

## **Antigen-induced human T cell help. Precursor frequency, radiation sensitivity, and allogeneic effects.**

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### **Research Article**

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FIGURE 2

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# Antigen-induced Human T Cell Help

## PRECURSOR FREQUENCY, RADIATION SENSITIVITY, AND ALLOGENEIC EFFECTS

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**ABSTRACT** We have recently noted marked differences between the in vitro responses of human B lymphocytes to stimulation with soluble antigens vs. stimulation with mitogens. In the present study, these differences were analyzed in terms of the precursor frequencies for the T cells and B cells involved and in terms of the radiation sensitivity of the T cells providing help in the two systems. Marked differences were found between antigen-induced and mitogen-induced systems with regard to T cell precursor frequencies and radiation sensitivity. In contrast, the precursor frequencies for the B cells involved in the two systems were approximately the same. In addition, having developed a system for the study of human antigen-specific B cell responses, we were interested in delineating the nature of the allogeneic effects that might be operative in this system. Marked allogeneic effects, both positive and negative, were noted in this system and will need to be taken into account in any studies that try to address the question of the genetic restriction, if any, that exists in human antigen-specific T cell-B cell collaboration.

Appreciation of the marked differences between the antigen-specific and mitogen-induced activation and immunoregulation of human B cell responses will be of importance in understanding the relationship between specificity and nonspecificity of antibody production in normal and disease states.

### INTRODUCTION

T cell regulation of B cell reactivity is an area of major interest and importance in both animal and human systems. With the recent availability of several systems

to study human B cell function in vitro, considerable interest has centered around the precise nature of the T cell regulation of human B cell responses in both polyclonal and antigen-specific assay systems. Unfortunately, there have been conflicting data among several of these systems (1-10). Of particular note has been the divergence of results with regard to the radiosensitivity of T cell help and the relative contributions of allogeneic effects in the mitogen-induced vs. antigen-induced human B cell systems. Disagreement with regard to this latter point has led to difficulty in delineating the precise nature of genetic restrictions in T cell-B cell collaboration in antigen-specific responses as well in appreciating the scope of allogeneic effects in co-culture experiments now commonly used in the study of patients with immune-mediated or immunodeficiency diseases.

Recently, in vitro systems have been developed for the study of antigen-induced, antigen-specific antibody production and secretion into culture supernatants by human B cells (11-14). The use of enzyme-linked immunosorbent assays (ELISA)<sup>1</sup> has proven to be extremely useful in these studies because of their simplicity, sensitivity, and specificity. These advances have allowed for a more precise study of the mechanisms involved in antigen-specific B cell responses.

One of the main features that we noted during the development of a system for the study of in vitro antigen-specific responses to the soluble protein antigens keyhole limpet hemocyanin (KLH) and tetanus toxoid was a marked dichotomy in the conditions required

<sup>1</sup> *Abbreviations used in this paper:* AET, 2-aminoethylisothiouonium bromide hydrobromide; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; KLH, keyhole limpet hemocyanin; PBMNC, peripheral blood mononuclear cell; PWM, pokeweed mitogen; (s)Ig, surface immunoglobulin.

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for stimulation with specific antigen vs. pokeweed mitogen (PWM) (14). In this study, we sought to determine if this dichotomy was reflected in the nature of the T cells and/or the B cells involved in specific as opposed to nonspecific B cell triggering. In fact, marked differences were found in terms of precursor frequency and radiosensitivity between antigen-induced and mitogen-induced T cell help.

In addition, having developed a system for the *in vitro* study of antigen-induced, antigen-specific antibody production in man, we were interested in delineating the nature of the allogeneic effects that might be operative in co-culture experiments of allogeneic T cells and B cells, with the ultimate purpose of addressing the question of the genetic restriction, if any, in T cell-B cell collaboration in the present system.

## METHODS

**Immunizations.** Normal subjects, aged 18–30 yr, received two subcutaneous injections of 5 mg KLH (Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, CA), which had been dialyzed against phosphate-buffered saline (PBS) and passed through a 0.45- $\mu$ m millipore filter (Milipore Continental Water Systems, Bedford, MA). Injections were spaced by 2 wk, and peripheral blood buffy coats were obtained 2–3 wk following booster injection. All procedures were carried out under peer-reviewed National Institutes of Health protocols.

**Cell separations.** Peripheral blood buffy coats were obtained by the technique of manual plasmapheresis. In this procedure, 450 ml of whole blood was removed into a sterile blood bag (Travenol Laboratories, Edison, NJ) that contained 2,500 U of heparin. While the donor's intravenous access line was kept open by a saline infusion, the blood bag was centrifuged at 2,400 rpm for 3 min in a Sorval model RC-2B refrigerated centrifuge (DuPont Instruments-Sorval Biomedical Div., DuPont Co., Wilmington, DE). The plasma layer and buffy coat were then transferred to a separate bag with the use of a plasma extractor, and the erythrocytes returned to the donor. The bag containing the buffy coat and autologous plasma was then spun at 600 rpm for 15 min, and the resulting cell pellet used as the source of peripheral blood mononuclear cells (PBMNC). For this series of experiments, these cell pellets were diluted with three parts of PBS and placed over Hypaque-Ficoll gradients in a standard fashion (15) to obtain unfractionated PBMNC. One unit of blood generally yielded  $5 \times 10^8$ – $1 \times 10^9$  PBMNC. The PBMNC obtained in this fashion were 10–30% monocytes, 60–80% lymphocytes, and 1–10% basophils and granulocytes by morphology and nonspecific esterase staining. Within the lymphocyte population, there were 5–10% B cells as determined by surface Ig (sIg) staining using a fluorescein-conjugated F(ab)<sub>2</sub> fragment goat anti-human Ig (Cappel Laboratories, Inc., Cochranville, PA) (16). Functionally, these cells have been found to be comparable to cells obtained from whole blood (unpublished observations).

T cell-enriched populations were obtained by a two-step procedure. Initially, cells were separated by the technique of rosette formation with S-2-aminoethylisothiuronium bromide hydrobromide (AET)-treated sheep erythrocytes (18) (Sigma Chemical Co., St. Louis, MO). Following lysis of the rosette layer with 10 ml of a solution containing 0.829 g% ammonium chloride (J. T. Baker Chemical Co., Phillipsburg,

NJ), 0.1 g% potassium bicarbonate (Sigma Chemical Co.), and 3.72 mg% EDTA disodium salt (Fischer Scientific Co., Pittsburgh, PA), the rosette-positive cells were passed through nylon wool columns (17). The percentage of T cells in these suspensions, as defined by erythrocyte rosette formation, was always >95%. In some experiments, the T cells that bore the OKT8 marker were eliminated by treatment of unfractionated T cells with the OKT8 monoclonal antibody (Ortho Pharmaceuticals, Raritan, NJ) and rabbit serum (Dutchland Laboratories, Denver, PA) as reported by others (6). Briefly, a pellet containing  $10 \times 10^6$  T cells (prepared as described above) was incubated with 50  $\mu$ l of the OKT8 reagent for 1 h on ice. The cells were then resuspended in 33% rabbit serum and RPMI 1640 medium (Flow Laboratories, McLean, VA), incubated for 1 h at 37°C, and then washed three times with RPMI 1640 medium. The resulting cell suspensions were >90% OKT4+ and <5% OKT8+, as determined by fluorescence-activated cell sorter analysis (3).

T cell-depleted populations (B cells and monocytes) were prepared by treatment of the non-AET rosetting cells with the anti-T cell hybridoma antibody Leu-1 (Becton, Dickinson & Co., Oxnard, CA) (19) and screened rabbit serum as previously described (14). Following this treatment, the cells were suspended at  $10^7$  cells/ml in RPMI 1640 medium containing 10% fetal calf serum (FCS; Gibco Laboratories, Grand Island, NY), and 2-ml aliquots were allowed to adhere to the bottom of plastic 60  $\times$  15-mm petri dishes for 1 h at 37°C in a 5% CO<sub>2</sub> incubator. The resulting nonadherent cell populations were 90–98% viable by trypan blue dye exclusion and contained 35–45% sIg-positive cells and 45–55% esterase-positive cells.

**Culture conditions.** Cultures for the measurement of supernatant Ig production were performed in 1 ml of RPMI 1640 medium containing 10% FCS in either 12  $\times$  75-mm round-bottomed test tubes (No. 2058, Falcon Labware, Div. of Becton, Dickinson & Co.), 24-well, flat-bottomed plates with 16-mm wells (No. 3524, Costar, Data Packaging, Cambridge, MA), or 96-well, flat-bottomed microtiter dishes (No. 3072, Falcon Labware), as indicated. Cultures contained various amounts of KLH or a 1:200 final dilution of PWM (Gibco Laboratories). Cultures were incubated in a 5% CO<sub>2</sub> atmosphere at 37°C and rocked at 6 cycles/min. Cell type and number in culture were varied as indicated, depending upon the experiment. Cultures were harvested on day 10, and supernatants were assayed immediately or were stored at 4°C in closed, sterile, 12  $\times$  75-mm test tubes until assay, usually within 1 wk.

**Irradiation of cell suspensions.** In some experiments, T cells were irradiated with 50–2,000 rad from a <sup>137</sup>Cs source (Gammator M, Isomedix, Inc., Parsippany, NJ). Following radiation, cells were washed once in RPMI 1640 and resuspended in fresh medium. AET-negative cells were irradiated with 3,000 rad to serve as a source of monocytes.

**Precursor frequency determinations.** The precursor frequencies for antigen- and mitogen-responsive B cell subpopulations were determined by limiting dilution analysis according to previously reported methods (20). In these experiments, cultures were set up in 96-well, flat-bottomed microtiter plates, using feeder layers of 350,000 T cells for antigen-stimulated cultures and 100,000 T cells for mitogen-stimulated cultures. Monocytes were added to the T cell suspensions to yield a monocyte density of 10%. B cells, prepared as described above, were plated at numbers ranging from 2,500 to 20,000 sIg-positive cells per well in replicates of 24. Cultures were incubated for 10 d at 37°C in 5% CO<sub>2</sub>, and supernatants were assayed for specific antibody production as described below. A well was considered positive

if it contained greater than three times the amount of specific antibody that was contained in the unstimulated control cultures as determined in the ELISA. The only experiments considered for statistical analysis were those in which there were <4 units of anti-KLH antibody production by the unstimulated cells, and 100% of the wells scored positive at the highest number of B cells plated. The precursor frequencies for antigen- and mitogen-reactive T cells were more difficult to ascertain accurately, owing to the enormous variability in this figure from donor to donor. Consequently, an estimate of precursor frequency was determined by the following method. Triplicate cultures were performed in 12 × 75-mm test tubes (Falcon Labware) containing 250,000 B cells (with monocytes) to which were added serial dilutions of T cells, ranging from  $1.6 \times 10^6$  to  $1.25 \times 10^3$ . Cultures were incubated in the presence of antigen or mitogen, supernatants assayed, and cultures scored as outlined above. The lowest number of T cells that were able to support antigen-specific antibody production in two-thirds of the cultures was chosen as the number most closely approximating the true precursor frequency.

**Assays.** Specific and total Ig production were measured using the ELISA, as has been previously reported (12, 21). All incubations were performed in volumes of 0.1 ml. Specific anti-KLH IgM was quantitated in terms of a hyperimmune reference serum. One DT<sub>M</sub> unit has been defined as the amount of KLH-specific IgM present in 1 ml of a 1:10,000 dilution of the reference serum (12).

**Statistics.** Data are expressed as geometric means times or divided by SEM where appropriate.

## RESULTS

**Precursor frequencies for antigen-induced vs. mitogen-induced T cell help.** In experiments designed to determine the precursor frequency of T cell help, triplicate cultures were set up at T cell concentrations ranging from 1,250 T cells/culture to 1,600,000 T cells/culture; all cultures contained 250,000 B cells with monocytes. The initial experimental point where two-thirds of the cultures manifested anti-KLH antibody production was chosen as the point most closely approximating precursor frequency for the desired helper T cell. Marked differences were seen between antigen-induced and PWM-induced systems in terms of helper T cell precursor frequency. The average precursor frequency for antigen-induced help was ~5% of the average precursor frequency for PWM-induced help (Table I). It is obvious that there is considerable variability in precursor frequency of helper T cells in human peripheral blood with antigen-induced T cell help ranging from 1:100,000 to 1:800,000 T cells and mitogen-induced T cell help ranging from 1:5,000 to 1:100,000 T cells. The variability from person to person may well reflect the dynamic state of the T cell repertoire in human peripheral blood. However, despite this variability, the differences between antigen-induced and PWM-induced T cell help are striking and represent a consistent phenomenon.

**Radiation sensitivity of antigen-induced and mi-**

TABLE I  
*Precursor Frequencies of Antigen and PWM-induced T Cell Help*

	Frequency of antigen-induced help	Frequency of PWM-induced help
Subject 1	1:100,000	1:5,000
2	1:800,000	1:10,000
3	1:400,000	1:12,500
4	1:800,000	1:100,000
5	1:200,000	1:25,000
6	1:800,000	1:6,250
7		1:5,000
8		1:2,500
9		1:20,000
Average	1:400,000	1:11,000

**togen-induced T cell help.** Having established a major difference between antigen- and mitogen-induced systems in terms of helper T cell precursor frequency, we next examined potential qualitative differences in the nature of the T cell help in terms of sensitivity to ionizing radiation. As shown in Fig. 1, following treatment with 1,000 R, T cells were unable to deliver help for antigen-induced, antigen-specific antibody production. This occurred despite the ability of these same irradiated T cells to provide not only adequate help, but in fact enhanced help in a system of PWM-induced antigen-specific antibody production.

To determine whether this dichotomy between antigen- and mitogen-induced T cell help was simply a reflection of the differences in precursor frequency noted above, graded numbers of irradiated or nonirradiated T cells were added at limiting numbers to cultures containing 250,000 B cells (plus monocytes). 8 to 16 times as many irradiated T cells as nonirradiated T cells were required to provide the same degree of T cell help in KLH-induced, anti-KLH antibody production (Fig. 2). Thus, when the substantial differences from donor to donor in terms of T cell precursor frequency were controlled for by performing cultures at limiting numbers, all six subjects studied showed a marked reduction in antigen-induced T cell help following treatment of the T cells with 1,000 rad. In contrast, PWM-induced T cell help was relatively radioresistant, even when studied at limiting numbers of T cells (Fig. 3). Following treatment with 2,000 rad, the ability of low numbers of T cells to provide help fell by only 50%. In addition, at higher numbers of T cells, the helper activity of irradiated T cells was substantially greater than that of the nonirradiated T cells. Thus, with respect to precursor frequency and radio-sensitivity, the T cells involved in antigen-induced, antigen-specific antibody production and those in-

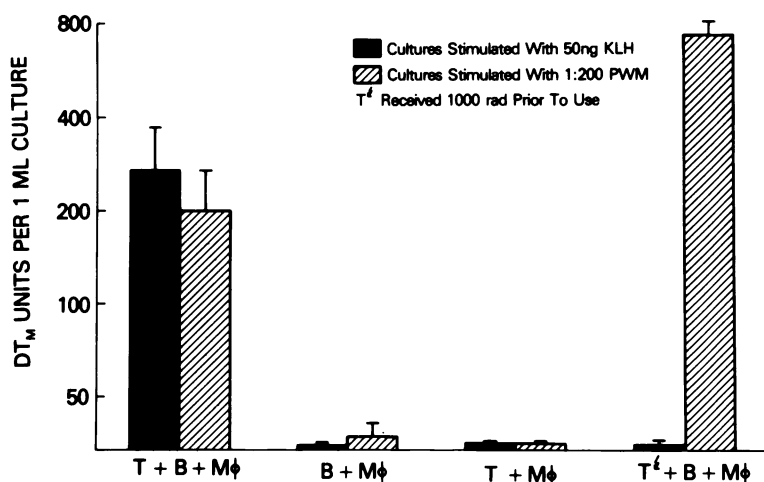


FIGURE 1 Apparent radiation sensitivity of antigen-induced T cell help. Triplicate cultures containing 250,000 B cells (with monocytes) and 250,000 untreated T cells or 250,000 T cells treated with 1,000 rad were stimulated with either 50 ng KLH or a 1:200 dilution of PWM.

involved in PWM-induced antigen-specific antibody production differ substantially.

**Antigen-specific B cell precursor frequency.** In contrast to the differences seen in the T cell precursor frequencies for antigen- and mitogen-induced systems, the B cell precursor frequencies for the two systems were approximately the same (Fig. 4). Furthermore, this precursor frequency was strikingly similar from subject to subject. In the six subjects studied in this manner, the average precursor frequency for both antigen-induced B cells and mitogen-induced B cells was 1:7,500 sIg-positive cells.

**Radiation sensitivity of antigen-induced T cell-mediated suppression.** We have demonstrated in the present system of antigen-induced, antigen-specific responses that low concentrations of antigen (1–200 ng/ml) induce substantial specific antibody production (12, 14). However, high concentrations of antigen (20 µg/ml) actually inhibit specific antibody production. This inhibition of specific antibody production by high-dose antigen has been shown to be specific for the antigen in culture, since polyclonal Ig responses were not inhibited and in fact were increased by stimulation with high concentrations of antigen (12).

A series of experiments were performed to determine whether this inhibition of specific antibody production by high concentrations of antigen could be reversed by irradiating the T cells. In 10 subjects studied, eight manifested good antibody responses to low concentrations of antigen with the expected suppression of specific antibody responses with high concentrations of antigen. Irradiation of T cells with 100 to

2,000 rad did not reverse this inhibition of specific antibody production (data not shown) and in fact only decreased the ability of the T cells to provide help. However, an interesting phenomenon was observed in the two remaining subjects. These two individuals were very poor responders to even low concentrations of antigen (concentrations that elicited maximal responses in the other eight subjects). As with the other eight individuals, higher concentrations of antigen did not elicit specific antibody responses in these subjects, but did result in polyclonal activation. In these two subjects, neither their unfractionated PBMNC nor cocultures of their purified T cells added back to their B cells (with monocytes) produced specific antibody in response to low concentrations of antigen. However, when their T cells were irradiated with 100 to 500 rad before culture, substantial antigen-induced, antigen-specific antibody responses were observed (Fig. 5). When the T cells were irradiated with higher doses of irradiation (>500 rad), the B cells were again unresponsive to low concentration antigen stimulation with respect to specific antibody production, presumably owing to the abrogation of the radiation-sensitive, antigen-specific helper T cells as demonstrated above. Thus, in a subpopulation of normal subjects, low concentrations of antigen appear to be capable of inducing a radiation-sensitive suppressor T cell population. Only by performing a radiation dose-response curve on the T cells could this be demonstrated in these individuals, since low doses of irradiation eliminate the suppressor cell function while higher doses of irradiation eliminate antigen-specific help. The latter situation would

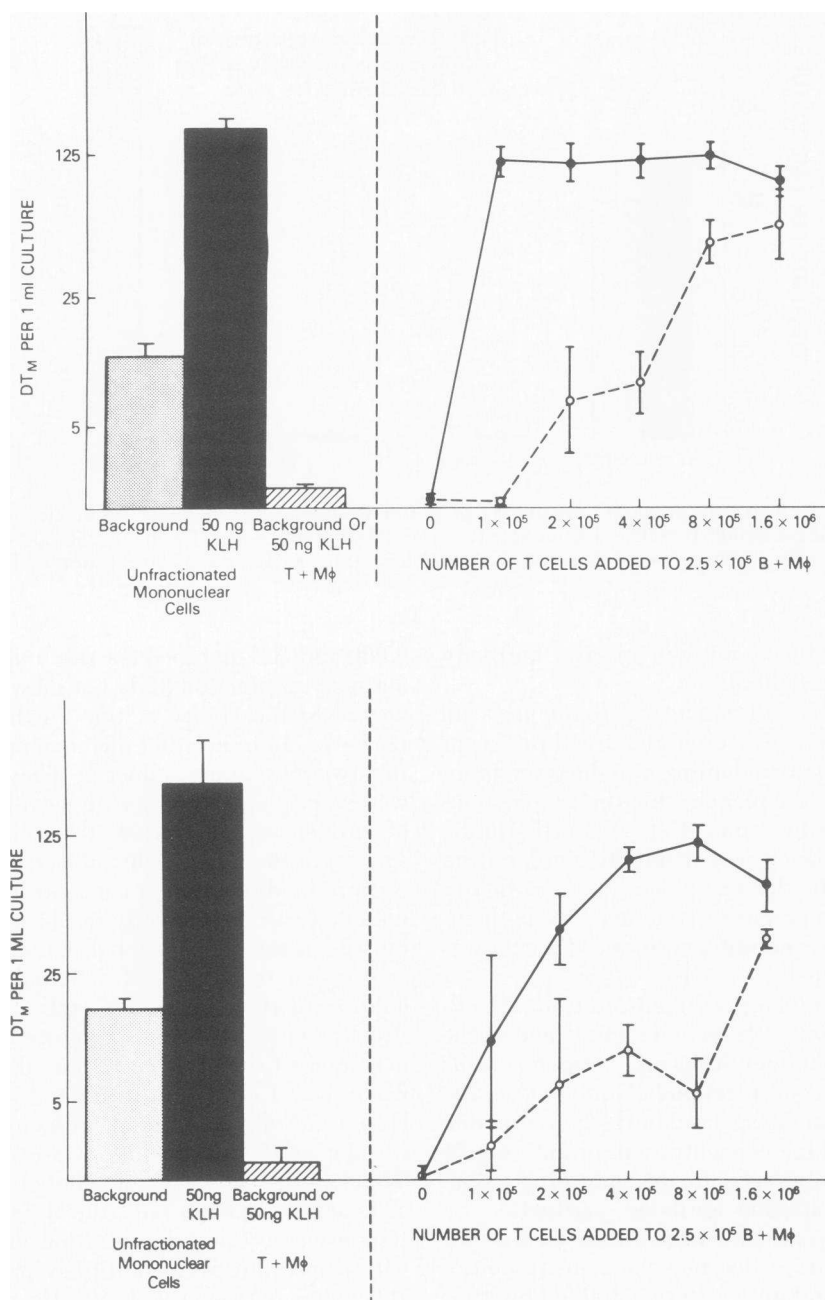
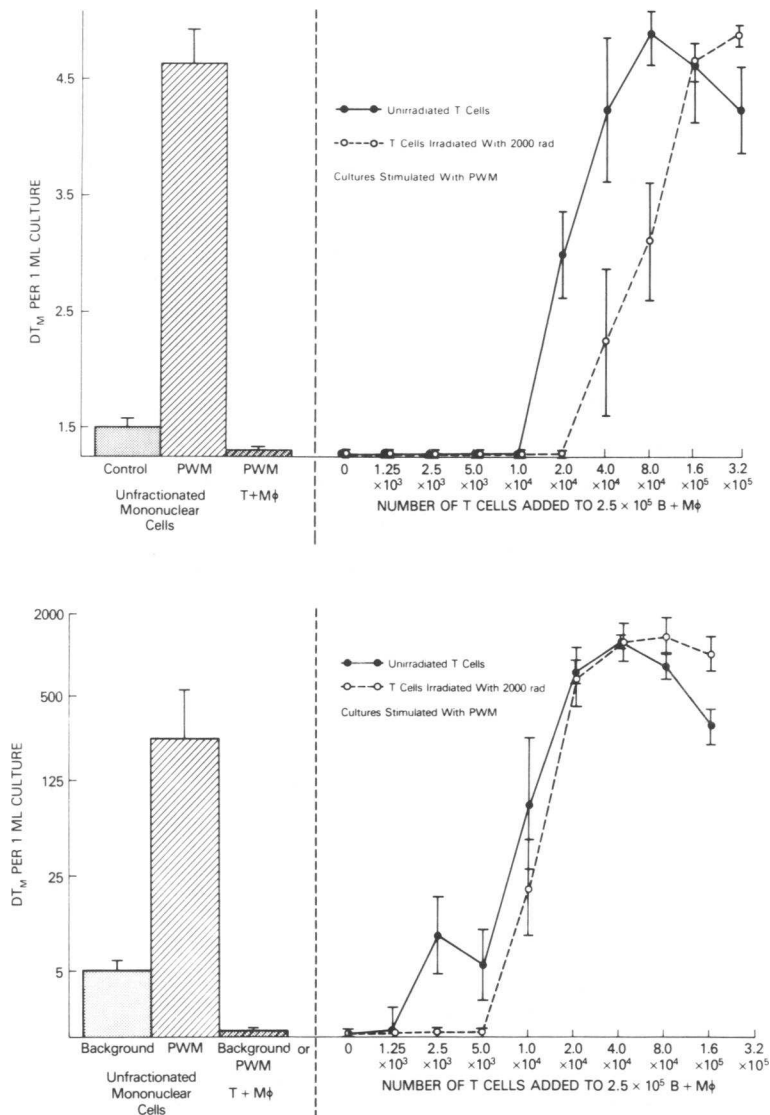


FIGURE 2 Radiation sensitivity of antigen-induced T cell help (*top* and *bottom*). Two separate experiments are shown. Varying numbers of irradiated or nonirradiated T cells were added to 250,000 B cells (with monocytes), and the cultures were stimulated with 50 ng KLH (*right*). The responses of unfractionated PBMNC are shown as a point of reference (*left*). (●), unirradiated T cells; (○), T cells irradiated with 1,000 rad.

lead to a state of unresponsiveness, which might be incorrectly interpreted as persistence of radiation-resistant suppressor T cells.

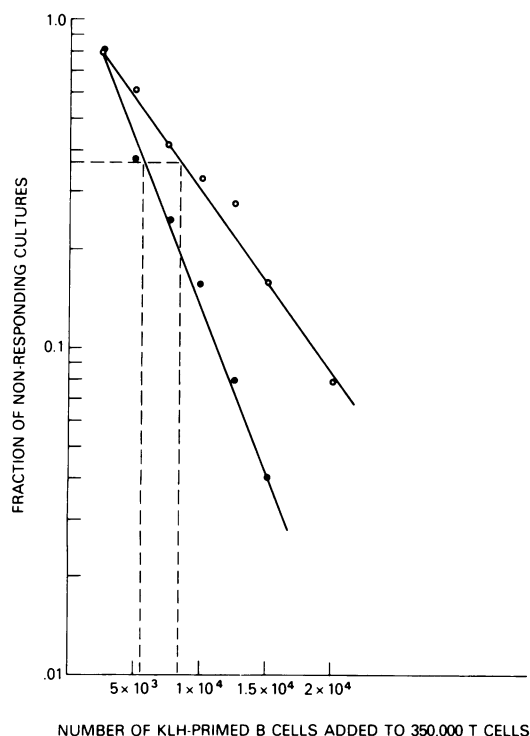
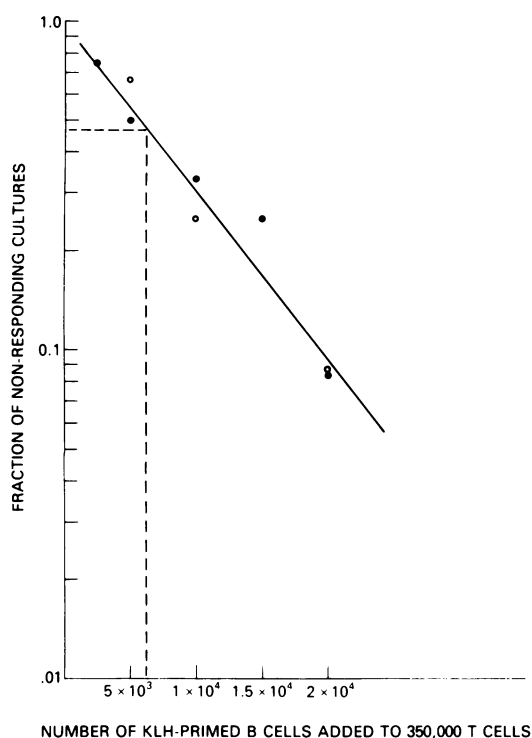
*Allogeneic effects in antigen-induced, antigen-specific antibody production.* Having established and characterized a system for examining antigen-in-



**FIGURE 3** Relative radioresistance of mitogen-induced T cell help (*top and bottom*). Two separate experiments are shown. Varying numbers of irradiated or nonirradiated T cells were added to 250,000 B cells (with monocytes), and the cultures were stimulated with a 1:200 dilution of PWM. Cultures were performed in triplicate with the responses of unfractionated PBMC shown as a point of reference.

duced, antigen-specific antibody responses in vitro, we sought to delineate the nature of the allogeneic effects that might be operative in co-culture experiments of allogeneic T and B cells. The ultimate goal of such co-culture experiments would be to eventually enable us to address the question of genetic restriction, if any, in human T cell-B cell collaboration in the present system.

In the first series of experiments, fresh, nonirradiated T cells from one subject (either immunized or nonimmunized to the antigen in question) were added to antigen-stimulated cultures of unfractionated PBMC of another unrelated or related immunized subject. Under these conditions, potent negative allogeneic effects were consistently seen (Fig. 6). This suppression of antigen-induced, antigen-specific anti-



body production by PBMC of one subject by the T cells of another subject occurred whether the donor of the T cells was totally unrelated, or a first degree relative. Furthermore, suppression occurred whether the donor of the T cells was immunized or nonimmunized to the antigen used in the cultures (KLH).

The next series of experiments were directed towards the elimination of these potent T cell-related negative allogeneic effects by prior treatment of the allogeneic T cells with either low-dose irradiation (500 R) or the antisuppressor T cell monoclonal antibody OKT8 and complement. Either low-dose irradiation or elimination of OKT8-positive cells completely abrogated the negative allogeneic effects demonstrated in Fig. 6 (data not shown). However, the remaining OKT8-depleted or irradiated allogeneic T cells exerted potent positive allogeneic effects when co-cultured with unfractionated PBMC or B cells from an immunized subject, and cultures produced substantial amounts of antigen-specific antibody, even in the absence of antigen (Fig. 7). These positive allogeneic effects were quite nonspecific in that they occurred regardless of the immune status of the T cell donor, whether the responding cell population consisted of unfractionated PBMC or simply B cells (with monocytes) alone, and with unrelated as well as mother-daughter pairs. In other words, the positive allogeneic effects created by the treated T cells-induced antibody production in the antigen-specific B cells via a polyclonal or nonspecific signal. Thus, the complex positive as well as negative allogeneic effects seen in allogeneic co-cultures precluded the examination of the genetic restrictions involved in human T cell-B cell collaboration in this system under the present conditions.

## DISCUSSION

The present study has clearly demonstrated marked differences between antigen- and mitogen-induced human T cell help in terms of precursor frequency and radiosensitivity. In addition, the complexities of the T cell-B cell interactions operative in human antigen-specific antibody production have been explored through the observations on radiosensitive suppressor T cells and the presence of both positive and negative allogeneic effects.

FIGURE 4 Precursor frequency analysis of antigen-inducible and mitogen-inducible B cells (*top* and *bottom*). Two separate experiments are shown. By Poisson distribution statistics (20), the precursor frequency is equivalent to the number of cells yielding 0.37 negative cultures (dotted line). (○), KLH-stimulated cultures, (●), PWM-stimulated cultures.



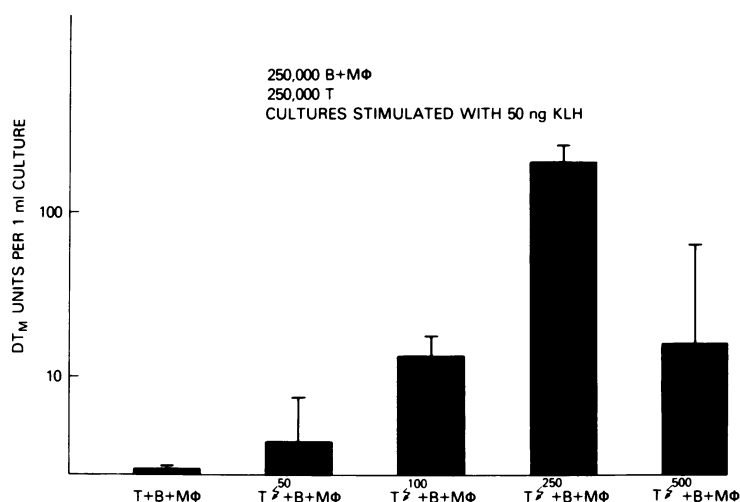


FIGURE 5 Radiation-sensitive T cell-mediated suppression. In this nonresponding immunized subject, T cells were treated with 0-500 rad before culture.

The ability of ionizing radiation to suppress humoral immune responses has been recognized for many years in both in vivo and in vitro systems (22). The earliest in vitro observations on this phenomenon suggested that the suppression was solely at the level of the B cell with T cell help relatively radioresistant (23). However, subsequent studies in the murine system examining the nature of T cell help at limiting num-

bers of T cells, or in the absence of the exquisitely radiosensitive suppressor T cells, have revealed that antigen-induced help is radiation sensitive (24). Similarly, the earliest human studies were felt to have demonstrated that the T cell help involved in mitogen-induced polyclonal Ig responses was quite radioresistant (4). However, more recent studies have indicated that even in the PWM-induced systems, there was a

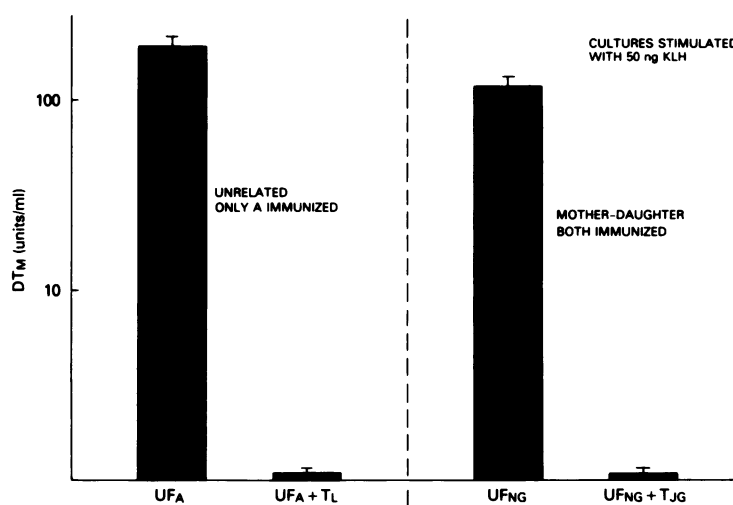


FIGURE 6 Allogeneic suppression of antigen-induced, antigen-specific antibody production. 250,000 T cells from a related or a unrelated donor were added to 500,000 unfractionated PBMNC (UF) of an immunized donor. Cultures were performed in triplicate and stimulated with 50 ng KLH. A, donor A; L, donor L; NG, donor NG; JG, donor JG.

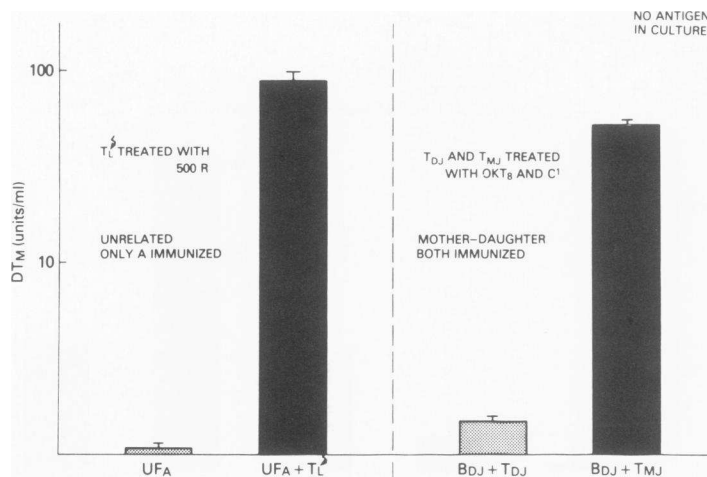


FIGURE 7 Allogeneic enhancement of antigen-specific antibody production. 250,000 T cells following either treatment with 500 rad (R), or OKT8 and complement, were added to 500,000 unfractionated PBMNC (UF) or 250,000 B cells and monocytes. Cultures were performed in triplicate, in the absence of antigen. A, donor A; L, donor L; DJ, donor DJ; MJ, donor MJ.

degree of radiosensitivity to the T cell help that could only be appreciated at limiting numbers of T cells (6). The previous studies on antigen-induced T cell help in the human system have been "conflicting," with some investigators noting radiation sensitivity and others noting a radiation resistance to antigen-induced T cell help (10, 25). The data present in this study using an antigen-specific system have confirmed the earlier observations on the effect of irradiation on PWM-induced T cell help and have provided evidence that the T cell involved in antigen-induced, antigen-specific antibody production is, in contrast to some earlier reports (8, 10, 25), exquisitely sensitive to radiation.

Irradiation can have a number of effects on the expression of cellular function. One of the most profound of these is on the ability of cells to proliferate. Radiation-mediated blockage of cellular proliferation is the most likely explanation for the differences in radiation sensitivity observed in the present study between antigen-induced and mitogen-induced T cell help. The simplest explanation for this phenomenon would be the fact that the antigen-induced helper T cell occurs at a much lower precursor frequency than the mitogen-induced helper T cell. In other words, indiscriminate elimination of a portion of the T cell pool with radiation might have left an adequate number of T cells to respond to mitogen, but an insufficient number of T cells to respond to antigen. However, when these differences in precursor frequency were controlled for by studying T cell dose-response curves at limiting numbers of irradiated or nonirradiated T cells, T cells involved in antigen-induced help were

still found to be at least eight times more sensitive to irradiation than mitogen-induced T cells. Hence, in this system, there are striking differences between antigen-induced and mitogen-induced T cell help with respect to both precursor frequency and radiosensitivity, and one cannot totally account for the other. This observation is consistent with earlier observations that T cells do not need to divide in order to provide help for the polyclonal B cell differentiation in response to PWM (4). In addition, it is consistent with the findings in the murine models that have demonstrated a need for T cell division in the generation of antigen-induced B cell responses (26). In other words, the peripheral blood lymphocyte pool contains, in relatively high numbers, T cells that have already differentiated into a type of helper effector cell that is able to be mitogen activated without undergoing proliferation. In contrast, T cells programmed to provide antigen-induced help appear to have a need for cell division before final differentiation into helper effector cells. This need for proliferation, which is at least partially independent of the need for expansion of the cellular pool, may be associated with the expression of essential new genetic material during the process of differentiation. This explanation would predict that once T cell help was generated by antigen it would then be relatively radiation resistant with respect to the effector phase of T cell help.

Another possibility is that the antigen-induced responses may involve a more complex series of T cell-T cell interactions than the mitogen-induced responses. If one of the cell types involved in such interactions

was exquisitely sensitive to radiation, even if it were not itself an antigen-induced cell, the overall T cell response would appear radiation sensitive. Similarly, if such a cell occurred at a relatively low precursor frequency, it could explain the differences noted in T cell precursor frequency between antigen- and mitogen-induced help as well as the differences noted between the precursor frequencies for antigen-induced T cell help reported here and antigen-induced T cell proliferation reported by others (27). Studies are currently underway to test these hypotheses.

The fact that some degree of antigen-induced T cell help was able to be generated by large numbers of irradiated T cells (Fig. 2) suggests that there may be two populations of antigen-induced helper T cells in the peripheral blood lymphocyte pool: an extremely radiation-sensitive one and a relatively radioresistant one. This latter population may represent a small subpopulation of antigen-reactive cells that have already been activated and generated *in vivo* and do not need to divide for their final differentiation into helper effector cells. Similar subpopulations of T cells have been described in the murine systems (28). Alternatively, the irradiated T cell may have some degree of helper activity, which is only seen at high cell numbers.

As mentioned earlier, previous studies have claimed that human antigen-induced T cell help is radiation resistant (10, 25). The reasons for the discrepancies between the data presented here and these earlier studies are unclear. However, possible reasons include a failure to conduct experiments at limiting numbers of T cells, prior *in vivo* activation of the antigen-specific helper T cell, or the presence of radiation-resistant polyclonal features in some of these systems.

Two of the subjects studied in this series of experiments exhibited an element of radiation-sensitive T cell suppression. In these subjects, low *in vitro* concentrations of antigen were unable to stimulate co-cultures consisting of nonirradiated T cells and B cells (plus monocytes) to produce specific antibody. However, when the T cells were treated with 500 R before co-culture with autologous B cells, the co-cultures produced substantial quantities of specific antibody *in vitro* in response to low concentrations of antigen. This phenomenon is probably due to the presence of suppressor T cells (presumably OKT8<sup>+</sup> cells) and is analogous to the phenomenon of low-zone tolerance, which has been well characterized in the mouse (29). Of note is the fact that it was seen in only two out of 10 subjects studied in this manner. In the remaining eight subjects, low-dose irradiation resulted in decreased specific antibody production. These experiments were initially designed to determine if the antigen-specific suppression of *in vitro* antibody production seen at high (10–20  $\mu\text{g/ml}$ ) *in vitro* concentrations of antigen was due

to the generation or activation of suppressor T cells. In contrast to the previously reported findings of others working in human antigen-specific systems (10, 30), we have been unable to demonstrate a T cell contribution to this suppressor phenomenon. In fact, our subsequent studies in this area suggest that not only are suppressor T cells not generated by high-dose antigen stimulation but that under these conditions more potent T helper effects are seen. The suppression of *in vitro* antibody production by high concentrations of antigen appears to be due to a direct effect on the B cell (31).

The present study has demonstrated that PWM-induced T cell help is able to be generated with  $\sim 1/20$  the number of peripheral blood T cells that are required for the generation of antigen-induced T cell help. This difference is presumably due to differences in precursor frequencies for these subsets of helper T cells and suggests that the signals for specific and nonspecific T cell-B cell collaboration may be delivered by different subpopulations of T cells. In contrast, the B cells involved in antigen-induced, antigen-specific antibody production and those involved in PWM-induced antigen-specific antibody production were found to occur with approximately the same precursor frequency. Thus, similar to the murine antigen-specific systems, and in contrast to the human influenza system (32), the antigen-induced helper T cell, which cooperates with antigen-specific B cells, is the limiting factor in the *in vitro* induction of antigen-specific antibody production. This difference from the human influenza system, where the B cell has been shown to be the limiting cell type with respect to the *in vitro* induction of specific antibody, may be due to differences between the two systems with respect to the composition of the peripheral blood lymphocyte pool, which is known to fluctuate greatly as a function of time following antigen exposure. In the influenza study (32), responses were contingent upon natural infection with influenza virus, while in the present study subjects were immunized with a soluble protein antigen, and studies were performed at defined time periods following immunization.

The discovery of at least a 20-fold difference between circulating numbers of antigen-inducible and mitogen-inducible T helper cells can explain our earlier observation that although low cell densities were adequate for the generation of PWM-induced antibody responses, higher cell numbers were generally required for the expression of antigen-induced responses. In addition, we have previously reported that at the higher cell numbers required for the induction of specific antibody production by antigen, PWM-induced responses were generally absent. This latter observation is presumably explained by the finding, demonstrated here in an antigen-specific system and reported by others in polyclonal systems (1,

5, 6), that excessive numbers of T cells suppress a PWM-driven response.

Alloreactivity is known to be able to give rise to either an enhancement or a suppression of the immune response (33, 34). Clearly, the dominant allogeneic effect in the two-way mixed lymphocyte reaction of this system is suppression. The generation of this negative effect is dependent upon the presence of a radiation-sensitive, OKT8<sup>+</sup> subset of T cells in culture and is able to completely override the stimulatory effects of low in vitro concentrations of antigen. This is similar to the findings in the murine system that radiation-sensitive, Lyt1<sup>+</sup>2<sup>+</sup> T cells can mediate a potent negative allogeneic effect on the capacity of primed cells to develop a secondary in vitro antibody response to antigen (33). In contrast to the findings of others (24, 35), who have noted negative, but not positive, allogeneic effects in human antigen-specific antibody production, we have consistently noted potent positive allogeneic effects independent of the presence of antigen in culture, which were only appreciated after the negative allogeneic effects were abrogated either by low-dose irradiation or elimination of OKT8<sup>+</sup> T cells. This effect is presumably similar to that seen by the addition of mixed lymphocyte reaction supernatants to unfractionated PBMNC (35, 36) or to T cell-depleted populations (36, 37). The potency of these allogeneic effects, seen even among first degree relatives, has hampered our efforts to address the question of the presence or absence of genetic restriction in the current system. It is hoped that the recent development of antigen-specific T cell clones (38) may make the answer to this fundamental question in human immunobiology more approachable.

The present study has thus demonstrated a marked dichotomy between antigen-induced and mitogen-induced T cell help with regard to precursor frequency and radiosensitivity. These differences provide strong evidence that in the human system specific and nonspecific T cell signals are delivered by different subpopulations of T cells. In addition, a complex set of both positive and negative allogeneic effects has been described, which must be taken into account in any experiments involving co-culture of cells from different donors. The further use of systems such as this should be of great value in probing the intricacies of human antigen-induced B cell activation and immunoregulation.

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