ANTIGEN PRESENTATION BY RESTING B CELLS

Radiosensitivity of the Antigen-Presentation Function and Two Distinct Pathways of T Cell Activation

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Typical T cells of the helper phenotype require that the foreign antigen be recognized in association with a molecule encoded within the I region of the mouse H-2 complex (1, 2), the Ia molecule (3, 4). Cells that bear such molecules and that are capable of activating T cells in the presence of antigen are known as antigen-presenting cells (APC).[1] Although much is known of the tissue distribution of Ia molecules, the nature of the cells responsible for antigen presentation is not completely clear (5). The adherent, nonspecific esterase positive, radioresistant macrophage has been considered to be the prototypic APC (6–8). However, studies by Steinman et al. (9) established that splenic and lymph node dendritic cells (Fc receptor negative, weakly adherent, nonspecific esterase negative, nonphagocytic cells) were capable of stimulating mixed lymphocyte responses. Sunshine et al. (10) further demonstrated that dendritic cells were capable of presenting soluble antigens to antigen-primed T cells, as assessed by T cell proliferation. Both hepatic Küpffer cells (11) and epidermal Langerhans cells (12) have also been shown to be capable of acting as accessory cells for T cell activation.

Attention has recently turned to the ability of B cells to present antigen to T cells (13, 14). It has been quite clearly shown that many B lymphomas possess APC activity (15–18). However, the antigen-presenting ability of normal B cells, particularly those in the resting state, remains uncertain. In 1981 Chesnut and Grey (19) investigated the ability of heavily irradiated (4,500 rads) macrophage-depleted spleen cells, prepared by passage over Sephadex G-10, to present antigen to antigen-primed T cells. They showed that T cells from donors immunized with rabbit immunoglobulin (Ig) proliferated in the presence of irradiated, syngeneic, macrophage-depleted spleen cells and rabbit anti-mouse Ig, but not with the original immunogen, normal rabbit Ig. In contrast, splenic adherent cells containing macrophages were capable of presenting both antigens equally well to the T cells. Since anti-Ig antibodies not only bind to mouse B

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[1] *Abbreviations used in this paper:* MHC, major histocompatibility complex; APC, antigen-presenting cell; HEL, hen egg lysozyme; Ig, immunoglobulin; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; OVA, ovalbumin; PPD, purified protein derivative.

cells, but also activate them, these results left open the question of the ability of resting B cells to act as APC. Indeed, Chesnut et al. (18) demonstrated that heavily irradiated LPS-activated B cell blasts were capable of presenting KLH to antigen-primed lymph node T cells and antigen-specific T cell hybridomas, whereas similarly irradiated normal B cells were not. More recently both Katz et al. (20) and Bandeira et al. (21) reported that normal B cells, treated either with mitomycin c or irradiation, failed to present ovalbumin (OVA) or minor histocompatibility antigens to specific T cell clones as measured by T cell proliferation. However, both groups demonstrated that untreated normal B cells themselves proliferated in the presence of specific T cells and antigen, implying that the B cells could act as APC for T cell activation leading to "recruitment" of B cell proliferation. These studies suggest that under some circumstances, resting B cells can present soluble antigen to antigen-specific, MHC-restricted T cells. However, the ability of untreated resting B cells to present antigen for T cell proliferation, still remains uncertain. Furthermore, little is known about the heterogeneity of the T cell response to B cells as APC.

In this paper we examine the ability of small resting B cells to act as APC to antigen-specific long-term in vitro T cell clones and lines as well as freshly explanted lymph node T cells. We report three new observations: (*a*) some, but not all, MHC-restricted T cells can be induced to proliferate (or to make interleukin 2 [IL-2]) in the presence of lightly irradiated B cells plus the appropriate foreign antigen, (*b*) in contrast to macrophages and dendritic cells, the antigen-presenting function of small resting B cells is very radiosensitive, and (*c*) APC can activate two distinct pathways in responding T cells, one that leads to proliferation of the T cell, and the other that leads to B cell activation and proliferation.

Materials and Methods

Animals. B10.A/SgSn (B10.A) mice were obtained from Harlan Sprague-Dawley (Madison, WI). B10.S(9R), B10.RIII, and B10.BR mice were the progeny of breeding pairs originally provided by Dr. Jack Stimpfling, McLaughlin Research Institute, Great Falls, MT. They were Caesarian-derived into our breeding colony by fostering on NIH General Purpose mice and subsequently propagated by brother-sister mating.

Antigen. The random terpolymer, poly-$(Glu^{60}Ala^{30}Tyr^{10})_n$ (GAT) was purchased from Vega Biochemicals, Tucson, AZ. Pigeon cytochrome *c* was purchased from Sigma Chemical Co., St. Louis, MO and purified on carboxymethyl cellulose as previously described (22). Cytochrome *c* cyanogen bromide cleavage fragment 81-104 was prepared as previously described (23) and provided by Dr. L. Samelson of our laboratory. Hen egg lysozyme (HEL) was purchased from Sigma Chemical Co.

Monoclonal Antibodies. Anti-Thy-1.2 was purchasd from New England Nuclear, Boston, MA. Anti-Lyt-1 (53.7.3) and anti-Lyt-2 (53.6.7) were prepared from hybridomas derived by Ledbetter and Herzenberg (24). MAR 18.5, a murine anti-rat kappa chain monoclonal antibody, was prepared from a hybridoma derived by Lanier et al. (25). The cells from which these antibodies were prepared were provided to us by Dr. John Kung of our laboratory.

Cell Preparations. Spleen cells from unprimed mice were incubated with anti-Lyt-1, anti-Lyt-2, and anti-Thy-1.2 for 30 min at 4°C. They were centrifuged and resuspended in guinea pig complement (1:4 dilution, Flow Laboratories, Rockville, MD) and the monoclonal antibody MAR 18.5, to increase the cytotoxicity of the anti-Lyt-1 and anti-Lyt-2 antibodies. The cells were incubated for 30 min at 37°C, then washed in RPMI 1640. This population is referred to as T-depleted spleen cells. After T cell depletion,

the cells were separated on a Percoll density gradient as previously described (26). The low density fraction (layering above the 50% Percoll layer, density <1.062 g/ml), has been shown to consist of ~50% IgM-bearing cells and 40–50% latex bead–ingesting cells. It is enriched in radioresistant APC activity. The high density cells (layering between the 60% and 70% Percoll layers, density between 1.074 g/ml and 1.086 g/ml) is composed of ~80% surface IgM-bearing cells, which have been shown to be resting B cells by several criteria (26). Stimulation of these cells with concanavalin A was performed as control for T cell depletion in every experiment and never caused any measurable proliferation in this cell population. These cells will be referred to as small resting B cells. In some experiments the high density cells were further purified by passage over columns of Sephadex G-10 (G-10–passed B cells) (27). Cells that did not bind to the Sephadex G-10 column were further purified in some cases by adherence at 4°C to petri plates (Corning, 100-mm tissue culture) coated with 10 μg/ml of affinity-purified goat anti-μ, 7 ml per plate (panned B cells) (28). Cytofluorometric analysis of these cells using goat anti-mouse light chain antisera demonstrated that the cells recovered after passage over Sephadex G-10 were ~95% Ig-bearing cells, and the cells recovered after panning were >99% Ig-bearing cells.

Radiation of Cells. All irradiation was delivered from a ^{137}Cs source (Gammator M; Isomedix Inc., Parsippany, NJ). Different doses were administered by varying the exposure time at a fixed distance from the source.

Immunizations. Immunizing antigens were emulsified in complete Freund's adjuvant (H37Ra, Difco Laboratories, Detroit, MI) and injected subcutaneously into the footpads and at the base of the tail in a total volume of 150 μl. Cells were obtained from the draining lymph nodes 7–10 d later and enriched for T cells by passage over nylon wool columns (29). T cells prepared in this manner for proliferation assays were further depleted of APC by treatment with two anti-Ia monoclonal antibody supernatants (10-2.16 (30) and M5/114 (31), anti I-A^k and I-E^k, respectively) plus complement. These antibodies were kindly provided by Dr. Gen Suzuki of our laboratory.

In Vitro Cell Culture. T cells obtained from immunized animals were cloned by limiting dilution and maintained in vitro by cycles of stimulation with antigen and irradiated (3,300 rads) syngeneic spleen cells, followed by a period of rest in the absence of antigen in the manner of Kimoto and Fathman (32). The cells were grown in a 1:1 (vol/vol) mixture of RPMI 1640 (Biofluids, Inc., Rockville, MD) and Eagle's-Hanks's amino acid (EHAA) medium prepared in the NIH media unit. This mixture was supplemented with 10% fetal calf serum, 4 mM glutamine, 100 U of penicillin, and 50 μM 2-mercaptoethanol (complete medium). The cells were not exposed to an exogenous source of IL-2 after they were cloned. The T cell clone 11.4 was established by soft agar cloning after the technique of Sredni and Schwartz (33). Unlike the other normal T cells used in these experiments, 11.4 was maintained solely with complete medium supplemented with 5% concanavalin A–stimulated rat spleen supernatant as a source of IL-2. Neither antigen nor filler cells were added. 11.4 responds to the antigen GAT in the context of the A^k_β:A^k_α Ia molecule. It is also alloreactive to *H-2*^{r}, but not to any Ia molecules encoded by the *H-2*^{s,d,q,u,b,f,t4} haplotypes.

T cell hybridomas were established as previously described (34). Briefly, antigen-stimulated T cell blasts were mixed with the hypoxanthine-aminopterin-thymidine–sensitive T cell line BW5147 in the presence of polyethylene glycol 1000 (Baker Chemical Co., Phillipsburg, NJ) 50% (vol/vol) for 8 min. 5×10^5 cells were then plated into 96-well Costar plates (#3596) containing 5×10^3 irradiated (2,500 rads) peritoneal wash-out cells. 6 d later hybridomas were picked and transferred to 24-well Costar plates (#3524), expanded, and tested for antigen-specific IL-2 production. All antigen-specific hybridomas were subcloned twice before being used in experiments.

T Cell Proliferation Assay. Varying numbers of T cells were incubated in 96-well flat-bottomed microtiter plates (Costar #3596), with different APC populations and antigen in a final volume of 200 μl. Assays were performed in Iscove's/F12 medium (35) supplemented with 10% fetal calf serum. After 48 h the cultures were pulsed with 1 μCi/well of tritiated thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA) and

harvested 16 h later (PHD cell harvester, Cambridge Technology, Inc., Cambridge, MA). Determinations were done in duplicate and the data expressed as the arithmetic mean. Standard errors of the mean were routinely <15%. All experiments included an irradiated T cell control to ensure that the measured tritiated thymidine incorporation was due to T cell uptake.

IL-2 Assay. The response of T cell hybridomas to stimulation was assessed by the ability of culture supernatants to cause the proliferation of an IL-2–dependent T cell clone, HT-2, as previously described (29).

B Cell Proliferation Assay. Irradiated T cells were cultured with various B cell populations in the presence or absence of additional filler cells and antigen in 96-well plates (Costar #3596). After 24 h the wells were pulsed with tritiated thymidine and harvested ~16 h later, as above. This roughly corresponds to the first round of replication of these cells. The optimum amount of irradiation required to abrogate T cell proliferation in the presence of antigen varied from 2,000 to 3,300 rads and had to be individually determined for each T cell clone. Assays were performed in duplicate in Iscove's/F12 medium supplemented with 10% fetal calf serum. Standard errors of the mean were routinely <15%.

Cell Size Analysis. Cell size profiles were analyzed using a Coulter Counter as described elsewhere (26).

Results

Small Resting B Cells Can Present Soluble Antigen to an MHC-Restricted T Cell Clone. We tested the ability of resting B cells to act as APC to an MHC-restricted T cell clone. Spleen cells from nonimmune B10.A mice were depleted of T cells by anti–T cell antibody and complement treatment and then fractionated by density on a discontinuous Percoll gradient. Two cell populations were removed from the gradient for analysis as APC: the high density cells that layer between the 60% and 70% layers of Percoll and that have been shown to be enriched for small resting B cells (26), and the low density, dendritic- and macrophage-enriched cells that layer above 50% Percoll. In these experiments the small resting B cells were then passed over a Sephadex G-10 column to further deplete them of any contaminating macrophages. The cells recovered from the Sephadex G-10 column were cultured with the T cell clone A.5.1 at a B:T ratio of 10:1 in the presence or absence of GAT. The B10.A-derived, long-term T cell clone, A.5.1, responds to APC bearing the A^k_β:A^k_α Ia molecule in association with the random terpolymer, GAT, by both proliferating and by causing polyclonal activation and proliferation of resting B cells (36, unpublished observation). B cell activation is conveniently measured by increase in cell volume.

Size profiles of the cell populations were determined after 24 h in culture (Fig. 1). The G-10-passed B cells themselves were of a relatively uniform size (median 114 μm^3), which did not appreciably change with the addition of either T cells (116 μm^3) or dendritic- and macrophage-enriched cells (120 μm^3) in the absence of GAT. However, the addition of GAT to the mixture of A.5.1 and syngeneic B cells was sufficient to cause a polyclonal enlargement of the B cells in culture (median of 152 μm^3) in the absence of added low density cells. The size histograms demonstrate that all of the cells in the culture were enlarged, ruling out the possibility that only the T cells responded. In addition, mixing experiments, in which T cells were activated separately from resting B cells and then added back just before size analysis, confirmed that at a ratio of 10:1 the contribution to the size enlargement made by the T cells was quite small (data not shown). It should

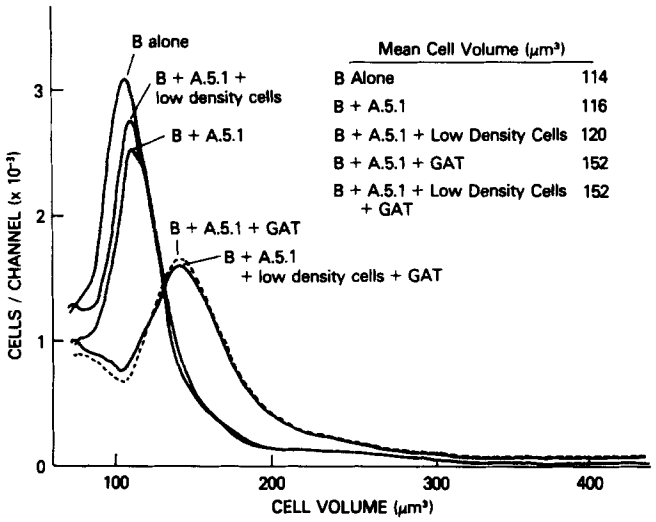

FIGURE 1. Size enlargement of small resting B cells induced by an antigen-activated T cell clone. G-10–passed B cells were isolated from B10.A spleen cells after T cell depletion and separation on a Percoll density gradient. All cells were unirradiated. Cell numbers used per well: small B10.A B cells, 1×10^6; T cell clone A.5.1, 1×10^5; B10.A low density APC, 5×10^4. Cell size was measured on a Coulter Counter 23 h after mixing the cells and antigen. GAT was used at a final concentration of 100 μg/ml.

be noted that in the presence of antigen the addition of 5×10^4 irradiated dendritic- and macrophage-enriched APC had no additional effect on the size enlargement of the B cells (152 μm^3). This indicates that either the culture was already saturated for antigen-presenting activity, or that the irradiated dendritic- and macrophage-enriched cells did not play a role in the presentation of antigen for B cell enlargement.

The ability of Sephadex G-10–passed B cells to act as APC for T cell activation resulting in their own recruitment was also assessed by measuring [^{3}H]thymidine incorporation by B cells. 2×10^4 irradiated A.5.1 T cells (2,000 rads) were incubated with 2×10^5 Sephadex G-10–passed B10.A B cells in the presence or absence of GAT (Table I*A*). The unirradiated B cells proliferated vigorously in the presence, but not in the absence, of GAT. The response was demonstrated to be due to B cell, and not T cell, thymidine incorporation by the observation that 1,000 rads delivered to the B cell population abrogated the incorporation of [^{3}H]thymidine.

The ability of the Sephadex G-10–passed resting B cells and the dendritic- and macrophage-enriched cells to act as APC for T cell proliferation was also examined (Table I*B*). Both lightly irradiated (1,000 rads) Sephadex G-10–passed B cells and heavily irradiated (3,300 rads) dendritic- and macrophage-enriched cells were capable of presenting GAT to A.5.1 as assessed by T cell proliferation. The T cell proliferation was dependent on the addition of a source of APC as well as the presence of GAT. Thus, small resting B cell populations, even after passage over Sephadex G-10, appeared to be able to present soluble antigen to T cells both for T cell proliferation and B cell recruitment.

Ability of B Cells to Present Antigen Is Radiosensitive. The observation that resting

TABLE I

Ability of Different APC to Present GAT to the T Cell Clone A.5.1 for T Cell and B Cell Proliferation

APC added			Antigen	
Sephadex G-10 B cells (unirradiated)	Sephadex G-10 B cells (1,000 rads)	Low density cells (3,300 rads)	GAT: −	GAT: +
A. B cell proliferation (T cells irradiated)			[^{3}H]Thymidine incorporation *cpm*	
2×10^5	—	—	3,500	37,200
—	2×10^5	—	ND*	1,600
B. T cell proliferation (T cells unirradiated)			*cpm*	
—	—	—	2,900	2,700
—	1×10^5	—	2,400	86,000
—	—	2×10^4	2,400	75,800

A. 2×10^4 A.5.1 T cells were given 2,000 rads and then incubated with the indicated number of Sephadex G-10–passed B cells in the presence or absence of GAT (100 μg/ml). Sephadex G-10–passed B cells were isolated from B10.A spleen cells after T cell depletion, separation on a Percoll density gradient ($p = 1.074$–1.086), and passage over a Sephadex G-10 column. B cell proliferation was measured by pulsing the wells with [^{3}H]thymidine after 24 h and harvesting them 16 h later.
B. 2×10^4 unirradiated A.5.1 T cells were incubated with the indicated number of APC in the presence or absence of GAT (100 μg/ml). Low density cells were isolated from the same source after T cell depletion and separation on the Percoll density gradient ($p \geq 1.062$). Sephadex G-10–passed B cells were isolated as in Part *A*. T cell proliferation was measured by pulsing the wells with [^{3}H]thymidine after 48 h and harvesting them 16 h later.
* Not determined.

B cells could present antigen to MHC-restricted T cells did not agree with earlier reports from Grey and colleagues (18, 19). Since one of the major differences between their assay systems and ours is the amount of irradiation delivered to the APC, we explored the radiation sensitivity of the antigen-presenting function of Sephadex G-10–passed B cells as compared to the known in vitro radioresistance of this function in dendritic- and macrophage-enriched cells (7, 17, 37). Table II contains the results of an experiment in which the T cell clone A.5.1 was assayed for its ability to proliferate when stimulated with GAT in the presence of either Sephadex G-10–passed B cells or dendritic- and macrophage-enriched cells that had been treated with varying doses of irradiation. The amount of [^{3}H]thymidine incorporation attributable to T cells was taken as the difference in uptake of cultures containing unirradiated and irradiated (2,500 rads) T cells.

A.5.1 T cells proliferated well in the presence of GAT when either G-10–passed B cells or low density cells were used as APC (Table II). It is also clear that the antigen-presenting ability of the B cells was radiosensitive; doses of irradiation in excess of 1,000 rads markedly reduced their stimulatory activity and 3,300 rads abolished it. In contrast, the antigen-presenting ability of the dendritic cell and macrophage-enriched population was only slightly affected by irradiation. The small decrease in antigen presentation by these cells at 2,000–3,300 rads may reflect the contribution of the B cells in this population to its overall antigen-presenting ability.

Although this experiment allowed us to conclude that the ability of B cells to

TABLE II
Effect of Irradiation on Different APC

Sephadex G-10 B cells	Low density cells	Irradiation to APC	Irradiated A.5.1 (2,500 rads)	T cell proliferation
		rads	*cpm*	Δ *cpm*
2×10^5	—	500	5,810	30,800
		1,000	1,600	29,600
		1,500	1,150	10,800
		2,000	1,000	2,200
		3,300	1,500	200
—	5×10^4	500	1,950	86,600
		1,000	1,050	88,000
		1,500	900	84,600
		2,000	1,200	68,600
		3,300	1,200	73,200

2×10^4 A.5.1 T cells were incubated with the indicated number of APC (see Table I for a description of the two APC populations) in the presence of GAT (100 μg/ml). As a control, A.5.1 cells were irradiated with 2,500 rads to determine how much of the total response was due to residual recruited B cell proliferation (which is expected at low doses of irradiation). T cell proliferation was calculated by subtracting this amount from the total amount of [^{3}H]-thymidine incorporated (Δcpm).

stimulate T cells to proliferate in the presence of antigen was radiosensitive, we could not determine from it the radiosensitivity of B cells as APC in the T cell–driven induction of B cell recruitment. In order to characterize the ability of B cells to present antigen for subsequent B cell recruitment, it was necessary to separate the B cell's ability to act as an APC when irradiated, from its ability to respond to a proliferative stimulus. In order to do this, use was made of the fact that antigen-activated T cells could cause B cell proliferation in an MHC-unrestricted manner. As described in the accompanying paper (36), the B10.A-derived, GAT-specific T cell clone, 11.4, when stimulated by GAT in the presence of syngeneic irradiated APC, causes B10.S(9R) B cells to enlarge and proliferate. Treating 11.4 T cells with 2,000 rads eliminates the T cell's ability to proliferate when stimulated with GAT in the presence of the appropriate APC, but not its ability to recruit B10.S(9R) B cells to proliferate. The proliferative response of the B10.S(9R) B cells was shown to be directly related to the number of 11.4 T cells and the number of syngeneic APC in the culture, as well as to the concentration of GAT (36). The B10.S(9R) B cells, in the absence of syngeneic B cells, do not proliferate, presumably because they can not function as APC to activate the T cell clone.

The effects of irradiation on the ability of syngeneic B cells to successfully present antigen to irradiated 11.4 T cells to in turn cause [^{3}H]thymidine incorporation by unirradiated allogeneic B10.S(9R) B cells is shown in Fig. 2. Panel *A* shows the effects of irradiation to the syngeneic B10.A B cell on its own proliferative response to LPS. 500 rads reduced the response by 80%; 1,000 rads almost totally eliminated it. Panel *B* shows a similar pattern when antigen-stimulated, irradiated, 11.4 T cells were used as the stimulus for the syngeneic B cell proliferation. These control experiments show that 1,000 rads is sufficient to eliminate most of the contribution of syngeneic B cells to any measured [^{3}H]-

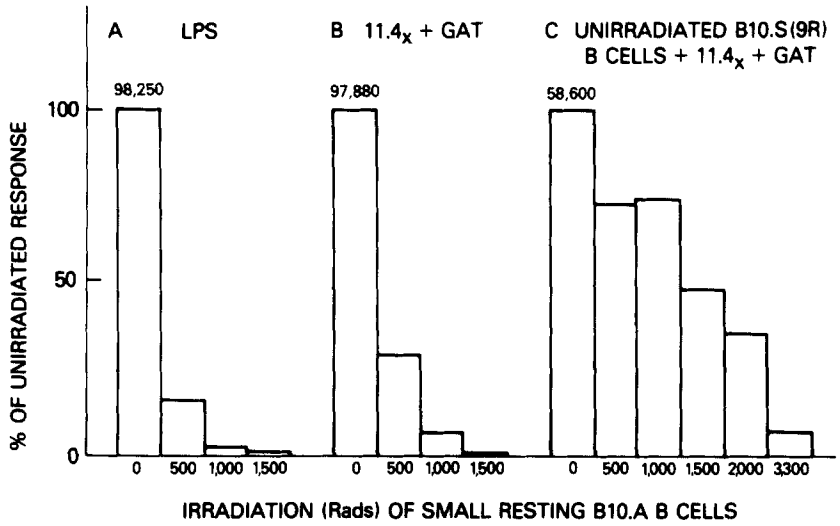

FIGURE 2. Effect of irradiation on B cell APC function. The [^{3}H]thymidine incorporation of stimulated B cells was determined as a function of the amount of irradiation delivered to syngeneic B10.A B cells. The amount of proliferation measured at each dose of irradiation is displayed as a percentage of the response of the unirradiated cells for each group. The numbers under each bar represent the amount of irradiation given to the B10.A B cells. The number over the first bar of each panel represents the CPM incorporated in the absence of any irradiation. Panel *A*. 2×10^5 B10.A small resting B cells were treated with different amounts of radiation and then incubated with LPS (50 μg/ml). Panel *B*. 2×10^5 small resting B10.A B cells were treated with different amounts of radiation and then incubated with 2×10^4 11.4 T cells (treated with 2,000 rads; 11.4_x) and GAT (100 μg/ml). Panel *C*. 2×10^5 small resting B10.A B cells were incubated with 2×10^5 allogeneic B10.S(9R) B cells in the presence of 2×10^4 irradiated 11.4 T cells and GAT (100 μg/ml). Varying doses of irradiation were given to the syngeneic B10.A B cells and not to the allogeneic B10.S(9R) B cells. The bars represent the amount of proliferation of the B10.S(9R) B cells only. This was calculated by subtracting the CPM incorporated by the B10.A B cells at the same amount of irradiation (panel *B*) from the total amount measured when the B10.A and B10.S(9R) B cells were mixed together. For example, the total CPM incorporated by the mixture of the two types of B cells, with no irradiation to the B10.A B cells, was 156,480.

thymidine incorporation. In panel *C*, irradiated syngeneic B10.A and unirradiated allogeneic B10.S(9R) B cells were mixed together in equal numbers in the presence of irradiated 11.4 T cells and antigen. The amount of allogeneic B cell [^{3}H]thymidine incorporation, calculated by subtracting the known small amount of B10.A B cell [^{3}H]thymidine incorporation induced by antigen-activated 11.4 T cells from the total [^{3}H]thymidine incorporation, is graphed as a function of the radiation dose given only to the syngeneic B10.A B cells. As the B10.A B cells, which were the only syngeneic APC in the culture, were given larger radiation doses, the proliferation of the allogeneic B cells decreased. By 3,300 rads there was >90% reduction in the amount of B10.S(9R) B cell [^{3}H]thymidine incorporation. Thus, the APC function of syngeneic B cells for allogeneic recruitment is also radiosensitive, although less so than the syngeneic B cell's own proliferative response. It is this difference in radiosensitivity that allowed us to assess the B cells APC function in the absence of its own response.

Overall, these data indicate that antigen presentation by small resting B cells for both T cell proliferation and initiation of B cell recruitment is a radiosensitive

function. This B cell trait is distinct from the radioresistance of dendritic cell and macrophage APC function. Therefore radiation dose provides a useful way to distinguish between the two cell types in antigen-presentation assays.

Highly Purified B Cells are Potent APC for B Cell Recruitment. It might be argued for syngeneic recruitment, where the B cells are not irradiated, that small numbers of contaminating macrophages and/or dendritic cells in the Sephadex G-10–passed B cell population were responsible for the antigen-presenting activity observed. To test this, highly purified panned B10.BR B cells, which were >99% Ig-bearing cells by flow microfluorometric analysis, were used to stimulate the T cell clone 11.4, which had been grown only on IL-2–containing supernatants. B10.BR cells possess the A^k_β:A^k_α Ia molecule for which 11.4 T cells are "cospecific," and thus should be capable of presenting GAT to 11.4 T cells. In addition, advantage was taken of the fact that 11.4 T cells are also alloreactive to *H-2*^{r}-encoded molecules, expressed by B10.RIII B cells. The experiment in Table III demonstrates that panned B cells, both B10.BR in the presence of GAT (Δcpm 37,100), and B10.RIII by themselves (Δcpm 8,700), were quite effective in inducing 11.4 T cells to cause proliferation of the stimulatory B cells in the absence of any other accessory cells.

In order to quantitate the ability of panned B cells to function as APC for T cell activation, 11.4 T cell–dependent [^{3}H]thymidine incorporation by B10.S(9R) B cells was measured as a function of the number of irradiated, panned B10.A B cells (Fig. 3, *A* and *B*). By utilizing the recruitment of B10.S(9R) B cells to measure the response, we were able to keep the number of 11.4 T cells and of the responding B10.S(9R) B cells constant while varying the number of panned B10.A B cell APC. The B10.A B cells were treated with 1,000 rads to abrogate their own proliferation but not their ability to function as APC.

The response of B10.S(9R) B cells depended upon the presence of GAT. Without antigen, [^{3}H]thymidine incorporation at each dose of panned B10.A B cells was between 8,500–10,000 cpm. At the dose of GAT used (100 μg/ml) the antigen-presentation capability of the panned B10.A B cells was maximal (94,000 cpm) at about 1×10^5 B cells per microtiter well. When the number of B10.A B cells was reduced below this value, the stimulated [^{3}H]thymidine incorporation of B10.S(9R) B cells fell with a slope of ~0.76 in panel *A* and 0.80 in panel

TABLE III

Purified B Cells Can Be Recruited without Additional APC

Mitogen	T cell	GAT	Proliferation (cpm) ± SEM of panned B cells	
			B10.BR	B10.RIII
—	—	—	1,100 (± 30)	1,200 (± 110)
LPS	—	—	94,300 (± 6,840)	91,900 (± 1,550)
Con A	—	—	700 (± 100)	600 (± 190)
—	2×10^4	—	3,800 (± 480)	12,500 (± 400)
—	2×10^4	+	40,900 (± 180)	13,600 (± 20)

Irradiated (2,000 rads) 11.4 T cells were incubated with panned purified B cells from either B10.BR mice (syngeneic at the I region) or B10.RIII mice (to which 11.4 is alloreactive). B cell proliferation was assessed by the incorporation of [^{3}H]thymidine. The proliferation measured in the presence of irradiated 11.4 T cells, GAT, and B cells treated with 1,000 rads was 316 cpm for B10.BR and 368 cpm for B10.RIII.

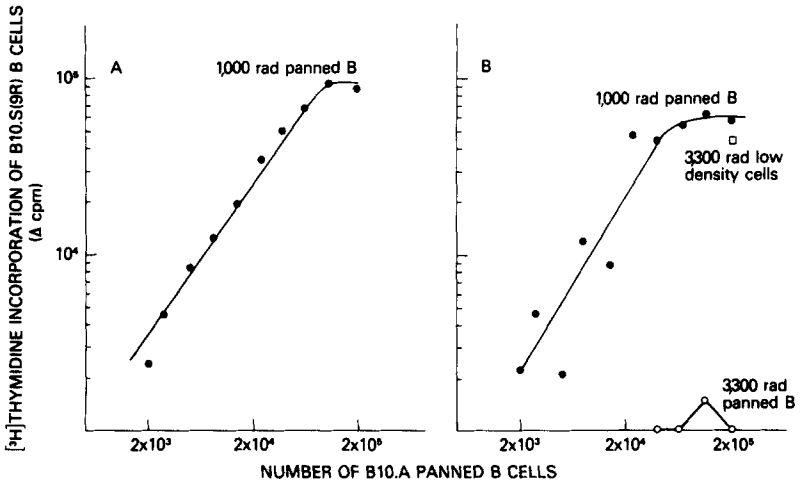

FIGURE 3. Allogeneic B cell proliferation induced by the T cell 11.4 in the presence of various numbers of lightly irradiated panned B10.A B cells. 2×10^4 11.4 T cells (2,000 rads) were incubated with 2×10^5 B10.S(9R) small resting B cells with varying numbers of panned B10.A B cells (1,000 rads) plus GAT (100 μg/ml). Culture of allogeneic B10.S(9R) small resting B cells with irradiated 11.4 T cells in the absence of GAT and syngeneic B10.A small resting B cells resulted in the incorporation of 11,560 cpm. Addition of GAT did not increase this number. Proliferation in the absence of GAT at each dose of syngeneic B cells added did not exceed 12,000 cpm. The slope of the linear regression line from 3×10^3 to 1.2×10^5 panned B cells is 0.76 in panel *A* and 0.80 in panel *B*. In panel *B* (○—○) is the proliferation observed when the panned B10.A B cells were irradiated with 3,300 rads. (□) is the proliferation observed when irradiated (3,300 rads) low density cells were used as APC.

B (determined from the linear regression line of a log-log plot of cell number vs. Δcpm). Detectable stimulation of [^{3}H]thymidine incorporation by B10.S(9R) B cells was observed with as few as 2×10^3 irradiated B10.A B cells. The fact that the slope of the log-lot plot of the dose-response curve was close to 1 indicates that only one limiting cell type in the B10.A B cell population was being titered into the culture. Panel *B* further illustrates that panned B10.A B cells treated with 3,300 rads failed to act as APC for the activation of 11.4. In contrast, low density cells treated with 3,300 rads were potent APC. Thus, the APC function of the panned B cells showed a similar radiosensitivity to that of the G10–passed B cells.

Finally, a quantitative comparison of APC function between lightly irradiated (1,000 rads), Sephadex G10–passed B cells, and heavily irradiated (3,300 rads) low density macrophage and dendritic cells is shown in Fig. 4. In this experiment the two populations were identical in their capacity to present GAT to 11.4 T cells for the stimulation of [^{3}H]thymidine incorporation by B10.S(9R) B cells. In three other experiments the two populations were also found to be comparable on a per cell basis. These results argue strongly that the APC activity of G10–passed B cells can not be due solely to contamination with a small number of low density cells. Furthermore, the complete radiosensitivity of the highly purified panned B cells argues strongly that the only APC in this population is a B cell.

B Cells Can Present Antigen for Stimulation of T Cell Proliferation to Some, But Not All, T Cell Clones. The T cell clone, A.5.1, could be stimulated both to prolif-

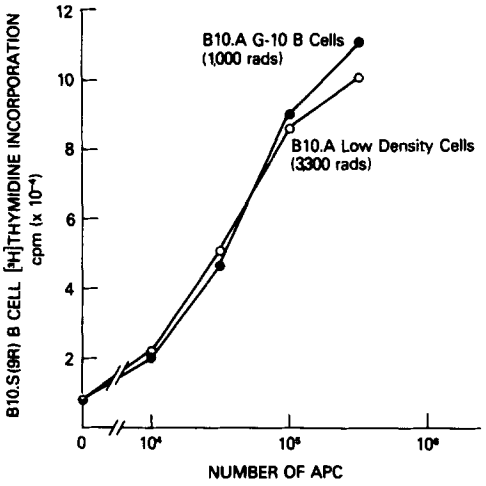

FIGURE 4. Heavily irradiated low density cells and lightly irradiated G-10-passed B cells are equally potent as APC for inducing allogeneic B cell recruitment by 11.4 T cells. 2×10^4 irradiated (2,000 rads) 11.4 T cells were incubated with 2×10^5 B10.S(9R) small resting B cells in the presence or absence of GAT (100 μg/ml). Allogeneic B cell proliferation was measured as a function of the number of B10.A APC titrated into culture. (○—○), Low density B10.A cells (3,300 rads), (●—●), Sephadex G-10-passed B cells (1,000 rads).

erate and to recruit B cells to proliferate when stimulated with antigen and purified B cells as APC. This was in contrast to the results of Katz et al. (20) and Bondeira et al. (21), who found that B cells could not induce a T cell proliferative response. In order to determine whether A.5.1 was an unusual T cell that had the capability of proliferating in the presence of its antigen and resting B cells (Tables I and II), a number of normal T cell clones as well as T cell hybridomas were assayed for such responsiveness (Table IV). 12 normal T cell clones and one T cell line were assayed for their ability to proliferate in the presence of the appropriate foreign antigen and lightly irradiated (1,000 rads) Sephadex G-10-purified small B cells or heavily irradiated (3,300 rads) dendritic- and macrophage-enriched cells. Because B cells are poorer as APC for T cell proliferation than macrophage-enriched cells (see below), the number of B cells used to stimulate the T cells was from 4–10-fold greater than the number of macrophage-enriched cells. All T cells tested were derived from B10.A mice. The T cell, 11.4, was grown in the presence of IL-2 containing, rat spleen Con A supernatant; the remaining cells were grown by serial stimulation with soluble antigen and irradiated (3,300 rads) spleen cells alternating with periods of rest in the absence of antigen.

Of the 13 T cell lines tested, 9 responded with substantial [^{3}H]thymidine incorporation when stimulated with antigen and Sephadex G-10-passed B cells (Table IV). That the response of these T cells was due to the APC activity of B cells was shown by the radiosensitivity of the presenting activity of this cell population (Table IV, Expt. 2). Four clones (numbers 10–13) showed little or no [^{3}H]thymidine incorporation in response to B cells, but did respond well when irradiated (3,300 rads) dendritic- and macrophage-enriched cells were used as APC. The very small amount of [^{3}H]thymidine incorporation observed in these

TABLE IV

Clonal Analysis of T Cells Capable of Proliferating in Response to Antigen Presented on Resting B Cells

	T cell	Sephadex G-10 B cells (1,000 rads): Antigen		Sephadex G-10 B cells (3,300 rads): Antigen		Low density cells (3,300 rads): Antigen	
		−	+	−	+	−	+
		cpm		*cpm*		*cpm*	
Expt. 1							
1	PC.3 ST	300	17,900	—*	—	560	23,180
2	PC.3.3	350	126,420	—	—	1,110	122,630
3	PC.3H	220	4,800	—	—	180	5,600
4	PC1.K	300	20,270	—	—	500	74,200
5	PC.3A	240	57,030	—	—	210	61,000
6	PC.3L	170	93,700	—	—	130	88,400
7	PC1B	320	24,000	—	—	140	162,000
8	PC1.1p	280	21,000	—	—	120	38,400
9	A1	810	86,600	—	—	210	50,160
10	GAT.25.58a	300	1,900	—	—	—	52,100
11	PC.3D	660	2,300	—	—	—	48,200
12	PC.3.1	220	470	—	—	200	6,940
13	11.4	330	870	—	—	160	15,600
Expt. 2							
1	PC.3 ST	240	15,150	100	550	600	42,800
2	PC.3.3	940	7,900	330	560	570	15,720
3	PC.3H	380	29,100	170	2,550	330	49,500
4	PC1.K	270	7,390	580	400	520	14,800
5	PC.3A	300	30,120	230	780	300	32,660
6	PC.3L	310	32,440	320	730	330	36,870
7	PC1B	480	8,300	580	1,100	210	61,000

* Not determined.

13 normal in vitro T cell lines were cultured at 2×10^4 cells per well in the presence of either Sephadex G-10–passed B10.A B cells (1,000 or 3,300 rads) or B10.A low density cells (3,300 rads). All cells were tested for a proliferative response to the relevant foreign antigen, either GAT or pigeon cytochrome *c*. The ratio of Sephadex G-10–passed B cells to low density cells was at least 4:1 and in most cases 10:1. The range of low density cells used was 3×10^4 to 1×10^5, while the range of Sephadex G-10–passed B cells used was 2×10^5 to 5×10^5. All T cells tested were cloned with the exception of A1, which is a T cell line.

four cultures in the presence of lightly irradiated B cells plus antigen was consistent with a small amount of B cell recruitment. Overall, the experiments on T cell clones suggest that there are two subpopulations of T cells: one that can proliferate when either B cells or low density, radioresistant macrophages and dendritic cells are used as APC and the other, which can only be stimulated to proliferate when marophages and dendritic cells are used as APC.

Four T cell hybridomas were also tested for their ability to respond to B cells as APC (Table V). Two measures of T cell activation were used: IL-2 secretion and recruitment of syngeneic B cell proliferation. The T cell hybridomas were treated with 10,000 rads so that they would not incorporate thymidine and interfere with the B cell proliferation assay. All the T cells produced IL-2 when dendritic- and macrophage-enriched cells were used as APC. Three of the four hybrids responded to small resting B cells in the presence of antigen (HEL or

TABLE V

Ability of T Cell Hybridomas to Respond to Different APC by the Production of IL-2 and the Recruitment of B Cells

APC:	Low density cells		Small B cells			
Responding cell:	HT-2		HT-2		B cell	
Antigen:	−	+	−	+	−	+
			cpm			
T cell hybridoma:						
B14	290	28,000	4,900	64,100	6,100	52,150
2H10	550	8,300	340	6,800	13,800	44,700
C10	270	54,600	1,500	54,900	11,500	79,000
2B4	600	41,100	3,300	3,400	6,000	7,000

Four different B10.A-derived T cell hybridomas were tested for their ability to respond to either low density APC or small resting B cell APC with the production of IL-2 or the induction of syngeneic B cell proliferation. 1×10^5 T cells were incubated with either 5×10^4 low density APC per well or 2×10^5 small resting B cells per well. Each T cell hybridoma was irradiated with 10,000 rads before assay. IL-2 production was assessed by removing supernatant from the stimulated T cell cultures at 24 h and testing its ability to cause proliferation of the IL-2–dependent T cell, HT-2. B cell proliferation was assessed by pulsing the original culture wells with tritiated thymidine as described in Materials and Methods. None of the APC in these assays were irradiated. The antigen used for B14 and C10 was HEL. The antigen used for 2H10 and 2B4 was pigeon cytochrome *c* fragment 81-104.

pigeon cytochrome *c*) for both IL-2 production and recruitment of B cell proliferation. The cytochrome *c*–specific hybrid 2B4 failed to respond to normal B cells and antigen in either assay. In order to determine whether the 2B4 hybrid could recruit B cells if adequately stimulated, 5×10^4 syngeneic low density APC treated with 3,300 rads were cultured with 2×10^5 syngeneic small resting B10.A B cells in the presence or absence of pigeon cytochrome *c*. Despite the fact that the 2B4 hybrid produced IL-2 (no antigen, 420 cpm of [^{3}H]-thymidine incorporated by HT-2; with antigen, 5,200 cpm), there was no recruitment of B cell proliferation in the presence of cytochrome *c* (no antigen, 1,700 cpm of [^{3}H]thymidine incorporated by the B cells; with antigen, 2,100 cpm).

The experiment shown in Table VI demonstrates that both Sephadex G-10–passed B cells and the more highly purified panned B cells were equally capable of inducing the T cell hybrid C10 to produce IL-2 in the presence of the appropriate foreign antigen (HEL). The 2B4 hybrid again failed to secrete IL-2 in response to either Sephadex G-10–passed or panned B cells as APC. It should also be noted that in the experiments displayed in Tables V and VI, all APC were unirradiated. Therefore it is not possible that the difference seen in the response of 2B4 cells to B cells compared to dendritic- and macrophage-enriched cells as APC was due to the differential radiation sensitivity of the antigen-presenting functions of these cells.

It appears that the 2B4 hybrid is both incapable of secreting IL-2 when B cells are used as APC and unable to produce B cell recruiting factor(s) when stimulated with low density APC plus antigen. This presumably reflects two distinct differences between 2B4 cells and the other T hybridomas tested: a failure to recognize

TABLE VI

2B4 T Cell Hybrid Fails to Respond to B Cells as APC

T cell hybrid	No. of APC	APC population: G-10 B cells Antigen: −	G-10 B cells Antigen: +	Panned B cells Antigen: −	Panned B cells Antigen: +	Low density cells Antigen: −	Low density cells Antigen: +
C10	1×10^4	3,100‡	83,500	3,000	18,000	—*	—
	5×10^4	6,100	256,000	3,800	264,000	2,300	250,000
	1×10^5	6,100	229,000	4,200	269,000	—	—
	2×10^5	3,100	205,000	3,300	235,000	4,100	191,000
2B4	1×10^4	2,400	2,800	3,000	4,500	—	—
	5×10^4	2,400	2,500	4,900	6,200	2,600	119,000
	1×10^5	—	2,900	4,600	6,700	—	—
	2×10^5	2,400	7,100	4,400	6,300	1,500	81,000
	4×10^5	—	2,700	—	—	—	—

The ability of the T cell hybrids C10 and 2B4 to secrete IL-2 in response to foreign antigen in the presence of different APC was tested. None of the APC were irradiated. The antigen used for 2B4 was the pigeon cytochrome *c* fragment 81-104 and that used for C10 was HEL.

* Not determined.

‡ Proliferation of the cell HT-2 as a measure of the amount of IL-2 secreted by the T cell hybridoma was performed as described in Materials and Methods and is expressed as cpm of [^{3}H]thymidine incorporated.

B cells as APC for lymphokine production, and a failure to produce B cell recruiting factor(s) even when successfully stimulated to produce IL-2.

Distinct Pathways in the T Cell Exist for B Cell–induced T Cell Proliferation and B Cell Recruitment. 11.4 and PC.3.3 cells, two of the normal T cell clones that exhibited different phenotypes, as judged by their ability to proliferate when B cells were used as APC (Table IV), were selected for further analysis. Fig. 5*A* presents an experiment in which various potential antigen-presenting populations were used in the presence of GAT to induce T cell proliferation of clone 11.4. Increasing numbers of T-depleted spleen cells were effective in causing 11.4 T cells to incorporate [^{3}H]thymidine. The low density dendritic- and macrophage-enriched cell population was much more potent than the T-depleted whole population (approximately eightfold on a per cell basis). The small resting B cells taken from the Percoll gradient, as well as B cells further purified over Sephadex G-10, failed to cause 11.4 T cells to take up [^{3}H]thymidine in the presence of GAT at any cell number tested, up to 3×10^5 per well. A simultaneous experiment with irradiated 11.4 T cells confirmed that 2×10^5 Sephadex G-10–passed B cells were quite effective at presenting antigen for their own recruitment (no GAT, 2,800 cpm; with GAT, 60,000 cpm). Furthermore, it should be noted that the inability of B cells to act as APC for the induction of proliferation of the T cell clone 11.4 is in striking contrast to the earlier experimental result shown in Fig. 4, namely, that the B cell–enriched and the macrophage- and dendritic cell–enriched populations were equivalent in stimulating 11.4 T cells to recruit B10.S(9R) B cell proliferation. Thus, 11.4 is capable of responding to B cell APC by producing B cell recruitment factor(s) but not by incorporating [^{3}H]-thymidine.

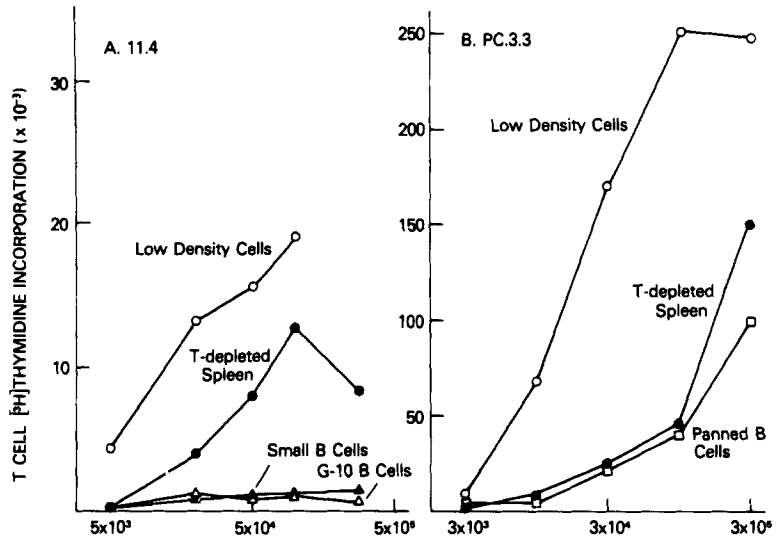

FIGURE 5. T cell PC.3.3 but not T cell 11.4 proliferates when stimulated with antigen in the presence of B cells. 2 × 10^4 T cells were incubated with various B10.A APC at varying numbers plus or minus antigen: GAT, 100 μg/ml for 11.4 in Panel *A* or pigeon cytochrome *c*, 30 μg/ml for PC.3.3 in Panel *B*. Low density APC (3,300 rads) (○—○), T-depleted spleen cells (3,300 rads) (●—●), small resting B cells taken from Percoll gradient (1,000 rads) (▲—▲), small resting B cells taken from Percoll and passed over Sephadex G-10 (1,000 rads) (△—△), panned B cells (1,000 rads) (□—□).

In contrast, the other B10.A-derived T cell clone, PC.3.3, was shown to proliferate quite well when panned B cells, the most highly purified B cells used in these experiments, were used to present antigen (Fig. 5*B*). This population presented antigen as well as T-depleted spleen, but was significantly less potent (~10-fold) than dendritic- and macrophage-enriched cells. Panned B cells caused a small but significant antigen-induced [^{3}H]thymidine incorporation by T cells (Δcpm 2,300) even at 3 × 10^3 B cells per well. This is similar to the minimum number of B cells required to obtain B10.S(9R) B cell recruitment by the 11.4 T cells (Fig. 3).

The inability of 11.4 T cells to proliferate when resting B cells were used as APC was not due to the unusual conditions under which these cells were grown. To show this, a variant of 11.4, 11.4F, was grown by alternating periods of stimulation with GAT plus 3,300 rads irradiated syngeneic spleen cells followed by rest with spleen cells and no antigen. No exogenous IL-2 was required to grow these cells. The comparative ability of different APC to cause proliferation of 11.4F T cells is shown in Table VII. The 11.4F cells failed to take up [^{3}H]-thymidine when B cells were used as APC, just as was true of the IL-2–dependent 11.4 T cells. At the same time, 11.4F T cells, when stimulated with GAT and purified B cells, were capable of causing B cell proliferation (Table VII).

Taken together, these data suggest that the pathways by which the cloned T cells are induced by antigen and APC to either proliferate or to produce activation factor(s) for B cell recruitment must be distinct.

B Cells Can Present Soluble Antigen to Primed Lymph Node T Cells. Although it has been shown that highly purified resting B cells can present antigen to in vitro

TABLE VII

11.4F T Cell Clone Responds to B Cells as APC to Give B Cell Recruitment But Not T Cell Proliferation

	GAT:	T cell no.	No. of APC	APC							
				T-depleted spleen (3,300 rads)		Low density cells (3,300 rads)		Sephadex G-10 (1,000 rads)		Panned B (1,000 rads)	
				−	+	−	+	−	+	−	+
T cell [^{3}H]thymidine incorporation (cpm)		11.4F (Unirrad.)									
		2×10^4	1×10^5	230	2,100	1,600	25,500	100	380	140	340
		2×10^4	2×10^5	490	5,500	2,300	15,800	100	340	330	210
		2×10^4	4×10^5	900	7,800	460	10,600	—	—	260	420
B cell [^{3}H]thymidine incorporation (cpm)		11.4F (2,000 rads)						Sephadex G-10 *(Unirradiated)*			
		3×10^5	2×10^5					3,400	5,700		
		1×10^4	2×10^5					4,260	9,670		
		3×10^4	2×10^5					4,930	21,820		

T cell proliferation: 2×10^4 11.4F T cells per well were cultured with varying numbers of APC in the presence or absence of GAT (100 μg/ml). T cell proliferation in the absence of any added APC was 160 cpm in the presence of GAT. B cell proliferation: 11.4F was treated with 2,000 rads and cultured at the numbers indicated in the presence of 2×10^5 Sephadex G-10–passed unirradiated B cells. The concentration of GAT was 100 μg/ml.

T cell clones maintained by a variety of different culture techniques, it might be argued that normal T cells freshly harvested from an immunized animal require activation signals that only macrophages or dendritic cells can provide. In order to test this possibility, T cells were isolated from the lymph nodes of purified protein derivative (PPD)–primed B10.A mice by passage over nylon wool columns and treatment with anti-Ia antibody plus complement. These T cells were then cultured with either lightly irradiated (1,000 rads) Sephadex G-10–passed B cells or heavily irradiated (3,300 rads) T-depleted whole spleen cells in the presence or absence of PPD (Fig. 6). Both lightly irradiated Sephadex G-10–passed B cells and T-depleted spleen cells were capable of inducing the purified lymph node T cells to proliferate in the presence, but not the absence, of PPD. The T cell response was directly related to the number of APC added. Although the Sephadex G-10–passed cells were clearly quite capable of presenting PPD for T cell proliferation, they appeared to be about two- to threefold less efficient than T-depleted whole spleen cells. The modest difference in potency of these two populations may reflect either the superiority of low density, radioresistant APC over B cells as APC for T cell proliferation, or the ability of only a subset of T cells in the whole population to utilize B cells for this function. The dramatic reduction (at least 30-fold) in the ability of heavily irradiated (3,300 rads) Sephadex G-10–passed B cells, in comparison to lightly irradiated (1,000 rads) B cells, to induce T cell proliferation eliminates the possibility that contaminating low density radioresistant cells (i.e., macrophages or dendritic cells) in the B cell population were responsible for the stimulatory activity. Experiments in which panned B cells were used to present PPD to antigen-primed T cells gave similar results. Thus, we conclude that purified small resting B cells are fully capable of presenting antigen even to T lymphocytes freshly explanted from the animal as long as the B cells have not been irradiated with >1,000 rads.

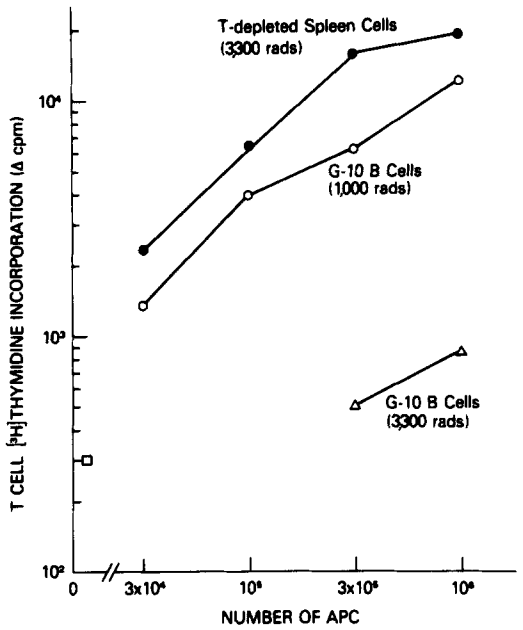

FIGURE 6. Small resting B cells can present soluble antigen to primed T cell populations. T cells were prepared from the draining lymph nodes of PPD-primed B10.A mice by passage over nylon wool and treatment with anti-Ia monoclonal antibodies plus complement. 4×10^5 T cells per well were incubated with varying numbers of different B10.A APC. The wells were pulsed with [^{3}H]thymidine at 72 h and harvested 16 h later. T-depleted spleen cells (3,300 rads) (●—●), Sephadex G-10–passed B cells (1,000 rads) (○—○), Sephadex G-10–passed B cells (3,300 rads) (△—△). The □ represents the Δ cpm in the absence of any added APC.

Discussion

In this report we have shown that highly purified small resting B cells from normal unprimed spleen are capable of presenting foreign antigen to a variety of MHC-restricted T cell clones to cause both T cell proliferation and T cell–dependent B cell stimulation, as measured by the latter's enlargement and proliferation. The ability of B cells to present foreign antigen to MHC-restricted T cells has been previously examined by a number of other investigators. In 1980 Kammer and Unanue (13) prepared nonadherent cells from whole spleen, passed them over a Sephadex G-10 column, and then selected for IgM-positive cells by cytofluorometric cell sorting. These cells were treated with mitomycin c and used as APC in a T cell [^{3}H]thymidine incorporation assay. KLH-primed lymph node T cells proliferated when incubated with these cells in the presence of KLH; however, peritoneal exudate cells were more than 10-fold better at inducing this activity. Although these data were consistent with the hypothesis that normal B cells could present antigen to T cells, the authors felt that because peritoneal exudate cells were so much more effective as APC than the cells in the B cell preparation, they could not definitely exclude the possibility that a small degree of contamination with macrophages or other accessory cells accounted for the apparent antigen-presenting activity of their B cells. This doubt has been eliminated in our studies by demonstrating the radiosensitivity of B cell

antigen presentation, a characteristic that dendritic cells and macrophages do not share.

Chesnut and Grey (19) compared 4,500 rads irradiated adherent spleen cells with 4,500 rads irradiated macrophage-depleted (Sephadex G-10–passed) spleen cells for their ability to present antigen to rabbit gamma globulin primed syngeneic T cell populations. They found that in in vitro T cell proliferation assays, rabbit Ig was effectively presented to T cells from rabbit Ig–immunized donors only by the splenic adherent cells. In contrast, the rabbit anti-mouse Ig stimulated comparable amounts of T cell proliferation whether splenic adherent cells or macrophage-depleted spleen cells were used as APC. This result was interpreted as implying that under certain circumstances (e.g. when the B cell immunoglobulin receptor could bind the antigen recognized by the T cell) B cells are capable of acting as APC for T cell proliferation. In a subsequent paper, experiments were presented in which LPS B cell blasts and B cell lymphomas, but not normal B cells, were capable of presenting the antigen KLH to either KLH-specific T cell hybridomas or KLH-primed normal T cell populations. Taken together, these reports would suggest that only under unusual circumstances do small resting B cells effectively present antigen to T cells for T cell proliferation.

The discrepancies between the conclusions reached in this paper and those cited above can be accounted for in a number of ways. We find that antigen presentation by small resting B cells is quite radiosensitive, in contrast to the presentation by low density splenic APC or B cell tumors (7, 17, 37). Radiation doses of as little as 1,500 rads significantly reduced the antigen-presenting capability of small resting B cells, and doses of over 2,000 rads totally abrogated it. Thus, experiments performed with heavily irradiated APC would not be expected to show that small resting B cells can present antigen. The finding that LPS blasts, but not resting B cells, are capable of presenting antigen to T cells might reflect an enhanced antigen-presenting capacity of activated B cells. Chesnut et al. (18) found that LPS-blasts bound ~10-fold more of the labeled antigen than did resting B cells. Another possible mechanism for enhanced antigen presentation by LPS B cell blasts is suggested by the observation that stimulation of B cell populations with LPS and dextran sulfate results in an increased expression of Ia molecules by these cells as they progress through the G_1 phase of the cell cycle (38). Finally, the antigen-presenting ability of LPS blasts may be less radiosensitive than that of normal B cells.

An apparent exception to the observation that heavily irradiated resting B cells cannot present antigen to T cells is the ability of B cells to present rabbit anti-mouse immunoglobulin to normal rabbit gamma globulin–primed T cells. However, in a recent report, Kakiuchi et al. (39) found that the presentation of rabbit anti-mouse immunoglobulin required prolonged exposure of the B cells to antigen, with the optimum attained at 16 h. It was also found that incubation of OVA-primed T cells (which contained 1% contaminating B cells) with rabbit anti-mouse immunoglobulin $F(ab')_2$ in the absence of additional APC allowed OVA to induce substantial T cell proliferation. This was interpreted to mean that the B cell APC required preactivation (in this case by rabbit anti-mouse immunoglobulin) before they could present antigen. If this is the case, the

antigen-presenting function of these cells may be more similar to that of LPS blasts than to that of the small resting B cells studied in this paper.

The mechanism by which irradiation impairs the ability of small resting B cells to act as APC is not clear. It appears that lymphocytes are very susceptible to radiation damage while in interphase. Anderson et al. (40) examined the effects of irradiation on the viability and migration in vivo of T and B lymphocytes. They found that 90% of unactivated T cells (thoracic duct lymphocytes from CBA mice) were killed by 300 rads, as compared to the death of 99% of B cells (thoracic duct lymphocytes from CBA nu/nu mice). They also observed that the in vivo migration of resting B cells to lymphoid organs was much more radiosensitive than that of resting T cells. Rahmsdorf et al. (41) studied the in vitro effects of gamma irradiation on T cells, B cells, and fibroblasts. They observed that the ability of fibroblasts to synthesize DNA was approximately fivefold more radioresistant than that of T cells, which, in turn, was twofold more radioresistant than that of B cells. The authors speculated that the differences in radiosensitivity between the cell types reflected differences in their ability to repair radiation-inflicted damage. However, measurement of single and double-strand DNA breakage and of unscheduled DNA synthesis (as a measure of the excision of modified bases from the DNA and their resynthesis) revealed no differences between the cell types examined. Overall, these data suggest that B lymphocyte viability, as well as at least some function, such as in vivo migration (and perhaps injury repair), is especially radiosensitive. The findings reported in this paper would now add antigen presentation to the list of radiosensitive B lymphocyte functions.

Reports by Katz et al. (20) and Bandeira et al. (22) have shown that normal B cells can indeed act as APC for the initiation of T cell function, but that they are only effective in inducing the T cells to recruit B cells. In the former case, long-term in vitro IL-2–dependent, OVA-specific, T cell clones were found to proliferate in the presence of OVA and mitomycin c–treated splenic adherent cells, but not mitomycin c–treated B cells recovered from anti-mouse Ig-coated plastic dishes. When the T cells and not the B cells were treated with mitomycin c, however, the B cells were found to proliferate. Bandeira et al. examined the ability of Sephadex G-10–passed cells to stimulate either antigen-specific T cell proliferation or syngeneic B cell proliferation. They found that irradiated (2,000 rads) B cells were incapable of promoting T cell proliferation, although irradiated T cells caused a vigorous recruitment of unprimed B cells. The results of Bandeira et al. might be explained by the radiosensitivity of the B cell antigen-presenting function. However, the observations of both papers might also be explained by our finding that there seem to be a number of different phenotypes exhibited by different T cell clones. The first, exemplified by the cells A.5.1 and PC.3.3, is that of a T cell that can proliferate well to the appropriate foreign antigen plus B cell APC. A second phenotype, seen in 11.4, is that of a T cell that does not proliferate when B cells are used as APC, even though B cells serve as excellent stimulators of both syngeneic and allogeneic B cell recruitment by the T cell. It is this phenotype that may have been studied by Katz et al. and Bandeira et al.

The existence of two T cell phenotypes suggests that there must be at least

two distinct activation pathways stimulated by the APC. One pathway leads the T cell to secrete a factor(s) that causes B cells to enlarge, proliferate, and, as described in the accompanying paper (36), differentiate into antibody-secreting cells. Although we have not yet demonstrated that the actual lymphokine(s) responsible for these effects are all T cell–derived, it is clear that in the system described, an MHC-restricted antigen-specific T cell is required at some point in the pathway that leads to polyclonal B cell activation. The other pathway initiated by the APC in the presence of the appropriate antigen leads to T cell proliferation. Although 11.4 T cells can clearly be stimulated to proliferate when the signal is delivered from a low density radioresistant cell, it is incapable of responding to a similar signal from a B cell. Whether this is due to a qualitative or quantitative difference between the low density APC and B cell signals is unknown. Clearly, however, many other T cells exhibit a phenotype distinct from 11.4 T cells, as they are quite capable of receiving the proliferation signal from B cell APC.

A third T cell phenotype, seen in the T cell hybridoma 2B4, is that of a cell that does not respond to B cell APC plus foreign antigen by producing IL-2. In addition, 2B4 does not seem to secrete any B cell recruiting factors, even when stimulated to make IL-2 by low density radioresistant APC. This cell may therefore exhibit two "defects": the failure to respond to B cell APC by producing IL-2, and the inability to make B cell recruiting factor(s) even when adequately stimulated to make other lymphokines. Whether this phenotype is truly a third subpopulation of T cells or only a somatic variant that arose in culture, remains to be determined.

Even though the resting B cells used in these experiments were extensively purified, it might be argued that a very small number of contaminating non-B cells present in the stimulating population were responsible for the antigen-presenting activity measured. We feel this is unlikely for several reasons. First, the cells in the purified B cell population that were found to present antigen could be distinguished from the more conventional APC present in the low density fraction of the Percoll gradient by their differential activation of several of the T cell clones assayed. As discussed above, the 11.4 T cell proliferated vigorously in the presence of foreign antigen and low density APC, but not at all to the B cell–enriched populations. In contrast, T cells PC.3.3 proliferated well when either population was used as APC. Second, the APC found in the B cell population were radiosensitive. This excludes the possibility that "traditional" APC such as macrophages or dendritic cells contaminate the B cell populations and are responsible for the antigen presentation. Such cells, which are present in both the T-depleted spleen cells and the low density cells taken from the Percoll gradient, were tested in parallel with the high-density B cell–enriched populations and shown to be very radioresistant. These data indicate that two distinct APC present in normal spleen are capable of presenting antigen to MHC-restricted T cells. Since the most purified cells used, those recovered from anti-immunoglobulin–coated dishes, were >99% B cells as determined by cytofluorometric analysis and as few as 2×10^3 of them were sufficient to elicit a

detectable T cell response (Figs. 3 and 5), the simplest interpretation of these observations is that resting B cells themselves constitute one of these APC populations.

We also observed that small resting B cells could present soluble antigen to an antigen-primed T cell population. Heavily irradiated T-depleted whole spleen cells were potent stimulators; lightly irradiated Sephadex G-10–passed B cells were only two- to threefold less efficient. The radiosensitivity of the B cell APC at 3,300 rads demonstrated that their antigen-presenting activity was not due to contaminating low density APC in the B cell population. This observation supports the notion that antigen presentation by resting B cells is not due to some unusual property of in vitro long-term T cell lines and clones, but in fact occurs with normal antigen-specific T cells freshly taken from the animal.

The observation that small resting B cells can act as APC for T cell activation implies that, in contrast to the results of Chesnut and Grey, unactivated cells can perform whatever antigen "processing" functions are required in order for T cells to recognize the antigen in association with class II restriction elements. Thus, the possibility that B cells are responsible for antigen-driven T cell activation must be taken into consideration in studies dealing with T cell–B cell collaboration. Apparently MHC-restricted T cell–B cell interaction may, in some systems, reflect MHC-restricted T cell activation by B cells rather than MHC-restricted T cell–induced B cell activation (36). Whether resting B cells can act as APC for resting (e.g. virgin) T cells has not been directly addressed in these experiments since it may be argued that none of the T cells used in these studies are truly resting (42).

Finally, the finding that two distinct pathways of T cell activation exist suggests that B cells and low density APC differ in at least some aspects of their antigen presentation. This might be due to different supplementary APC signals (such as lymphokines), which initiate different T cell functions (i.e., T cell proliferation and lymphokine release). The difference observed in the ability of the different APC to initiate distinct T cell activation pathways may reflect quantitative differences in either the ability of the APC to deliver these signals or of the individual T cell clones to receive them. Further studies will be required to differentiate between these possibilities.

A question that is still not completely answered is whether macrophage and dendritic cells alone can serve as stimulators for T cell–driven B cell recruitment. Attempts to completely purify these cells away from surface immunoglobulin-positive cells have so far been only partially successful. One observation that suggests that they may indeed be capable of this activity is that low density cells treated with 3,300 rads did cause allogeneic B cells to proliferate when used as stimulators for antigen-specific T cells (Fig. 4). Since B cell antigen-presenting function was shown to be eliminated by this dose of irradiation (Table II and Fig. 2), the remaining antigen-presenting activity is likely to be attributable to dendritic cells or macrophages. This question, as well as the evaluation of what soluble factors may be involved in T and B cell stimulation, is currently being pursued.

Summary

In this report we have examined the ability of small resting B cells to act as antigen-presenting cells (APC) to antigen-specific MHC-restricted T cells as assessed by either T cell proliferation or T cell–dependent B cell stimulation. We found that 10 of 14 in vitro antigen-specific MHC-restricted T cell clones and lines and three of four T cell hybridomas could be induced to either proliferate or secrete IL-2 in the presence of lightly irradiated (1,000 rads) purified B cells and the appropriate foreign antigen. All T cell lines and hybridomas were stimulated to proliferate or make IL-2 by macrophage- and dendritic cell–enriched populations and all T cells tested except one hybridoma caused B cell activation when stimulated with B cells as APC. Furthermore, lightly irradiated, highly purified syngeneic B cells were as potent a source of APC for inducing B cell activation as were low density dendritic and macrophage-enriched cells. Lymph node T cells freshly taken from antigen-primed animals were also found to proliferate when cultured with purified B cells and the appropriate antigen. Thus, small resting B cells can function as APC to a variety of T cells. This APC function was easily measured when the cells were irradiated with 1,000 rads, but was greatly diminished or absent when they were irradiated with 3,300 rads. Thus, the failure of some other laboratories to observe this phenomenon may be the result of the relative radiosensitivity of the antigen-presenting function of the B cells. In addition, this radiosensitivity allowed us to easily distinguish B cell antigen presentation from presentation by the dendritic cell and macrophage, as the latter was resistant to 3,300 rads. Finally, one T cell clone that failed to proliferate when B cells were used as APC was able to recruit allogeneic B cells to proliferate in the presence of syngeneic B cells and the appropriate antigen. This result suggests that there are at least two distinct pathways of activation in T cells, one that leads to T cell proliferation and one that leads to the secretion of B cell recruitment factor(s).

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