGG-primed thymocytes (upper panel) and spleen cells (lower panel) on day 3 (see legends for Fig. 2).

TABLE III

Suppression of Anti-DNP Secondary Antibody Response by KLH-Primed Thymocytes and Spleen Cells

Immunization		Cells* transferred	PFC/spleen on day 3‡	
Primary	Secondary		Direct	Indirect
DNP-KLH	DNP-KLH + BPV	None	236,000 ×/÷ 1.51	128,000 ×/÷ 1.34
		KLH-primed Th	115,000 ×/÷ 1.79	21,000 ×/÷ 2.58
		KLH-primed Spl	174,000 ×/÷ 1.58	21,800 ×/÷ 1.27
		" anti-θ treated	321,000 ×/÷ 1.06	110,000 ×/÷ 1.51
DNP-KLH	DNP-KLH	None	110,000 ×/÷ 1.50	54,400 ×/÷ 1.30
		KLH-primed Th	43,150 ×/÷ 1.23	3,850 ×/÷ 1.51

* 5×10^7 thymocytes or spleen cells were passively transferred simultaneously with the secondary challenge with DNP-KLH.

‡ Geometric means and standard deviations calculated from more than six similarly treated animals.

the use of pertussis vaccine with the secondary challenge with DNP-KLH enhanced the magnitude of both IgM and IgG antibody responses, the degree of suppression produced by carrier primed cells was comparable in both immunizing regimens (Table III).

DISCUSSION

The present experiments demonstrated the differential effect of carrier-specific suppressor T cells on hapten-specific IgM and IgG antibody formations

depending primarily on the time when they were administered to the recipient animals that were undergoing primary and secondary antibody responses upon immunization with a hapten coupled to the homologous carrier. It was found that if the suppressor T cells were given simultaneously with or in the very early stage of the primary immunization, the suppressive effect was preferentially directed to late-coming IgG antibody response rather than the early IgM antibody response, although the latter was also suppressed in the later phase of the primary antibody response. However, if the suppressor T cells were given 3 days after the primary immunization of the recipient, at a time when IgM antibody response was already at its maximum, they rapidly terminated the pre-existing IgM antibody response, while being no more strongly effective in suppressing IgG antibody response which was at this time just beginning to make an appearance. These results indicate that the susceptibility of B cells to the suppressive effect of T cells is inherently different depending on their maturation stages and on the immunoglobulin classes they are destined to produce.

Such disparity in the inert sensitivity of IgM and IgG B cells to T cells' suppression may probably be related to their different dependency on T-cell functions with respect to their differentiation into antibody-forming cells (19–24). The observed relative insensitivity of early IgM antibody response in both primary and secondary antibody responses may reflect the fact that these responses are largely T-cell independent. By contrast, at least some processes of the development of IgG antibody-forming cells, as well as a part of IgM antibody-forming cells, from their precursors have been shown to be strongly T-cell-dependent. Thus the IgG antibody response and the later phase of IgM antibody response may have been greatly suppressed by the carrier-specific T cells.

These interpretations appear to fit the recent concept of differentiating events occurring in B cells following antigenic stimulation. Accumulating evidence claims that B cells having μ-chain determinants on their surface are the precursors of both IgM and IgG forming cells (25, 26). Such virgin B cells, termed Bμ cells, would differentiate either directly into IgM-forming cells or into the cells having both μ- and γ-chains on their surface (B$\mu\gamma$ cells) that consequently become Bγ cells, the precursors of IgG forming cells (27, 28). The former, the direct process of maturing into IgM forming cells may be largely T-cell independent, while the latter, the process of becoming Bγ cells may be T-cell dependent. If we assume that the inert sensitivity of B cells to suppressive influence of T cells is related to such T-cell dependency of B cells, we would be able to guess the sites of action of suppressor T cells. Suppressor T cells given on day 0 or 2 were unable to suppress the direct process from Bμ cells to IgM-forming cells, while being able to prevent the indirect processes from converting into B$\mu\gamma$ and Bγ cells, which perhaps take place in the early stage of immunization. This would naturally result in the preferential suppression of both IgG and late IgM antibody responses. However, after the critical time of day 3, suppressor T cells preferentially inhibited the T-cell-dependent part of IgM

antibody response resulting in the rapid termination of the antibody formation, while being unable to effectively inhibit IgG antibody formation, probably due to the relative insensitivity of the late maturation process from Bγ cells to IgG-forming cells.

Such interpretations may be possible in the secondary antibody response too. The secondary antibody response is characterized by a quick response of greater magnitude which reflects the presence of a pool of memory B cells. Since the degree of suppression in secondary antibody response was much less than that in the primary antibody response, the considerable part of memory B cells may be present in a state insensitive to the suppressive influence of T cells. However, the stronger suppression of IgG antibody response than of IgM antibody response suggests that a sufficiently overt number of memory B cell for IgG antibody response is present in a sensitive state to the suppressor T cells, which is most likely to be the Bμ memory cell. Such Bμ memory cells would either directly mature into IgM-forming cells in a T-cell-independent pathway or indirectly differentiate into IgG forming cells under the influence of T cells, and the latter process may have been suppressed by primed T cells.

These observations are clearly analogous to the findings made by Gershon and Kondo (12, 13) that the antigen-specific suppression produced by T cells obtained from tolerant animals preferentially affected the 2-mercaptoethanol-resistant IgG antibody formation of the host. Although at the present time it is not known whether the active suppressor cells induced by tolerization and hyperimmunization are the same or not, the higher sensitivity in IgG antibody formation than in IgM antibody formation to the T cells' suppressive effect seems to accord with the widely recognized preponderant effect of neonatal thymectomy on IgG rather than IgM antibody response (19–21). Thus, it is probable that the process of becoming Bγ cells is more easily altered by T cells' inductive or suppressive influence than the T-cell-independent process of becoming IgM-forming cells.

The other important point considered critical for the effect of suppressor T cells is the rigid specificity of T and B cells. It was found that to elicit effective suppression, hapten must be present on the same carrier molecule to which the specificity of suppressor T cells is directed. Thus even if T cells primed with unrelated antigen were concurrently stimulated with this second antigen, no effective suppression was induced in the antibody response to the hapten-carrier conjugate. This claims that the observed suppression is not due to the antigenic competition between two different populations of helper T cells. The results are analogous, though inversely, to those reported by Mitchison (29) who showed that in the adoptive secondary antibody response hapten-specific B cells were effectively stimulated by hapten-carrier conjugate in the presence of T cells whose specificity was directed to the homologous carrier, and that the hapten must be present on this same carrier molecule. It is also noteworthy that thymocytes themselves exerted the antigen-specific suppressor effect, while they

are usually considered to be out of stimulation pathways after antigen injection. However, recent reports from several laboratories have proven that the thymus is a source of specific suppressor T cells (3, 11–14).

Although these observations do not deny the possible role of non-specific mediators from primed T cells, they certainly favor the possibility of specific mediation of T- and B-cell-interaction in both induction and suppression of antibody responses. Such specific mediators from T cells are now being recognized in both the augmentation (30–32, footnote 2) and suppression (15, 33) of antibody responses, and we are currently exploring the presence of such mediators using the present experimental system.

Since our current studies indicated that suppression by T cells was antigen-specific and preferentially induced in certain subpopulations of B cells, it is obvious that T cells somehow interferred with the selection process in the B-cell population. Such selective pressure of T cells on B-cell response has been suggested by Gershon and Paul (34) and Taniguchi and Tada (35) who showed that affinity of produced antibody was influenced by the number of antigen-stimulated T cells present in immunized animals. From this point of view, the above findings appear to have some bearing on the different affinity for antigen in precursors of IgM and IgG antibody-forming cells. It has been claimed that, in general, IgM antibodies have a lower affinity than IgG antibodies to the same antigens (36–38). Thus the important question raised at this point is what subpopulation of B cells in terms of their affinity to antigen is preferentially suppressed by carrier-specific T cells. A resolution of this question will be presented in the accompanying paper.

SUMMARY

Passively transferred thymocytes and spleen cells from donors primed with keyhole limpet hemocyanin (KLH) exerted differential suppressive effect on IgM and IgG antibody responses of syngeneic recipients immunized with DNP-KLH depending primarily on the time when KLH-primed cells were transferred. This was demonstrated by the decrease in the numbers of DNP-specific direct and indirect PFC in the spleen of the recipients given KLH-primed cells at different times during primary and secondary immunization. Whereas the cell transfer simultaneously with or 2 days after the primary immunization produced only slight suppression of the peak IgM antibody response, it caused profound suppression of late IgM and IgG antibody responses. By contrast, the cell transfer 3 days after the immunization produced immediate suppression of the ongoing IgM antibody response resulting in its earlier termination, while being unable to prevent the induction of IgG antibody response. KLH-primed cells could moderately suppress the secondary anti-DNP antibody response, in

[2] Taniguchi, M., and T. Tada. 1974. Regulation of homocytotropic antibody formation in the rat. X. IgT-like molecule for the induction of homocytotropic antibody response. *J. Immunol.* Manuscript accepted for publication.

which IgG antibody response was found to be slightly more sensitive than IgM antibody response to the suppressive influence of KLH-primed cells.

The suppressive effect of the KLH-primed spleen cells was completely eliminated by the in vitro treatment of the cells with anti-θ and C before cell transfer, indicating that cells responsible for the suppression are, in fact, T cells. The suppression of DNP-specific antibody response by KLH-primed T cells was achieved only if the recipients were immunized with DNP-KLH but not with DNP-heterologous carrier, suggesting that direct interaction between T and B cells is necessary for the suppression of the antibody response. It is concluded that susceptibility of B cells to the specific suppressive influence of T cells is inherently different depending on the differentiation stage of B cells and on the immunoglobulin class they are destined to produce.

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