ANTIGEN-SPECIFIC, *H-2*-RESTRICTED HELPER T CELL HYBRIDOMAS*

By NEAL W. ROEHM,‡ PHILIPPA MARRACK, and JOHN W. KAPPLER§

From the Department of Medicine, National Jewish Hospital and Research Center, Denver, Colorado 80206

The role of helper T cells in B cell responses to antigen has been debated for many years. Generally, two types of activities have been attributed to helper T cells. The first somehow directly involves the action of the T cell receptor for antigen either as a cell-free mediator or in a cell-bound state (1–3) and has usually been demonstrated in responses to protein-bound antigens. Two proposals have been made for the mode of action of this antigen-specific activity. The first and oldest proposal is that in combination with antigen the T cell receptor is involved in the presentation of a particularly stimulatory antigenic array to the B cell (1). The second proposal stems from the finding that many helper T cells have an apparent specificity for both antigen and a product of the major histocompatibility complex, usually an *I* region product in the mouse (4–6). It has been suggested that interaction of the helper T cell receptor(s) for antigen/*I* delivers some stimulatory signal directly to the cell bearing them, which in some cases may be a B cell (4, 5).

In this regard it is of interest that there has been a longstanding controversy over the role of B cells and/or macrophages (Mϕ)[1] in the presentation of antigen to helper T cells during the induction of B cell responses to antigen. Whereas there is universal agreement that antigen presentation by Mϕ is crucial (7, 8), presentation by B cells does not always seem to be required (9), although many groups have found that it is (4, 10, 11). This dichotomy may depend upon the subset of B cells being studied (12). Of course, any model proposing that some essential activating signal is delivered by a helper T cell to a B cell directly through recognition of antigen/*I* on that B cell surface in fact predicts that B cell antigen presentation should be required for the successful induction of response.

A second type of activity that has been implicated in the action of helper T cells is the production of a group of nonspecific factors. For example, it has been shown that factors of this kind are sufficient to drive B cell responses to erythrocyte (RBC)-bound antigens (13, 14), although the question of which, and how many, of these factors actually are required is still being investigated (15, 16).

A series of experiments from a number of laboratories have suggested that the two

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[1] *Abbreviations used in this paper:* α-MM, alpha-methyl-D-mannoside; CFA, complete Freund's adjuvant; Con A M, concanavalin A media; Con A SN, supernatant of Con A-activated spleen cells; HGG, human gamma globulin; HRBC, horse erythrocytes; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; Mϕ, macrophage; OVA, chicken ovalbumin; PFC, plaque-forming cell; RBC, erythrocyte; TNP, trinitrophenol.

types of helper activity noted above, one antigen- and perhaps *I* region-specific, and the other nonspecific, are both required by B cells responding to protein-bound haptens. Anti-trinitrophenol (TNP) protein responses are absolutely dependent on the presence of carrier-primed helper T cells (13). However, synergy has been reported (17, 18) between this kind of T cell and others capable of producing nonspecific activities.

Several obstacles have prevented a complete understanding of how the helper T cells are operating in these responses. First, it has been difficult to evaluate directly the effects of helper T cell recognition of antigen/*I* or *I* alone on B cell surfaces for a number of reasons. These include experimental problems encountered when trying to couple antigen to B cells by means other than via the immunoglobulin receptor, the fact that the use of mixed populations of T cells has rendered effects hard to evaluate, and the knowledge that recognition of allogeneic *I* by T cells leads to production of many nonspecific helper factors that may mask other effects. Secondly, it has been hard to unequivocally test the synergistic effects of antigen-specific helper T cells and nonspecific helper factors, because addition of the latter may always have effects on the former as well as, or instead of, effects on the B cells under study.

In this paper we describe experiments using antigen-specific, *I* region-restricted T cell hybridomas as a source of antigen-specific helper activity. These cells, originally selected by their ability to secrete interleukin 2 (IL-2) in response to antigen/*I*, have proved to be potent sources of antigen-specific, *I* region-restricted helper activity. For effective help to be delivered by these hybridomas, antigen presentation by the responding B cells was required. In addition, the helper effects of several of the hybridomas were dependent upon the addition to responding cultures of nonspecific factors, in the form of supernatants of concanavalin A-activated spleen cells (Con A SN). Because the T cell hybridomas were presumably unaffected by the presence or absence of nonspecific factors, this documented a requirement for both antigen-specific help and nonspecific factors in the B cell response to protein-bound antigens. Finally, our experiments suggested that stimulatory signals might be delivered to B cells independently of their immunoglobulin receptors by T cell recognition of antigen/*I* or *I* alone on B cell surfaces.

Materials and Methods

Mice. B10.D2nSn mice were purchased from The Jackson Laboratory, Bar Harbor, ME. C57BL/10SgSn (B10) and B10.M mice were bred in our facility from breeding triplets purchased from The Jackson Laboratory. B10.Q mice were bred from triplets kindly provided by Dr. David Sachs, National Institutes of Health.

Antigens and Other Reagents. Complete Freund's adjuvant (CFA) and *Escherichia coli* lipopolysaccharide were purchased from Difco Laboratories, Detroit, MI; 2,4,6-trinitrobenzenesulfonic acid (TNP) from Pierce Chemical Co., Rockford, IL; α-methyl-D-mannoside (α-MM), Con A, and chicken ovalbumin (OVA) from Sigma Chemical Co., St. Louis, MO; and keyhole limpet hemocyanin (KLH) from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA. Human gamma globulin (HGG) was purified from American Red Cross Cohn Fraction II by DEAE cellulose chromatography. Horse erythrocytes (HRBC) obtained from an individual animal were purchased from Colorado Serum Co., Denver, CO. These antigens were prepared and used in their TNP-conjugated or unconjugated forms as previously described (19, 20).

Immunizations. Animals were immunized for OVA-, KLH-, or HGG-specific helper T cells by injection with 100 μg antigen emulsified in CFA at the base of the tail. Para-aortic and

inguinal lymph nodes were taken 7 d later (21). Splenic B cells were immunized for anti-TNP responses by intraperitoneal injections of 10 μg TNP-LPS 7 d before use.

Cell Preparations. Carrier-primed T cells were isolated from lymph node suspensions by passage over nylon fiber columns (22). T cell-free, B cell/macrophage preparations were made by treating spleen cell suspensions with monoclonal anti-Thy-1 (kindly provided by Ian Trowbridge, The Salk Institute, La Jolla, CA) plus rabbit anti-mouse T cell serum (M. A. Bioproducts, Walkersville, MD, absorbed as in [23]) and then rabbit serum as the complement source (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY).

Cell Culture. 3×10^6 B cell/Mϕ were cultured in 0.7 ml of medium in Linbro 76-033-05 24-well culture trays (Linbro Chemical Co., Hamden, CT). The indicated number of carrier-primed lymph node T cells or irradiated (4,000 rad) hybridoma cells were added to each well. Culture conditions were modified from those of Mishell and Dutton (7, 24). Antigen concentrations were TNP-KLH 0.2 μg/ml, TNP-HGG 2.0 μg/ml, and TNP-OVA 1.0 μg/ml, except where otherwise indicated.

Assay for Plaque-forming Cells (PFC). On day 4 of culture, anti-TNP-direct PFC were assayed by a slide modification of the hemolytic plaque assay using TNP-conjugated HRBC (24). In some cases, helper activity is expressed as the expected anti-TNP PFC response per culture per 10^6 T cells based on a linear regression equation fitting the observed anti-TNP PFC response following titration of the helper cell population.

Preparation of Con A SN. Spleen cell suspensions were cultured for 24 h with 4 μg/ml Con A. Supernatants were then harvested and stored at −20°C until use. To prevent effects due to residual Con A in SN, α-MM was added to the Con A SN before use to give a final concentration of 10 mg/ml in culture. To test the effects of residual Con A, control medium containing Con A and α-MM (Con A M) was prepared as described above except that it was not incubated with spleen cells.

T Cell Hybridomas. The antigen-specific, *H-2*-restricted T cell hybridomas used in these experiments were prepared as previously described (25, 26). The origin and properties of the hybridomas are listed in Table I.

Results

Helper Activity of T Cell Hybridomas. To test the helper activity of antigen/*I*-specific T cell hybridomas, we used a system previously developed to measure the activity of normal carrier primed T cells (3), in which the cells to be assayed were titrated into cultures containing B cells, Mϕ, and TNP-coupled protein antigen. In previous experiments (17, 29), we had shown that the generation of optimal antibody responses to soluble antigens required nonspecific helper factors in addition to an antigen-specific T cell signal. Because we were not sure that the T cell hybridomas could provide all these nonspecific signals, and because we wished to measure their antigen-specific activity under conditions where nonspecific factors were not limiting, we

TABLE I

Hybridoma	Normal T cell parent		Fusion partner	Hybridoma produces IL-2 when stimulated with:
	Strain	Antigen specificity		
AODK 10.4	B10.D2 (*H-2^d^*) →	KLH	A040.10AG1	Con A; KLH + $I\text{-}A^d$
AODH 3.4	DBA/2 ($H\text{-}2^d$)	HGG	A040.10AG1	Con A; $H\text{-}2^b$; OVA + $I\text{-}A^k$
AODH 7.1	DBA/2 ($H\text{-}2^d$)	HGG	A040.10AG1	Con A; HGG + $I\text{-}E^d$
BDK 11.1	B6D2F1 ($H\text{-}2^{b \times d}$)	KLH	BW5147	Con A; KLH + $I\text{-}A^b$
A040.10AG1	B10.A ($H\text{-}2^a$)	OVA	FS6 14.13	Con A; $H\text{-}2^b$; OVA + $I\text{-}A^k$
FS6 14.13	B6D2F1 ($H\text{-}2^{b \times d}$)	None	BW5147	Con A

tested the hybridomas for help in the presence or absence of Con A SN, added as a source of the nonspecific factors (17).

Fig. 1 shows typical results with two hybridomas, AODK 10.4, specific for $I\text{-}A^d$/KLH, and AODH 7.1, specific for $I\text{-}E^d$/HGG, each cultured with TNP-KLH or TNP-HGG and with B10.D2 B cell/Mφ. Hybridoma cells were irradiated with 4,000 rad before testing.

As shown in Fig. 1A, in the presence of Con A SN, AODK 10.4 allowed the generation of an anti-TNP PFC response by B10.D2 B cells; its activity was significantly greater than that of KLH-primed lymph node T cells on a per cell basis. The PFC response generated with AODK 10.4 was absolutely dependent on the nonspecific factors present in Con A SN, although these factors alone did not stimulate a response. The HGG-specific hybridoma AODH 7.1 was not able to mediate this helper function using TNP-KLH as antigen in the presence of Con A SN.

However, AODH 7.1 mediated significant helper function using TNP-HGG as antigen with B cell/Mφ from the same pool (Fig. 1 B). Again the helper function of AODH 7.1 was significantly greater than that of HGG-primed lymph node T cells on a per cell basis. In contrast to AODK 10.4, the helper activity of AODH 7.1 upon stimulation with its specific antigen was not totally dependent on the addition of Con A SN, although the PFC responses were consistently greater when the nonspecific factors were added (see also Fig. 2). Confirming the KLH-specific helper function of AODK 10.4, significant PFC responses were not generated with this T cell hybridoma upon stimulation with TNP-HGG.

We were interested in finding out how general the dependence was on Con A SN factors of T cell hybridoma helper activity. Therefore, six different T cell hybridomas were tested, each titrated into cultures containing TNP-antigen and B cell/Mφ

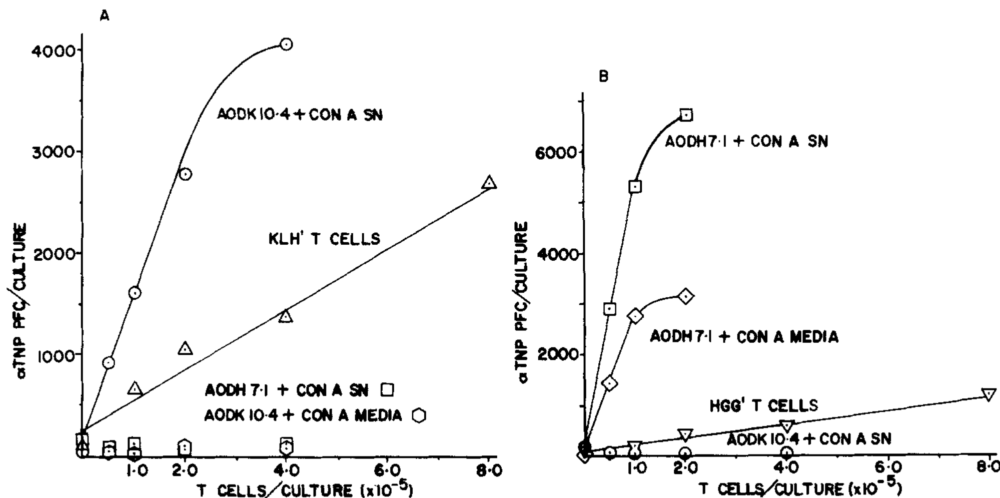

FIG. 1. Carrier-specific helper function of T cell hybridomas. Helper T cells in the form of either carrier-primed B10.D2 lymph node T cells or irradiated T cell hybridomas, AODK 10.4 (KLH, $I\text{-}A^d$) or AODH 7.1 (HGG, $I\text{-}E^d$), were added to duplicate Mishell-Dutton cultures of TNP-LPS-primed B10.D2 B cell/Mφ stimulated with either TNP-KLH (panel A) or TNP-HGG (panel B) as antigen. Hybridomas were assayed for T cell help in the presence of either Con A SN as a source on nonspecific factors or Con A M as a control. Anti-TNP PFC measured on day 4 using a Jerne hemolytic plaque assay.

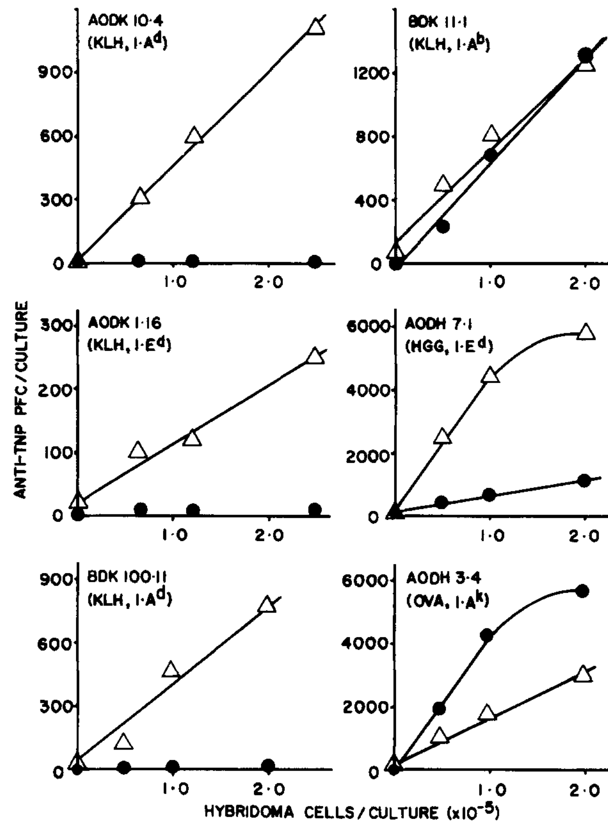

FIG. 2. Nonspecific factor dependence of anti-TNP PFC response generated using T cell hybridomas as the source of antigen-specific help. The indicated T cell hybridomas were titrated into duplicate cultures containing TNP-LPS-primed B cell/Mϕ of the appropriate *I* region haplotype and TNP conjugated to the indicated carrier as antigen. The duplicate cultures were stimulated in the presence of Con A SN as a source of nonspecific factors (△) or Con A M (●) as the control, and the anti-TNP PFC response was determined on day 4 of culture.

bearing the *I* region haplotype for which the T cell hybridomas were specific. As shown in Fig. 2, in the presence of Con A SN all hybridomas delivered helper activity, as measured by the development of anti-TNP PFC by B cells in the cultures. Interestingly, the PFC responses varied considerably in their dependence on Con A SN. The appearance of hapten-specific PFC was absolutely dependent on added nonspecific factors when help was provided by three T cell hybridomas, AODK 10.4 (see also Fig. 1A), AODK 1.16, and BDK 100.11. A fourth hybridoma, BDK 11.1, was active whether or not Con A SN was added. The response seen with AODH 7.1 was again improved by Con A SN and the activity of one hybridoma, AODH 3.4, was actually greater in the absence of Con A SN. Not shown is the fact that in every case the hybridomas, under optimal conditions, had greater helper activity on a per cell basis than conventional antigen-primed T cells, and in addition, the fact that in every case, help was both carrier and *H-2* specific. We attribute the differential dependence on nonspecific factors of the PFC response to the different abilities of the T cell hybridomas themselves to secrete needed lymphokines. A more complete analysis of these phenomena is in progress.

H-2 Restriction of Antigen-specific Help. The availability of cloned T cell hybridomas with antigen-specific helper function offered an ideal opportunity to test requirements for *H-2* identity between collaborating helper T cells and B cells in the absence of allogeneic effects. The T cell hybridomas AODK 10.4 and BDK 11.1 both produce IL-2 in response to the specific antigen KLH, although AODK 10.4 is $I\text{-}A^d$ restricted and BDK 11.1 is $I\text{-}A^b$ restricted. We therefore used these two hybridomas as a reciprocal pair in a test of the *H-2* restriction of their helper activities. As shown in Fig. 3, the $I\text{-}A^d$-restricted hybridoma, AODK 10.4, allowed the generation of an anti-TNP PFC response by nonirradiated $H\text{-}2^d$ B10.D2 B cells, but not by $H\text{-}2^b$ B10 B cells, even in the presence of functional irradiated $H\text{-}2^d$ (B10.D2) antigen-presenting cells. Conversely, the $I\text{-}A^b$-restricted hybridoma, BDK 11.1, allowed the generation of an anti-TNP PFC response by nonirradiated $H\text{-}2^b$ B10 B cells, but not $H\text{-}2^d$ B10.D2 B cells, even in the presence of functional irradiated $H\text{-}2^b$ (B10) antigen-presenting cells. In each case controls demonstrated that the presence of irradiated *H-2*-disparate B cell/Mϕ did not inhibit the PFC responses generated by nonirradiated B cells and the appropriate T cell hybridoma. We should also mention that after irradiation with 4,000 rad, the T cell-depleted B cell/Mϕ used in this experiment were demonstrated to function normally as antigen-presenting cells in the production of IL-2 by the appropriate T cell hybridomas (data not shown), but were no longer able to develop into PFC. We concluded that the delivery of the antigen-specific helper signal in response to low concentrations of soluble antigens was controlled at least by *H-2* antigens expressed by B cells.

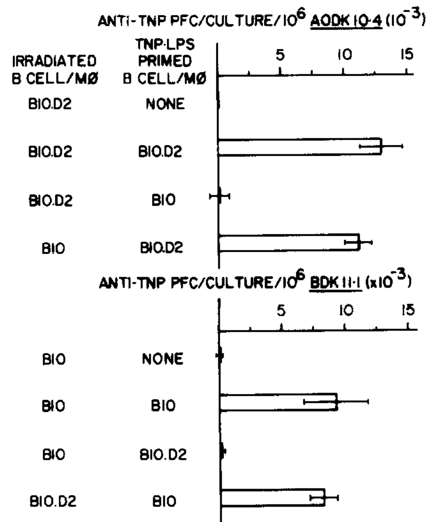

FIG. 3. Delivery of antigen-specific helper signal by T cell hybridomas restricted by *H-2* antigens on B cells. Irradiated AODK 10.4 (KLH, $I\text{-}A^d$) or BDK 11.1 (KLH, $I\text{-}A^b$) T cell hybridomas were titrated into Mishell-Dutton cultures stimulated with TNP-KLH in the presence of Con A SN. TNP-LPS-primed T cell-depleted B cell/Mϕ from B10.D2 ($H\text{-}2^d$) or B10 ($H\text{-}2^b$) mice were assayed for their anti-TNP PFC response in the presence of irradiated (4,000 rad) B10.D2 or B10 B cell/Mϕ as a source of antigen-presenting cells. In control experiments, the irradiated B10.D2 and B10 B cell/Mϕ were shown to functional normally as KLH antigen-presenting cells in the production of IL-2 by AODK 10.4 and BDK 11.1, respectively. Data are expressed as the expected anti-TNP PFC per culture per 10^6 hybridoma cells ± SE.

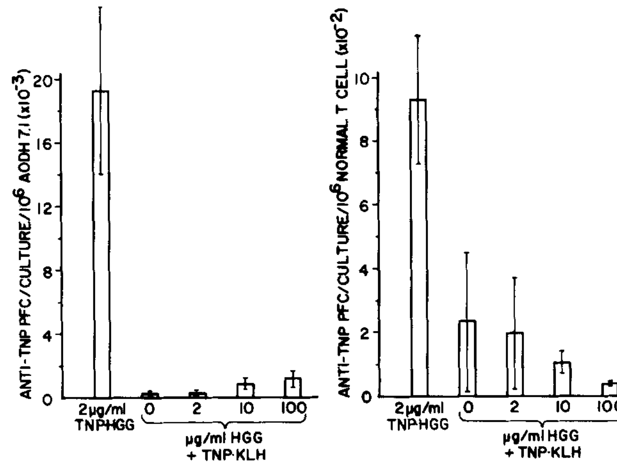

FIG. 4. Requirement for linked recognition at low concentrations of carrier antigen. HGG-specific helper T cells in the form of irradiated AODH 7.1 hybridoma cells (plus Con A SN) or normal HGG-primed lymph node T cells were titrated into duplicate cultures of TNP-LPS-primed B10.D2 B cell/Mϕ. Cultures were stimulated with either TNP conjugated to the specific carrier HGG as the positive control or TNP conjugated to an irrelevant carrier KLH plus increasing concentrations of free HGG. Data are expressed as the expected anti-TNP PFC response per culture per 10^6 hybridoma cells ± SE. Immunogenicity of the TNP-KLH demonstrated using normal KLH-primed lymph node T cells (3,050 ± 475 anti-TNP PFC/culture/10^6 KLH-primed T cells).

Requirement for Linked Recognition in Antigen-specific Helper Function at Low Antigen Concentrations. Linked recognition is a characteristic that has been associated with carrier-specific help in anti-hapten antibody responses, i.e., collaboration between carrier-primed T cells and hapten-primed B cells is often not observed upon stimulation with the specific carrier and conjugates of the hapten and an unrelated carrier (27).

In the experiment presented in Fig. 4, we tested whether linked recognition was required for the antigen-specific helper function of the HGG specific hybridoma AODH 7.1. Positive controls using TNP-HGG as antigen demonstrated the antigen-specific helper activity of AODH 7.1- and HGG-primed lymph node T cells. Stimulation of these same populations with TNP-KLH and free HGG in the range of 2.0–100 μg/ml resulted in very small responses compared with those observed with conjugates of TNP and the specific carrier HGG. That the TNP-KLH was in fact immunogenic was demonstrated by the response elicited using KLH-primed lymph node T cells (see legend to Fig. 4).

We observed similar results using the KLH-specific T cell hybridoma AODK 10.4 stimulated with TNP-HGG and free KLH (0.2–100 μg/ml) (data not shown). We concluded that within this antigen dose range, the delivery of the antigen-specific helper signal required linked recognition of the hapten-carrier conjugate.

B Cell Activation in the Absence of Linked Recognition. One of the shortcomings of the linked recognition experiments described in the preceding section was that the antigens tested, KLH and HGG, could not be used as bystander antigens at concentrations >100 μg/ml without nonspecifically suppressing B cell responses (data not shown). To test even higher bystander antigen concentrations, we used the antigen OVA (which is not inhibitory, even at 10 mg/ml), and the T cell hybridoma AODH

3.4, specific for OVA and $I\text{-}A^k$. In these experiments, we compared responses obtained with TNP-OVA to those obtained with free OVA.

Fig. 5 shows the dose-response profile of TNP-primed B10.A B cells stimulated with AODH 3.4 and either TNP-OVA or free OVA. Using the TNP-carrier-linked antigen, an optimal anti-TNP PFC response was seen in the 0.1–10.0 μg/ml range. With free OVA, no significant anti-TNP response was seen in this experiment below 100 μg/ml, but responses equivalent to those obtained with TNP-OVA were observed in the 1–5 mg/ml range. Two additional points are worth noting. First, the anti-TNP PFC response seen with free OVA did not depend on the addition of TNP antigen to the cultures. In fact, addition of TNP-KLH at 0.2 μg/ml actually suppressed this bystander response somewhat (data not shown). Second, no anti-TNP PFC response was seen with either TNP-OVA or with free OVA using B cells from unprimed or LPS primed mice (see below). Thus, under conditions where very high concentrations of free carrier could be used, carrier-specific helper T cells collaborated with hapten-primed B cells in the generation of a specific antibody response without apparent involvement of the B cell's immunoglobulin receptor.

Because these and other (10) experiments suggested that T cell recognition of antigen/*I* on B cells could deliver a helper signal directly to that B cell, we reasoned that T cell recognition of *I* alone might have the same effect. AODH 3.4 was again useful in approaching this question, because in addition to its OVA/$I\text{-}A^k$ reactivity, it was reactive to $I\text{-}A^b$ in the absence of antigen.

As shown in Fig. 6, irradiated AODH 3.4 hybridoma cells were titrated into cultures of TNP-LPS-primed B cells of different *H-2* haplotypes. In the absence of antigen, AODH 3.4 allowed the generation of anti-TNP PFC responses with B cells of the $H\text{-}2^b$ haplotype (B10), but not with B cells of the $H\text{-}2^d$ (B10.D2), $H\text{-}2^f$ (B10.M), or $H\text{-}2^q$ (B10.Q) haplotypes. That these B cells were in fact able to respond was shown using TNP-OVA as antigen and OVA-primed lymph node T cells syngeneic with the B cells.

The anti-TNP PFC response of the $H\text{-}2^b$ B cells did not require the addition of

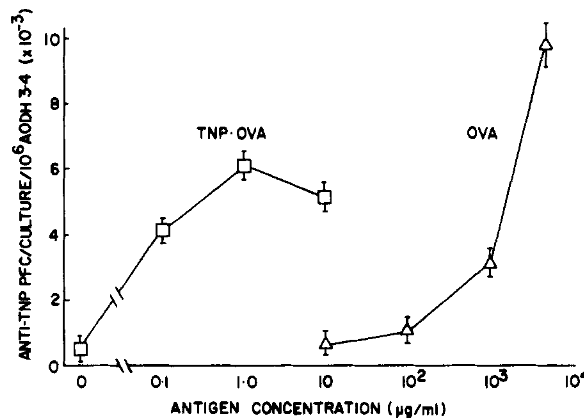

FIG. 5. B cell activation in the absence of linked recognition. Helper activity of AODH 3.4 as a function of antigen concentration. Irradiated AODH 3.4 (OVA, $I\text{-}A^k$) were titrated into Mishell-Dutton cultures of TNP-LPS-primed B10.A B cell/Mϕ. Cultures were stimulated with the indicated concentration of either TNP-OVA or free OVA, and the data are expressed as the anti-TNP per culture per 10^6 AODH 3.4 hybridoma cells ± SE.

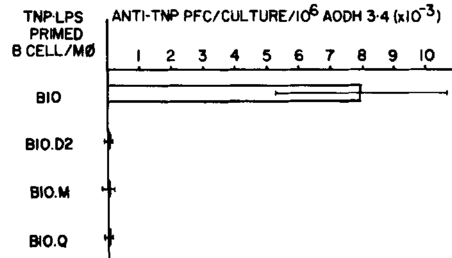

Fig. 6. Direct recognition of hapten primed B cell *I* region antigens by T cell hybridoma results in B cell activation. Irradiated AODH 3.4 ($I\text{-}A^b$; OVA + $I\text{-}A^k$) hybridoma cells were titrated into cultures of TNP-LPS-primed B cell/Mφ of B10 ($H\text{-}2^b$), B10.D2 ($H\text{-}2^d$), B10.M ($H\text{-}2^f$), or B10.Q ($H\text{-}2^q$) mice without added antigen. Data expressed as the anti-TNP PFC per culture per 10^6 AODH 3.4 ± SE. The ability of the TNP-LPS primed B cells to generate anti-TNP PFC was demonstrated in control cultures using syngeneic OVA-primed lymph node T cells and TNP-OVA as antigen. The calculated anti-TNP PFC per culture per 10^6 syngeneic lymph node T cells ± SE were: B10, 662 ± 70; B10.D2, 750 ± 22; B10.M., 837 ± 30; and B10.Q, 669 ± 36.

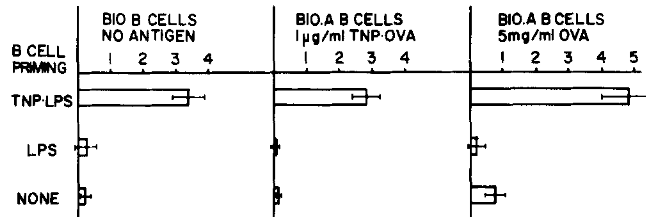

Fig. 7. Requirement for hapten priming in the generation of B cell anti-TNP PFC. B10 ($H\text{-}2^b$) or B10.A ($H\text{-}2^a$) mice were primed with either TNP-LPS (10 μg), LPS (10 μg), or left unprimed. Irradiated AODH 3.4 hybridoma cells were titrated into separate cultures of T cell depleted B cell/Mφ of the B10 mice without antigen or B cell/Mφ of the B10.A mice using either TNP-OVA (1 μg/ml) or OVA (5 mg/ml) as antigen. Data are expressed as the anti-TNP PFC per culture per 10^6 AODH 3.4 ± SE.

TNP antigen to the cultures. Thus, primed B cells could be triggered to develop into PFC using a T cell hybridoma that directly recognizes the B cell's surface *I-A* in the absence of overtly added antigen.

As demonstrated in Fig. 7, the generation of an anti-TNP PFC response using AODH 3.4 and B10 ($H\text{-}2^b$) B cells without antigen or B10.A ($I\text{-}A^k$) B cells and either microgram quantities of TNP-OVA or milligram quantities of OVA alone, required priming of the B cell donor with TNP-LPS. Significant anti-TNP PFC responses were not observed in these systems using unprimed or LPS-primed B cell donors.

Discussion

A number of points dealing with how helper T cells work are addressed by the data in this paper. First, over the years it has been difficult to reconcile data concerning the effects of nonspecific helper factors, in this and many other papers defined as those present in the Con A-activated supernatants of normal spleen cells, and the effects of antigen-specific T cells. Although it is well established that B cell responses to certain antigens, most notably RBC bound, can be driven by nonspecific factors alone, the same does not seem to be true for responses to protein-bound antigens (13, 17), except under special circumstances (28). However, several recent experiments (17, 29) have suggested that nonspecific factors might play a necessary, but not sufficient

role in this type of response. In the past such a role has been difficult to evaluate because any source of nonspecific helper factors added to mixtures of B cells and antigen-specific helper T cells might affect either one or both cellular component(s) of the mixture. In the experiments described in this paper, for the first time, effects of nonspecific helper factors could be evaluated in an experimental system in which we could be fairly sure that the target of the factors was not an antigen-specific T cell. AODK 10.4, for example, was unable to stimulate an anti-TNP PFC response of *H-2*^{d} B cells and Mϕ in the absence of Con A SN. Con A SN alone was equally ineffective. In the presence of Con A SN, however, AODK 10.4 stimulated a good anti-TNP PFC response, thus demonstrating unequivocally a need for both antigen-specific T cells and nonspecific helper factor(s) in the response. The target of the Con A SN was most probably the responding B cell, as these nonspecific factors have no effect on the growth, secretion of IL-2, or viability of AODK 10.4 (data not shown). We cannot exclude the fact that Con A SN may induce AODK 10.4 or Mϕ to secrete some other nonspecific factor not present in the putative inducing reagent; however, the fact that direct effects of materials in Con A SN on B cells have been demonstrated (15, 30, 31) suggests that this is not the most likely mode of action for the mixture.

Three of the six tested T cell hybridomas described in this paper required that Con A SN be added to demonstrate help. Con A SN had no effect with a fourth, improved the response with a fifth, and actually suppressed the response with the sixth. We postulate that this is due to the fact that each of our T cell hybridomas is capable of producing a different combination of lymphokines when challenged with antigen plus antigen-presenting cells. Although in the case of all the hybridomas tested in this study one of these lymphokines is IL-2, we do not yet know what other factors are made by these hybrids, nor do we know which lymphokines are needed for B cell IgM responses to protein-bound antigens. By analogy with antibody responses induced with anti-immunoglobulin or RBC, more than one nonspecific factor may be needed, including Mϕ-derived factors, IL-2, B cell growth factor, and thymus-replacing factor (15, 16, 30–36). Further experiments are planned to study this. In any case, experiments with the three hybrids that showed helper activity only in the presence of added lymphokines clearly support earlier findings (17, 18, 29) that B cell responses to protein antigens need nonspecific helper signals as well as an antigen-specific signal from helper T cells.

Hybridoma T cells apparently behaved very much as normal T cells do in the induction of antibody responses. The experiments in this paper showed that at moderate or low antigen concentrations, the hybridomas had to recognize carrier linked to the hapten against which the B cell response was measured for B cell stimulation to be observed. Moreover, the hybridoma T cells were restricted for help by *H-2* products, and these had to be borne by the responding B cells in the cultures, a finding we (7, 10) and others (4, 11) have consistently made in the past.

Two observations in this paper were surprising, however. First, we unexpectedly found that at very high carrier concentrations, anti-TNP PFC were stimulated in vitro even in the absence of deliberately added hapten. Three explanations for this were possible. Antigen might have been carried over from the mouse during preparation of B cells, as responding B cells had to be obtained from TNP-primed animals. Alternatively, the B cells may have been responding to an in vitro antigen, in the fetal calf serum for example, that cross-reacted with TNP. If either of these hypotheses

were true, the activity of the unsuspected TNP antigen was only apparent at very high carrier concentrations. A third, and more interesting possibility is that at very high carrier concentrations, the protein was taken up, processed, and presented to hybridoma T cells by the responding B cells themselves, in the absence of a hapten/immunoglobulin receptor-mediated concentrating mechanism. In fact, previous studies from this laboratory have suggested that B cells could do exactly this (7, 10). More recent experiments from our own (37, 38) and other (39) laboratories have confirmed this idea using B cell tumor lines.

If this were indeed the correct explanation, it would suggest that B cells could be stimulated by interaction of T cells with antigen/*I* region products on their surface in the absence of a concomitant signal delivered through their immunoglobulin receptors, an idea proposed in the past by others (40) and supported by data from several laboratories (41, 42). This hypothesis is also supported by other data in this paper dealing with the consequences of recognition of allogeneic *I* region products on B cell surfaces by the hybridoma AODH 3.4. Our experiments showed that under these circumstances B cells could be driven to yield anti-TNP PFC in the absence of overtly added TNP, providing the responding cells came from TNP-primed animals.

An extension of this theory would propose that the phenomenon of linked recognition, at low hapten-carrier concentrations, occurs because the binding of hapten to surface immunoglobulin causes the concentration and uptake of carrier by hapten-specific B cells. The carrier will then be presented to T cells by hapten-specific B cells, which might thereby be stimulated. In this case, the role of surface immunoglobulin would be to concentrate and internalize hapten-carrier rather than to act as an intermediary for the delivery of antigen-specific signals to the B cell.

Another surprising finding in this study was that B cells had to be taken from TNP-primed animals. This was true even when the cells were responding in the apparent absence of TNP. Others who have observed polyclonal B cell stimulation under conditions rather reminiscent of ours did not find the same requirements (41). Over the years, however, we have consistently found that IgM anti-TNP protein responses in vitro are elicited most satisfactorily in hapten-primed B cells. This could either be because our culture conditions and T cells are insufficient to drive virgin B cells to full differentiation, or perhaps because of some failure of antigen presentation by virgin B cells in our cultures.

Finally, it was striking that out of six randomly chosen antigen/*I*-specific T cell hybridomas, all demonstrated carrier-specific, *H-2*-restricted helper activity. The implication of this result is that the presence of an antigen/*I*-specific receptor(s) on a T cell is sufficient for it to demonstrate such activity providing the necessary lymphokines are also present, either secreted by the T cell or added exogenously.

Summary

We have examined the carrier-specific helper activity of a number of antigen-specific, *I* region-restricted T cell hybridomas prepared in our laboratory. The hybridomas were assayed for helper activity in the presence or absence of exogenously added nonspecific factors found in the concanavalin A-activated supernatants of normal mouse spleen cells. Of six hybridomas tested, all six could stimulate the IgM anti-hapten responses of hapten-primed B cells in the presence of the appropriate hapten-carrier conjugates. At low or moderate carrier doses, the response was depend-

ent upon hapten-carrier linkage and the ability of the hybridoma cells to interact with carrier in association with *H-2* products of the responding B cells themselves. Plaque-forming cell responses stimulated by some of the hybridomas were absolutely dependent upon the addition of nonspecific factors, suggesting that anti hapten-protein responses require both an antigen specific *I* region restricted signal from the T cell hybridomas and nonspecific helper factors, made either by the T cell hybridomas or added exogenously.

Under two sets of circumstances, B cells were stimulated in the absence of a simultaneous signal delivered through their immunoglobulin receptor. This occurred either when hapten-primed B cells were stimulated with an ovalbumin/$I\text{-}A^k$-specific hybridoma in the presence of very high concentrations of ovalbumin, or when $H\text{-}2^b$ B cells were incubated with a hybridoma specific for $I\text{-}A^b$ alone. This was interpreted to mean that B cells can be stimulated by reaction of T cells with surface *I* molecules.

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