Brief Definitive Reports

HAPTEN-SPECIFIC STIMULATION OF SECONDARY B CELLS INDEPENDENT OF T CELLS*

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Cooperative interactions between thymus-dependent lymphocytes (T cells) and antibody-forming cell precursors derived from the bone marrow (B cells) are important for the stimulation of B cells by most soluble antigens (1–3). This is most clearly exemplified in the secondary immune response to hapten-carrier complexes where T cells specific for the carrier markedly increase antibody production by B cells specific for the hapten. Alternative mechanisms of secondary B cell stimulation are implied, however, by the finding that hapten on heterologous or poorly immunogenic carriers is also able to stimulate antibody formation (4–6). Recent studies have indicated that such stimulation can be the result of interactions between B and T cells both recognizing haptenic determinants (6–8). This demonstration of "hapten self-help" questions the notion of direct stimulation of secondary B cells independent of T cell participation. In most studies, except those using very high antigen concentrations (9) or large polymeric antigens (10, 11), B cell stimulation in the absence of specific T cells has not been demonstrable (3, 12).

Previous reports from this laboratory indicated that isolated secondary B cells in splenic fragment cultures can be stimulated to form foci of antibody-producing cells by stimulation with hapten on heterologous carriers (13, 14). This report extends these findings by demonstrating similar results after treatment of donor cell suspensions before transfer with anti-theta serum and complement. Thus, in the apparent complete absence of T cells, secondary B cells can be stimulated by low concentrations of soluble hapten-carrier conjugates, including conjugates in which the carrier is heterologous or nonimmunogenic.

Materials and Methods

Antigens.—2,4-dinitrophenyl conjugates of *Limulus polyphemus* hemocyanin (DNP-Hy), bovine gamma globulin (DNP-BGG), bovine serum albumin (DNP-BSA), and poly-L-lysine (75,000 mol wt) (DNP-PLL) were prepared as previously described (5, 13, 14). DNP-BSA contained 37 mol of DNP per mol of BSA, DNP-BGG contained 20 mol of DNP per mol of

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BGG, DNP-PLL contained 34 mol of DNP per mol of PLL, and DNP-Hy contained 10 mol of DNP per 100,000 mol of Hy.

Immunization.—Male BALB/c mice, to be used as spleen cell donors, were injected intraperitoneally with 0.1 mg of DNP-Hy in complete Freund's adjuvant at 8–12 wk of age. Carrier-immunized BALB/c mice were obtained by intraperitoneal injection of 8-wk old males with 0.1 mg of Hy in complete Freund's adjuvant 4–8 wk before use (14).

Cell Transfers.—4–7 mo after immunization, mice were killed by cervical dislocation. Spleen cell suspensions were prepared in Dulbecco's modified Eagle's medium with a Teflon tissue homogenizer. Viability of cell preparations, as measured by trypan blue dye exclusion, was greater than 90%.

Anti-Theta Serum Treatment of Donor Cells.—Anti-theta serum was obtained from AKR mice injected with thymocytes from C3H mice according to a modification of the method of Reif and Allen (15). The method of treatment of donor spleen cell suspensions was similar to that described by Wigzell (16) and is presented in detail in another publication (8). 1 ml of anti-theta serum diluted 1:10 in Dulbecco's modified Eagle's medium, 0.15 M for D-fructose, was added per 10^8 cells. After 10 min incubation at 37°C, complement in the form of fresh guinea pig serum (GPC) (0.5 ml/10^8 cells) was added and incubation was continued for 30 min at 37°C. After the addition of 5 U/ml of sodium heparin, cells were centrifuged and suspended for injection. Control studies showed that greater than 90% of radioactivity was released from BALB/c thymocytes labeled with chromium 51 by this treatment, while 30% of ^{51}Cr was released from spleen cells similarly treated (8).

Spleen Fragment Cultures.—The spleen fragment culture system used for these studies, as well as the radioimmunoassay of culture fluids, has been previously reported (5, 13, 14).

RESULTS

Table I demonstrates the effect of anti-theta serum treatment on the response of transferred secondary spleen cell populations. As previously reported (5) when 2×10^7 spleen cells from an immune donor are transferred to an irradiated syngenic recipient, fragment cultures of the recipient's spleen respond maximally when relatively high concentration of the homologous antigen is used for in vitro stimulation. This response requires the presence of carrier-specific T cells (8). The effectiveness of anti-theta serum treatment is readily apparent from the 90% diminution of the response to homologous complexes. That anti-theta serum treatment has no effect on B cell responsiveness per se

TABLE I

Effect of Anti-Theta Serum Treatment on Response of Transferred Secondary B Cells in Organ Fragment Culture

Treatment of donor cells*	Recipient primed with	Maximum amount of antibody released per culture per day‡
None	—	20 ± 4 ng
Anti-theta serum + GPC	—	2 ± 1 ng
None	Hy	48 ± 6 ng
Anti-theta serum + GPC	Hy	50 ± 7 ng

* 2×10^7 spleen cells from DNP-Hy-immunized mice injected into all recipients.

‡ All fragments stimulated with 10^{-6} M DNP determinant concentration on Hy.

is indicated by the normal response of anti-theta-treated cells transferred to carrier-immunized recipients. Furthermore, the number of secondary B cells that give rise to clones in fragment cultures derived from carrier-immunized recipients is not diminished by anti-theta serum treatment (8).

Table II shows the response obtained when 1–2 $\times$ 10^6 donor cells with and without anti-theta serum treatment were transferred to nonimmune irradiated recipients. Treatment with anti-theta serum reduced by only 30–40% the number of foci stimulated by the hapten on the homologous carrier and did

TABLE II

Effect of Anti-Theta Serum Treatment on the Monofocal Response to Hapten on Homologous and Heterologous Carriers

Treatment of donor cells*	Antigen and DNP-lysyl determinant concentration	Foci/10^6-injected cells producing		
		0.7-3 ng of antibody per day	>3 ng of antibody per day	Total and standard error‡
None	DNP-Hy 10^{-7} M	0.8	0.7	1.5 ± 0.1
Anti-theta serum + GPC	DNP-Hy 10^{-7} M	0.8	0.2	1.0 ± 0.3
None	DNP-BSA 10^{-7} M	0.7	0.3	1.0 ± 0.2
Anti-theta serum + GPC	DNP-BSA 10^{-7} M	0.9	0.2	1.1 ± 0.3
None	DNP-BGG 10^{-7} M	0.9	0.2	1.1 ± 0.3
Anti-theta serum + GPC	DNP-BGG 10^{-7} M	0.8	0.2	1.0 ± 0.3
None	DNP-PLL 10^{-9} M	0.9	0	0.9 ± 0.3
Anti-theta serum + GPC	DNP-PLL 10^{-9} M	0.8	0	0.8 ± 0.3

* 1–2 $\times$ 10^6 spleen cells from DNP-Hy-immunized mice injected per recipient.

‡ Data represent at least 5 million injected cells for analysis of each antigen in a total of three or more separate experiments. The standard deviation reflects variance in values obtained from separate experiments.

not lower at all the frequency of foci stimulated with hapten on heterologous carriers. Thus, in spite of the fact that anti-theta treatment reduces by 90% the amount of antibody produced when 2 $\times$ 10^7 cells are transferred to irradiated non-carrier-primed recipients, the number of isolated precursor cells responding in such recipients shows only a minimal dependence on theta-sensitive cells when the hapten is presented on the homologous carrier. No dependence on theta-sensitive cells is observed when the hapten is presented on heterologous carriers. Significantly, after anti-theta serum treatment the frequency of foci stimulated by the homologous antigen and the amount of antibody produced by such foci are similar to that obtained with heterologous carriers, including PLL, a poor immunogen in mice (17).

DISCUSSION

The studies reported here confirm the notion that hapten on heterologous carriers can directly stimulate secondary B cells without the participation of T cells specific for the antigen by demonstrating clonal stimulation with hapten on a relatively nonimmunogenic carrier, PLL, and by showing that clonal stimulation by hapten on heterologous carriers is not diminished by the elimination of transferred T cells. Thus, when spleen cells from a mouse immunized to DNP-Hy were transferred to nonimmune irradiated recipients, one fragment produced anti-DNP antibody per 10^6 injected cells after stimulation with DNP-BSA, DNP-BGG, or DNP-PLL. This number was the same even after the donor cells were treated with anti-theta serum and complement. Such treatment, however, could be shown effective in reducing by 90% the degree of stimulation obtained when large numbers of cells (2×10^7) were transferred and did reduce the number of isolated B cells stimulated by the homologous antigen to a level equivalent to that achieved by hapten on heterologous carriers. Thus, in the absence of specific T cells, isolated secondary B cells are stimulated equally by hapten on homologous, heterologous, or nonimmunogenic carriers.

The finding that after anti-theta serum treatment of spleen cells heterologous antigens stimulate as many B cells as does the homologous antigen indicates that few, if any, B cells recognize a haptenic determinant in conjunction with determinants in its environment on the carrier used for immunization. Thus, the so called "local environment" (18) of a hapten appears to play little role in B cell recognition. The reduction by anti-theta serum in the frequency of B cells stimulated by the homologous antigen does, however, indicate that suspensions of secondary spleen cells contain occasional carrier-specific T cells that can elicit stimulation of B cells if both reside in the same fragment. Since collaboration with specific T cells results in an increase in the amount of antibody produced by a stimulated clone (14), the occurrence of clones producing large amounts of antibody as a result of stimulation with the homologous antigen may also result from the occasional presence of carrier-specific T cells. This T cell enhancement of the clonal response is consistent with the finding that the elimination of T cells results in most foci producing only small amounts of antibody regardless of the antigen used for stimulation.

Since anti-theta serum did not decrease the number of foci stimulated by hapten on heterologous carriers, it is unlikely that T cells recognizing haptenic determinants influence the number of stimulated foci when small numbers of cells ($1–2 \times 10^6$) are transferred. This would imply that the number of hapten-specific T cells in the spleen of an immunized mouse is significantly smaller than the number of carrier-specific T cells.

The data presented in this paper clearly demonstrate that secondary B cells can be stimulated by hapten-carrier complexes in the absence of T cells. While

the mechanism of such stimulation is not known, we have previously postulated that the receptors of secondary B cells, being multivalent, could be cross-linked by multiply substituted antigens at low concentrations and that such receptor interlinkage is necessary for stimulation (14). The inability of B cells from nonimmune mice to be stimulated in the absence of specific T cells (14) would thus be consistent with the postulate that the monovalence of primary B cell receptors limits their interlinkage (14).

The amount of antibody produced per stimulated focus is far lower in the absence of specific T cells than that obtained by foci in fragments from carrier-primed recipients (14). Furthermore, only 10% of the precursors stimulated in carrier-primed recipients are stimulated in the absence of specific T cells (14). In spite of the fact that only a fraction of maximum stimulation is obtained in the absence of carrier specific T cells, the mechanism of T cell-independent B cell stimulation may be important in early defense against viruses and other agents presenting repeating determinants, especially when antigen concentration is low.

SUMMARY

Treatment of spleen cell suspensions from immunized mice with anti-theta serum and complement before transfer to nonimmune irradiated recipients reduced the degree of in vitro stimulation by hapten-homologous carrier complexes by 90%, but did not decrease at all the number of isolated precursor cells stimulated by hapten on heterologous carriers. Thus, secondary B cells can be stimulated by low concentrations of multiply substituted hapten-carrier complexes in the apparent complete absence of specific T cells.

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