PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 194–198 (1979)

Modulation of Hapten-Specific Antibody Responses with Anticarrier Antibody: I. Differential Effects of IgM and IgG Anticarrier on Primary Direct and Indirect Hapten-Specific Plaque-Forming Cells (40645)[1]

ELLEN WHITED COLLISSON, BIRGER ANDERSSON, AND E. W. LAMON[2]

Veterans Administration Hospital, Departments of Surgery and Microbiology, Cancer Research and Training Center, School of Medicine, University of Alabama in Birmingham, Birmingham, Alabama 35294 and the Department of Tumor Biology, Karolinska Institute, S 104 01 Stockholm 60 Sweden

Antibody responses against a variety of antigens require the participation of T cells as helper cells. Such thymus-dependent antibody responses have been extensively studied in hapten-carrier systems. Evidence from a number of these studies suggest that T cells having carrier specificity are necessary for optimal hapten antibody responses. For example, passive transfer experiments demonstrate helper activity when carrier-primed T cells combined with hapten-primed B cells are transferred to irradiated donors receiving hapten-carrier conjugates (1). However, Kirov and Parish have recently shown that carrier-specific B cells participate in the helper effect (2). A 40 to 50% decrease in hapten-specific direct plaque-forming cells (PFC)[3] and 85 to 90% decrease in hapten-specific indirect PFC responses were observed after radioactive antigen suicide of the carrier-specific B cells. Thus, carrier-specific antibody may modulate the helper activity of T cells. Indeed, previous experiments have shown that passively administered antibody alters subsequent humoral responses to thymus-dependent antigens (3–8). Antibody-mediated modulation of thymus-dependent antibody responses has included both potentiation and suppression depending on the class or subclass of antibody used (3–5). For example, Henry and Jerne have shown that 19 S antisheep erythrocyte (SRBC) antibody potentiates whereas 7 S antibody suppresses the direct plaque-forming cell (PFC) response to SRBC (3). Some studies have shown a modulation of serum antihapten levels by passive administration of anticarrier antibody (5–8).

Since receptors for the Fc region of both IgG and IgM have been demonstrated on T cells (9, 10), it is conceivable these might bind to immunoglobulins complexed to carrier erythrocytes which are T-cell dependent. Such complex binding could thus initiate regulation of hapten antibody-producing responses through helper or suppressor T cells.

The following investigation evaluated *in vivo* fluctuations of a primary hapten antibody response induced by IgG and IgM antibodies specific for T-cell-dependent carrier erythrocytes. The hapten response was examined at the isolated single-cell level using Jerne's plaque-forming cell (PFC) assay enumerating modulations in both direct and indirect PFC (11).

Materials and methods. Homologous anti-bovine erythrocyte (ORBC) sera were prepared from the pooled sera of at least 20 mice 5 days after primary or tertiary immunization with 0.2 ml of 50% ORBC. The sera were fractionated by ammonium sulfate precipitation and Sepharose 6B column chromatography. The leading half of the 19 S peak from primary sera and the trailing half of the 7 S peak from hyperimmune sera were collected for use. Immunodiffusion with class-specific antisera revealed no cross-contamination of M and G and the latter contained both G_1 and G_2. Complement-dependent hemolytic titers of the fractions were determined using twofold dilutions of the antiserum fractions and an excess of guinea pig complement. To make comparisons between experiments using different pools of antisera, calculations

[1] Credit is given to Project 5132-02 from the Veterans Administration Hospital, Birmingham, Ala. Also, this investigation was supported by Grant NO1-CA 17273-04, awarded by the National Cancer Institute, DHEW, National Institutes of Health, and a grant from the Swedish Cancer Society.

[2] Recipient of a Research Career Development Award from the National Cancer Institute.

[3] Abbreviations used in this paper: PFC, plaque-forming cell; ORBC, bovine erythrocytes; TNP, 2,4,6-trinitrophenyl; SRBC, sheep erythrocytes.

0037-9727/79/090194-05$01.00/0

were made using "hemolytic units." One hemolytic unit is defined as the amount of antibody in 0.1 ml at the highest dilution producing complement-dependent hemolysis of a 1% erythrocyte suspension.

Six- to eight-week-old CBA mice of both sexes were used with 3 to 5 animals per group. The animals were immunized i.p. with 0.2 ml of 10% TNP-ORBC immediately followed by i.p. injection of the appropriate anticarrier antibody. One milliliter of ORBC was coated with 60 mg TNP for immunization (13, 14). On Days 3, 5, 7, and 10, as indicated in the results section, TNP-specific antibody-producing cells from spleens were enumerated using the Jerne plaque assay (11) with TNP-SRBC target cells (13). Indirect plaques were developed with 1:400 dilution of rabbit anti-mouse globulin (Cappel Laboratories). Sheep erythrocytes alone were used as controls and SRBC background was subtracted from the TNP-SRBC counts. The numbers of PFC are represented as $\log_{10}$ mean ± SE per spleen. Background levels of TNP-specific PFC from unimmunized animals were 2.52 ± 0.08 (38 animals) for direct PFC and 2.30 ± 0.05 (33 animals) for indirect PFC. Log_{10} means have been converted to geometric means, as indicated in the results section. Haptenic inhibition of direct and indirect PFC was accomplished by adding varying concentrations of TNP coupled to glycine (12) to the spleen cells as they were mixed with the TNP-conjugated erythrocytes. Probabilities were determined using a Student's *t* test.

Results. Kinetics experiments indicate both the direct and indirect TNP-specific immune responses peak in the mouse 5 days after injection of TNP-ORBC conjugates (Fig. 1). The Day 5 responses in Fig. 1 represent the geometric mean TNP-PFC/spleen from 28 animals and the other days represent geometric means from 6 mice. The effects of IgM and IgG anti-ORBC on the hapten response at different times after injection are shown in Table I. Both classes of antibody potentiated direct and indirect PFC on Day 3 after injection. However, at the peak of the response the two classes of anticarrier antibodies had opposite effects on the TNP-PFC. Thus, on Day 5 in this experiment IgM anti-ORBC potentiated the direct response by >23,800 PFC and potentiated the indirect response by >110,000 PFC. In this experiment IgG anti-ORBC inhibited the indirect response by >43,000 PFC but had no significant effect on the direct response. Since the optimal TNP-PFC response was on Day 5 and because of the dramatic and opposite effects of the two classes of antibody on the antihapten response at this time, Day 5 was chosen for further analyses.

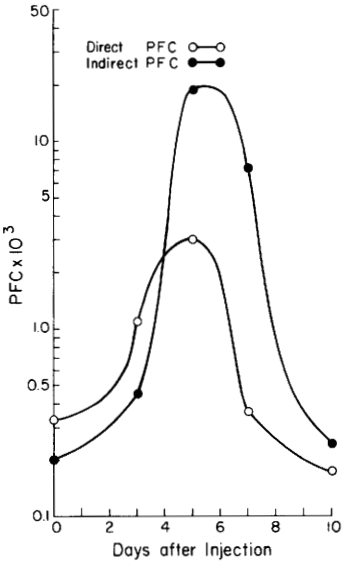

FIG. 1. Kinetics experiment of the primary PFC response specific for TNP. Spleens from CBA mice were assayed for PFC after being injected i.p. with 0.2 ml, 10% TNP-ORBC. Each point represents the geometric mean PFC/spleen from the following numbers of animals. Day zero represents data from 38 and 33 animals for direct and indirect PFC, respectively, Day 5 shows data from 28 mice for both direct and indirect PFC, and Days 3, 7, and 10 each from 6 animals each.

The results from multiple experiments in which animals were simultaneously injected with relatively high concentrations of IgM or IgG anticarrier antibody and antigen are summarized in Table II. IgM increased the geometric mean of the TNP-specific PFC by 5297 direct plaques and 47,448 indirect plaques. High concentrations of IgG mediated essentially no direct PFC suppression but reduced the mean number of indirect plaques by 16,671 PFC. Similar results have been obtained with several strains including CBA, C3H/HeJ, BALB/c, and (C56 B1/6 × DBA/2)F_1 mice and using either bovine or horse erythrocyte carriers and IgM or IgG

anticarrier antibodies. Haptenic inhibition studies in which varying concentrations of hapten coupled to glycine were added to the PFC overlay confirmed the TNP specificity of these PFC (data not shown here). Furthermore, the concentration of hapten (5 m*M*) required to inhibit 50% of the direct TNP-PFC was at least 10-fold greater than required to inhibit 50% of the indirect plaques indicating a higher average avidity of the indirect PFC. The IgM-potentiated direct and indirect PFC had the same low and high avidities, respectively, as the PFC from animals receiving antigen only.

TABLE I. *In Vivo* Kinetics of Modulation of TNP-PFC by Anticarrier Antibodies

Day after injection	Control[a]	Anti-ORBC IgM[b]	Anti-ORBC IgG[c]
	Direct PFC	Direct PFC	Direct PFC
3	2.73 ± 0.43[d] (573)	4.01 ± 0.12 (10,233)	3.78 ± 0.07 (6,026)
5	3.57 ± 0.10 (3,715)	4.44 ± 0.10 (27,542)	3.65 ± 0.07 (4,467)
7	2.37 ± 1.08 (234)	2.14 ± 0.60 (138)	3.25 ± 0.03 (1,778)
10	2.79 ± 0.26 (617)	3.27 ± 0.11 (1,862)	3.05 ± 0.09 (1,122)
	Indirect PFC	Indirect PFC	Indirect PFC
3	2.51 ± 0.55 (324)	3.83 ± 0.01 (6,760)	3.56 ± 0.32 (3,631)
5	4.55 ± 0.15 (45,709)	5.20 ± 0.05 (158,489)	3.53 ± 0.29 (3,388)
7	4.03 ± 0.07 (10,715)	NT[e]	3.24 ± 0.21 (1,738)
10	2.06 ± 0.27 (115)	3.12 ± 0.05 (1,318)	3.02 ± 0.33 (1,047)

[a] Control animals were injected with 0.2 ml, 10% TNP-ORBC.
[b] Animals received antigen and passive anticarrier IgM with 24 hemolytic units (defined in the text).
[c] Passive IgG anti-ORBC with 32 hemolytic units was injected with the antigen.
[d] Log_{10} mean ± SD of PFC/spleen from three animals tested separately. Numbers in parentheses represent the geometric mean.
[e] Not tested.

Dose–response experiments conducted on Day 5 with passive anticarrier IgM and IgG are shown in Figs. 2 and 3, respectively. Potentiation of both direct and indirect antihapten PFC was produced by IgM with carrier specificity. The maximum potentiation observed was three- to fourfold greater than controls receiving TNP-carrier conjugates alone (Fig. 2). The direct response was increased from 1940 PFC/spleen from animals receiving antigen alone to 7356 PFC by IgM anticarrier antibody. The number of indirect PFC was elevated from a control mean of 8481 to 30,528 plaques by passive IgM anti-ORBC. The potentiation in both direct and indirect PFC was dose responsive, i.e., decreased with decreasing concentrations of IgM.

As shown in Fig. 3, IgG anticarrier antibody had no significant effect on direct antihapten PFC. In contrast, higher concentrations of IgG anti-ORBC affected a 10-fold suppression of indirect TNP-specific PFC. It is conceivable that the hyperimmune sera used as a source of IgG might contain anti-

TABLE II. Anticarrier Antibody-Mediated Modulation of TNP-PFC Measured 5 Days After Injection

Group	PFC	No. experiments	No. mice	Log_{10} $\bar{x}$ ± SD	Geom $\bar{x}$	Δ PFC[a]	*P*
Control[b]							
Antigen only	Direct	9	28	3.48 ± 0.27[c]	3,020		
	Indirect	9	28	4.27 ± 0.33	18,621		
IgM[d]							
Anti-ORBC	Direct	5	14	3.92 ± 0.35	8,317	+5,297	<0.025
	Indirect	5	15	4.82 ± 0.26	66,069	+47,448	<0.0005
IgG[e]							
Anti-ORBC	Direct	9	31	3.42 ± 0.25	2,630	−390	NS[f]
	Indirect	9	31	3.29 ± 0.09	1,950	−16,671	<0.0005

[a] Calculated by subtracting the geometric means of the control PFC/spleen from the experimental means.
[b] Mice injected with 0.2 ml, 10% TNP-ORBC.
[c] Interexperimental variation.
[d] IgM hemolytic units (defined in the text) for each experiment ranged from 24 to 128, log_{10} $\bar{x}$ ± SD = 1.70 ± 0.28, geometric $\bar{x}$ = 50.
[e] IgG hemolytic units for each experiment ranged from 32 to 64, log_{10} $\bar{x}$ ± SD = 1.67 ± 0.11, geometric $\bar{x}$ = 47.
[f] Not significant.

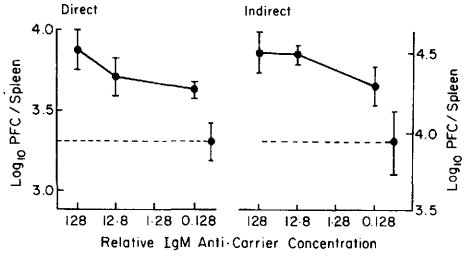

FIG. 2. Modulation of TNP-specific PFC by passively administered anti-ORBC IgM. Spleens from at least four CBA mice per group were assayed for PFC 5 days after being injected i.p. with 0.2 ml, 10% TNP-ORBC and 0.1 ml anti-ORBC IgM (——). Control spleens from animals receiving only TNP-ORBC were also assayed (---). PFC were assayed with TNP-SRBC. Antibody concentrations are represented in hemolytic units as defined in the text. Maximal potentiation of TNP-specific direct and indirect PFC was significant at $P < 0.005$ and $P < 0.025$ levels, respectively.

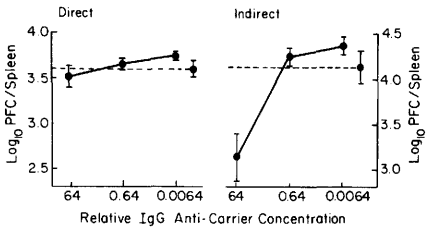

FIG. 3. Modulation of TNP-specific PFC by passively administered anti-ORBC IgG. Spleens from at least four CBA mice per group were assayed for PFC 5 days after being injected i.p. with 0.2 ml, 10% TNP-ORBC and 0.1 ml anti-ORBC IgG (——). Control spleens were assayed from animals receiving only TNP-ORBC (---). PFC were assayed with TNP-SRBC. Antibody concentrations are represented in hemolytic units as defined in the text. Significant ($P < 0.0125$) suppression of indirect hapten-specific PFC was observed with the highest concentrations of IgG anti-ORBC.

idiotypic antibody against carrier-specific receptors. Thus, idiotype suppression (15) could be a mechanism involved in the IgG-induced suppression of hapten-specific PFC. We, therefore, evaluated whether anti-ORBC IgG bound to the carrier would produce this suppression. Injection of TNP-ORBC conjugates, pretreated with IgG anticarrier and then washed, resulted in comparable suppression of the indirect PFC against TNP (data not shown). Thus, IgG with carrier specificity alone is sufficient to produce this suppression.

Discussion. The data presented here show that passive antibody specific for a T-dependent carrier can modulate the *in vivo* primary PFC response to a defined haptenic determinant. An increase in the numbers of both direct and indirect TNP-specific antibody-producing cells in mouse spleens was produced by both classes of anticarrier antibodies 3 days after injection. However, at the peak response, 5 days after injection, IgM continued to produce potentiation of both direct and indirect PFC. Anticarrier IgG had little effect on the 5-day direct hapten-specific PFC. However, the indirect hapten responses at 5 days were consistently suppressed by anticarrier IgG at relatively high antibody concentrations.

One possible explanation for these data is that IgM anticarrier activates helper T cells, potentiating both the direct and indirect PFC responses to hapten; and IgG, in relatively high concentrations, activates suppressor cells which either inhibit helper activity or directly suppress the indirect PFC response to hapten. Inhibition of indirect PFC could also be a result of IgG anticarrier antibody covering sites necessary for helper-cell recognition. Inhibition by simple blocking of antigenic determinants seems unlikely, however, since $F(ab)_2$ fragments of anticarrier IgG have been shown to be much less efficient than intact IgG in antibody-mediated suppression (4, 16). The structures necessary to interact with antibody to produce help or suppression of a thymus-dependent response would be receptors on T cells for IgM Fc (10) and IgG Fc (9), respectively. In support of such a model (although their system was not antigen specific), Moretta *et al.*, using human lymphocytes, have recently shown that T cells bearing IgM Fc receptors can function as helper cells and T cells bearing IgG Fc receptors can function as suppressor cells (17). Furthermore, T cells have been found essential in antibody-mediated potentiation of immune responses to erythrocytes (18) as well as in a hapten-carrier system where the hapten response was potentiated by anticarrier antibody (19). Also, as indicated above, the Fc region of IgG has been shown to be important in IgG-mediated suppression of antibody responses (4, 16).

Since T cells have receptors for the Fc regions of immunoglobulins, it is reasonable to assume immune complex binding to lymphocytes has a functional role that may well

be of a regulatory nature. The present study suggests activation of helper or suppressor T cells by IgM and IgG anticarrier antibodies, respectively, which bind to the carrier through their antigen combining sites and to the T cells via IgM or IgG Fc receptors.

The experiments presented here provide no direct evidence for the involvement of T cells in this antibody-induced potentiation or suppression. However, evidence from a number of studies make it seem probable that T cells are involved: 1. The responses studied are thymus dependent; 2. it has been demonstrated that antibody-mediated modulation of such responses requires T cells (18, 19); 3. finally, the demonstration of immune complex receptors on T cells (9, 10) would predict some functional significance for these structures. Ultimate resolution of this problem will require passive transfer experiments with purified populations of cells.

Conceivably, the observed modulations in the hapten-specific responses could involve an indirect effect on helper or suppressor cells via amplifier T cells (20). Alternatively, antigen–antibody complexes could activate macrophages to modulate the hapten-specific responses (21).

Whatever the mechanism involved, the magnitudes of the observed *in vivo* modulations are very large and statistically highly significant. As much as 10-fold suppression of indirect PFC was produced by IgG and 4-fold potentiation of both direct and indirect PFC was produced by IgM. These anticarrier antibody-mediated modulations may represent *in vivo* immunoregulatory phenomena since anticarrier IgM and IgG are components of the host primary immune response.

Finally, these findings may offer promise in terms of pragmatic manipulation of the immune response; helper activity against undesirable antigens might be potentiated by attaching IgM to another (carrier) portion of the molecule. Specific suppression also may be activated using IgG anticarrier antibodies.

Summary. Immunization of mice with TNP coupled to ORBC resulted in a TNP-specific antibody response. TNP-specific direct and indirect PFC in the spleens reached a maximum level 5 days after injection. Passively administered anticarrier antibodies injected with the hapten-carrier conjugates were found to modulate the primary PFC response to hapten. Three days after injection, both classes of carrier-specific antibody induced potentiation of hapten-specific direct and indirect PFC. On Day 5, however, IgM and IgG had opposite effects on the TNP-specific response. Carrier-specific IgM produced a three- to fourfold increase in both direct and indirect hapten-specific PFC over animals injected with the hapten-carrier conjugates alone. In contrast, carrier-specific IgG, at relatively high concentrations, suppressed the hapten-specific indirect PFC but had little effect on direct hapten-specific PFC.

1. Mitchison, N. A., Eur. J. Immunol. **1,** 18 (1971).
2. Kirov, S. M., and Parish, C. R., Scand. J. Immunol. **5,** 191 (1976).
3. Henry, C., and Jerne, N. K., J. Exp. Med. **128,** 133 (1968).
4. Gordon, J., and Murgita, R. A., Cell. Immunol. **15,** 392 (1975).
5. Pearlman, D. S., J. Exp. Med. **126,** 127 (1967).
6. McBride, R. A., and Schierman, L. W., J. Exp. Med. **131,** 377 (1970).
7. Pincus, C., Miller, G., and Nussenzweig, V., J. Immunol. **110,** 301 (1973).
8. Takatsu, K., Hamaoka, T., and Kitagawa, M., Immunology **26,** 233 (1974).
9. Yoshida, T. O., and Andersson, B., Scand. J. Immunol. **1,** 401 (1972).
10. Lamon, E. W., Andersson, B., Whitten, H. D., Hurst, M. M., and Ghanta, V., J. Immunol. **116,** 1199 (1976).
11. Jerne, N. K., and Nordin, A. A., Science (Wash.) **140,** 405 (1963).
12. Okuyama, T., and Satake, K., J. Biochem. **4,** 454 (1960).
13. Rittenberg, M. B., and Pratt, K. L., Proc. Soc. Exp. Biol. Med. **132,** 575 (1969).
14. Kettman, J., and Dutton, R. W., J. Immunol. **104,** 1558 (1970).
15. Hart, D. A., Wang, A. L., Pawlak, L. L., and Nisonoff, A., J. Exp. Med. **135,** 1293 (1972).
16. Chan, P. L., and Sinclair, N. R., Immunology **21,** 967 (1971).
17. Moretta, L., Webb, S. R., Grossi, L. E., Lydyard, P. M., and Cooper, M. D., J. Exp. Med. **146,** 184 (1977).
18. Dennert, G., Proc. Soc. Exp. Biol. Med. **143,** 889 (1973).
19. McBride, R. A., and Schierman, L. W., J. Immunol. **110,** 1710 (1973).
20. Feldmann, M., Beverley, P. C., Woody, J., and McKenzie, I. F., J. Exp. Med. **145,** 793 (1977).
21. Pierce, C. W., and Knapp, J. A., *in* "Immunobiology of the Macrophage" (D. S. Nelson, ed.), pp. 1–33. Academic Press, New York (1976).

Received February 23, 1979. P.S.E.B.M. 1979, Vol. 162.