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FUNCTIONAL ANALYSIS OF HUMAN T CELL SUBSETS DEFINED BY MONOCLONAL ANTIBODIES

I. Collaborative T-T Interactions in the Immunoregulation of B Cell Differentiation[1]

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T-B and T-T interactions involved in the regulation of PWM-triggered human B cell differentiation were studied *in vitro*. Functionally distinct human T cell subsets were isolated by C-mediated lysis by using the monoclonal antibodies OKT4 and OKT8. Graded numbers of either untreated or irradiated T cell subsets were added to autologous B cells, and total antibody synthesis was measured after 5 to 6 days of culture by using a highly sensitive reverse hemolytic plaque assay. The data indicate that a) the helper activity that is exclusively contained within the OKT4$^+$ population is radiosensitive. Only at high T/B ratios can this radiosensitivity be overcome; b) the OKT8$^+$ population contains radiosensitive cells important in suppressing B cell differentiation, and c) the suppression induced with OKT8$^+$ cells requires the presence of radiosensitive OKT4$^+$ cells. Thus, OKT8$^+$ cells added to cultures containing B cells and irradiated OKT4$^+$ cells do not suppress the PFC response. Addition of unirradiated OKT4$^+$ cells to these cultures permits reexpression of suppression by OKT8$^+$ cells. It is concluded that two radiosensitive cells, one within the OKT4$^+$ population and the other within the OKT8$^+$ population, collaborate to induce suppression. Possible mechanisms for this suppressive interaction including induction of suppressor precursor cells within the OKT4$^+$ population or inhibition of OKT4$^+$ helper cells by OKT8$^+$ cells are discussed.

Both the induction and homeostatic regulation of B cell differentiation is mediated, in large part, by T cells (1–3). During the last several years evidence has accumulated that, in addition to T-B interactions, precise interactions among functionally distinct T cell subsets ultimately determines the net outcome of the T cell immunoregulatory influence on B cells (4–8). To date, the most definitive means of assessing these subtle T-T interactions have come from murine studies in which functionally distinct T cell sets have been isolated. Particularly informative have been the studies of T cell sets isolated by virtue of reactivity with alloantibodies (i.e., Lyt1 and Qa-1 systems) that mark cells at discrete stages of T cell differentiation (5, 6). An important example of T-T interaction to emerge from these studies is the capacity of inducer T cells (Lyt1$^+$, Qa-1$^+$) to interact with regulatory T cells (Lyt1, 2, 3$^+$, Qa-1$^+$), thereby generating suppressor T cells (Lyt2, 3$^+$).

Although there is evidence that distinct human T cell sets can either help or suppress B cell differentiation (9–12), the evidence that T-T interactions are involved in these immunoregulatory functions is less secure. Two distinct types of experimental approaches have been useful in addressing this complex issue. First, previous studies have demonstrated that the radiosensitivity of different T cell populations may be used to functionally distinguish helper from suppressor functions (13–16). These studies have suggested that helper functions are radioresistant and suppressor functions radiosensitive. Second, functionally distinct human T cells subsets have been isolated by a variety of criteria including relative binding to specific Ig isotypes, lectin affinity, and reactivity with specific heteroantibodies (11, 17–21). More recently, the production of hybridoma monoclonal antibodies to human T cells at functionally different stages of differentiation has provided a powerful tool to isolate immunoregulatory T cell sets (10, 22, 23).

The strategy employed in the experiments to be reported below involved the isolation of distinct human T cell sets and quantitative assessment of their effects on B cell differentiation alone, or in collaboration with one another. This approach was dependent, in part, on the relative radiosensitivity of distinct immunoregulatory functions of cells contained within these sets. To isolate T cell sets, we utilized two monoclonal antibodies that have been shown to specifically react with T cells. One antibody, termed OKT4, identifies 50 to 60% of human T cells and the other, OKT8, identifies 30 to 40%. Both antibodies are C-fixing and, importantly, C-mediated lysis of unselected T cells when using these two antibodies yields mutually exclusive subsets. Together, these two subsets account for >95% of the peripheral human T cell pool. In previous studies, evidence has been provided that the OKT4$^+$ population contains cells capable of inducing the differentiation of B cells and precytotoxic T cells (10, 22). The reciprocal population, OKT8$^+$, contains cytotoxic effector cells but no helper cells. The role of this latter population in regulating B cell differentiation has not been definitively studied.

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In this report, B cell differentiation triggered by pokeweed mitogen (PWM)[2] was quantitatively assessed by using a highly sensitive reverse hemolytic plaque assay (see References 28, 29). By investigating the radiosensitivity of the immunoregulatory functions mediated by the isolated $OKT4^+$ and $OKT8^+$ subsets, evidence was obtained that the $OKT4^+$ population contains potent and highly radiosensitive helper cells and immunoregulatory cells. In addition, we found that collaborative interactions between radiosensitive $OKT4^+$ and $OKT8^+$ cells are required to suppress B cell differentiation. The relationship between the radiosensitive OKT4 cells involved in inductive functions and those involved in suppression is discussed.

MATERIALS AND METHODS

I. *Lymphocyte preparation and isolation of human T and B cells.* Fresh peripheral blood lymphocytes were isolated from consenting healthy human volunteers by Ficoll-Hypaque density gradient centrifugation. Highly enriched populations of T and B cells were then isolated by methods previously described in detail (24). In brief, human mononuclear cells were washed in minimum essential medium (MEM) (Grand Island Biological Co., Grand Island, NY) containing 5% fetal calf serum (FCS) (Microbiological Assoc., Bethesda, MD) and then passed through a Sephadex G-200 rabbit anti-human $F(ab)_2$ column in the presence of 2.5 mM EDTA. Lymphocytes that bind to the immunoabsorbent columns and can subsequently be recovered by elution with soluble Ig were >90% surface $SmIg^+$; and <10% formed E rosettes. These highly enriched B cell populations were further purified by C-mediated lysis with rabbit anti-human T cell sera ($R\alpha T_H$) or OKT3, a monoclonal antibody (see below) to eliminate any residual T cells (25, 26). The effluent cell population (nonretained) contained <2% cells bearing surface immunoglobulin, and 70 to 85% of these cells formed spontaneous rosettes with sheep erythrocytes (E rosettes). This population was further fractionated into highly purified T cell populations by the formation of E rosettes with sheep erythrocytes (SRBC) and the subsequent isolation by Ficoll-Hypaque sedimentation of pelleted and interface cells. This population, which represents the unfractionated T cell population in the current studies, is >95% E rosette positive and <1% surface membrane immunoglobulin positive.

II. *Production and characterization of monoclonal antibodies to human T cell sets.* Production, growth, and characterization of the hybridoma-secreting monoclonal antibodies, OKT3, OKT4 and OKT8, were previously described in detail (26). In brief, 8-wk-old female CAF_1/J mice (Jackson Laboratories, Bar Harbor, ME) were immunized i.p. with 2×10^7 human T lymphocytes in PBS at 14-day intervals. Four days after the third immunization, the spleens were removed, single cell suspensions were prepared, and cell fusion was carried out according to the procedure described by Kohler and Milstein (27). Thus, 1×10^8 splenocytes were fused with 2×10^7 myeloma cells in media containing 35% polyethylene glycol (PEG), 5% dimethyl sulfoxide (DMSO), and RPMI 1640 (Grand Island Biological Co., Grand Island, NY), as previously described. Subsequently, growing cells were selected in HAT (see Abbreviations) medium at 37°C with 5% CO_2 in a humid environment. Supernatants from growing hybridoma cells were tested for reactivity on isolated T, B, and null cells, both by indirect immunofluorescence and by C-mediated microcytotoxic assays. The monoclonal OKT3, OKT4, and OKT8 antibodies were shown to be highly specific for human T cell populations. The OKT3 antibody reacts with approximately 85 to 95% of E rosette positive cells. The OKT4 antibody reacts with 50 to 60% of peripheral T cells whereas the OKT8 antibody reacts with 30 to 40% of human T cells.

III. *Isolation of human T cell sets by complement-mediated lysis utilizing the monoclonal antibodies OKT4 and OKT8.* In order to isolate T cell populations highly enriched in either OKT4 reactive or OKT8 reactive cells, the unfractionated T cells were resuspended at 50×10^6 cells/ml in RPMI 1640 media containing 5% FCS. To 0.2 ml T cells was added 0.2 ml of either OKT3, OKT4 or OKT8 at a dilution of 1:250, and the cells were then incubated for 45 min at room temperature. After incubation, fresh rabbit C was added at a final dilution of 1:10 and incubation was further carried out for 1 hr at 37°C in a humid atmosphere. This entire procedure was repeated twice in order to thoroughly deplete the T cell population of the designated T cell subset. Analysis of the resulting populations showed that the OKT4-treated population contained >90% $OKT3^+$ cells, >90% $OKT8^+$ cells, and <2% $OKT4^+$ cells, whereas the OKT8-treated population contained >90% $OKT3^+$ cells, >90% $OKT4^+$ cells, and <5% $OKT8^+$ cells. Because of these results, we used the notation $OKT4^+$ to signify a population of T cells remaining after treatment with OKT8 plus C and $OKT8^+$ to signify the reciprocal population remaining after treatment with OKT4 plus C.

IV. *Culture conditions used for the polyclonal induction of a PFC response by human PBL.* The final medium for all cultures consisted of RPMI 1640 (Grand Island Biological Co.), supplemented with 1% penicillin-streptomycin, 200 mM L-glutamine, 2.5 mM HEPES buffer, 0.05 sodium bicarbonate (Microbiological Associates), and 12% heat inactivated FCS. 2×10^5 B cells were suspended in 2 ml of final media and cultured in 10 x 25 mm tissue culture tubes (3207, Falcon Plastics, Oxnard, CA). To these B cell cultures were added graded numbers (see Results) of unselected T cells, $OKT4^+$ cells, or $OKT8^+$ cells in addition to 10 μg of PWM (Grand Island Biological Co.). In some experiments, T cells, or T cell subsets, were irradiated with 1250 R by using the Model M38-1 gammator emitter. Control cultures consisted of 2×10^6 B cells cultured alone or in the presence of PWM without added T cells. All cultures were incubated at 37°C in 5% CO_2 humid atmosphere for 5 to 6 days and then assayed for antibody production using the reverse hemolytic plaque assay.

V. *Reverse hemolytic plaque assay for the enumeration of antibody-secreting cells.* The preparation and purification of rabbit anti-human Ig antibodies has been previously described in detail (28). Purified antibodies eluted from human Ig-coated Sepharose 4B columns were used to coat SRBC by the chromium chloride method as described by Eby *et al.* (29). On the day of assay, cells were thoroughly washed in RPMI 1640 medium and resuspended. Then, 50 to 100 μl aliquots were added to 0.9 ml of 0.5% liquid agarose (SeaKem Agarose, Marine Colloids, Rockland, ME) containing 100 μl of a 11% suspension of antibody-coated sheep erythrocytes. This mixture was layered on a 60 x 15 mm Petri dish previously coated with 5 ml of 0.5% liquid agarose and allowed to gel. The dishes were incubated for 1 hr at 37°C, in a humid atmosphere containing 5% CO_2, 95% air. One milliliter of a 1/100 dilution of rabbit anti-human IgG antisera was then added for an additional hour of incubation. Finally, the antisera were removed and 1 ml of 1/10 dilution of absorbed guinea pig C (Cedarlane Laboratories,

[2] Abbreviations used in the paper: HAT, hypoxanthine aminopterine thymidine; PWM, pokeweed mitogen; PBL, peripheral blood lymphocytes; PFC, plaque-forming cells; $R\alpha T_H$, rabbit anti-human T cell sera; RαHIg, purified rabbit anti-human immunoglobulin.

Hicksville, NY) was added for an additional hour. Plaques were enumerated in triplicate and the results were expressed as the mean PFC/10^6 B cells in original culture. The standard error of the mean was always <20%. In addition, cell counts and viability (by dye exclusion) were performed on all cell cultures at the time of assay.

RESULTS

I. *The radiosensitivity of human immunoregulatory T cells: analysis of unselected T cells.* In initial experiments we found that only small numbers (as few as 1%) of autologous T cells were required for significant PFC formation by highly purified B cells triggered by PWM. Unexpectedly, we observed that the inducing or helper capacity of these small numbers of T cells was radiosensitive. Since many laboratories have reported opposite findings, i.e., that the helper cell in PWM driven B cell differentiation is radioresistant (14–16), we analyzed the radiosensitivity of the inducing T cell population in a quantitative manner. Thus, graded numbers of untreated or irradiated (1250R) T cells were added to a constant number of autologous B cells. PWM was added to the cultures and the number of PFC were enumerated 5 days later. Most striking, the unirradiated T cells were clearly more efficient than irradiated T cells in inducing B cell differentiation over a wide range of T cell concentrations (Fig. 1). With further addition of untreated T cells, the PFC production plateaued and then declined. Only at relatively high T/B cell ratios did we observe greater PFC production with the irradiated T cell population. We concluded from these studies that at least one T helper population is highly radiosensitive. The apparent radioresistance of helper function observed at high T/B cell ratios was more difficult to interpret. One conventional interpretation is that irradiation eliminates the function of a suppressor cell population (14). To explore this idea, in addition to the possibility of T-T cell interactions in this system, we first enriched for functionally distinct T cell subsets using the monoclonal antibodies, OKT4 and OKT8.

II. *Analysis of immunoregulatory T cell subsets by using monoclonal antibodies to T cell surface differentiation antigens.* Graded numbers of unselected T cells, OKT4$^+$, or OKT8$^+$ were added to a constant number of autologous B cells before culture with PWM (Fig. 2). As can be seen, OKT4$^+$ cells are dramatically enriched in helper function. In contrast, and as previously shown, the OKT8$^+$ cells do not generate helper activity (10).

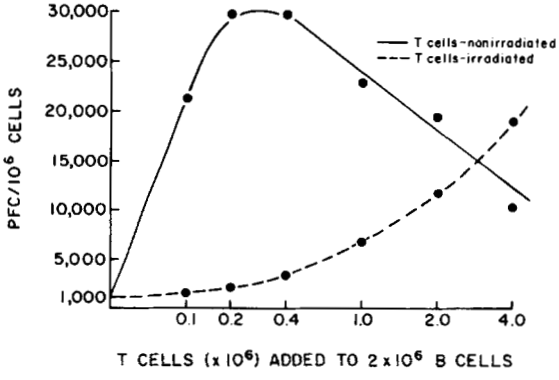

Figure 1. Effect of irradiation on the helper activity of the unfractionated T cell population. 2 × 10^6 B cells were cultured in the presence of PWM and graded numbers of untreated T cells (——) or irradiated T cells (-----). After 5 days, cultures were harvested and assayed for PFC activity.

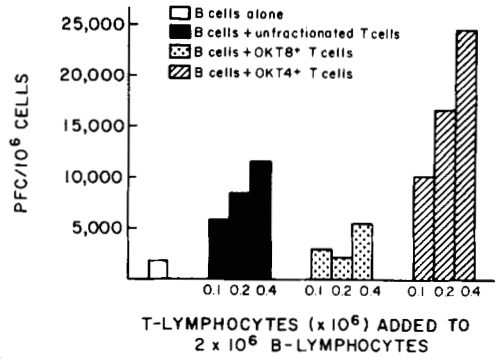

Figure 2. OKT4$^+$, but not OKT8$^+$ enriched populations, induce B cell differentiation. 2 × 10^6 B cells were cultured in the presence of PWM and either unfractionated T lymphocytes (*solid bars*), OKT8$^+$ lymphocytes (*stippled bars*), or OKT4$^+$ T lymphocytes (*diagonal line bars*). After 5 to 6 days, cultures were harvested and assayed for PFC activity.

To further assess these T cell subsets for immunoregulatory functions, graded numbers of either unselected T cells, OKT4$^+$, or OKT8$^+$ cells were added to cultures containing both autologous B cells plus OKT4$^+$ cells (Fig. 3). The further addition of OKT4$^+$ cells enhanced B cell PFC production. In sharp distinction, the addition of small numbers of OKT8$^+$ cells suppressed B cell differentiation. Increasing numbers of OKT8$^+$ cells resulted in increased suppression. Taken together, these results demonstrate that the OKT4$^+$ population contains inducer cells whereas the reciprocal population of OKT8$^+$ cells contain a population of T cells important in suppression.

III. *Analysis of the radiosensitivity of isolated OKT4$^+$ and OKT8$^+$ cell functions.* The data presented in Figure 1 demonstrated that the effects of irradiation on immunoregulation in unselected T cell populations are complex and highly dependent on the number of T cells present. To help resolve this complexity, the functional radiosensitivity of OKT8$^+$ or OKT4$^+$ populations was examined. In the first set of experiments, graded numbers of untreated or irradiated OKT8$^+$ cells were tested for their capacity to suppress the PFC response of B cells cultured with unirradiated OKT4$^+$ cells (Table I). As expected, and consistent with results in Figure 3, the addition of unirradiated OKT8$^+$ cells suppressed PFC formation. Irradiation of these OKT8$^+$ cells (1250R) markedly reduces this suppressor function at all cell concentrations tested. Thus, the suppressor function mediated by OKT8$^+$ cells is radiosensitive.

To investigate the effects of irradiation on the helper function mediated by OKT4$^+$ cells alone, graded numbers of either untreated or irradiated OKT4$^+$ cells were added to a constant number of B cells (Fig. 4). Over a wide range of OKT4$^+$ concentrations (0.02 × 10^6 to 0.2 × 10^6), the helper function was markedly radiosensitive. Interestingly, at high concentrations of unirradiated OKT4$^+$ cells, the PFC response observed plateaued and subsequently declined, similar to the findings with unfractionated T cells. In contrast, addition of irradiated OKT4$^+$ cells induced B cell PFC only at high T/B ratios.

It is unknown whether the abrogation by irradiation of helper function at low OKT4$^+$ concentrations and the apparent radioresistance of help at high concentrations is a reflection of distinct radioresistant and radiosensitive subsets of T cells within the OKT4$^+$ population. However, these results, taken together with the observed plateau and subsequent decline of helper function noted in the unirradiated OKT4$^+$ population are compatible with the idea that the OKT4$^+$ population contains a distinct radiosensitive immunoregulatory cell. Further evidence that radiosensitive OKT4$^+$ cells participate in im-

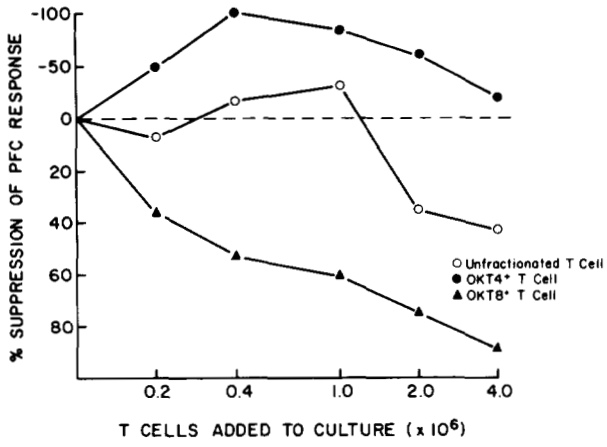

Figure 3. OKT8$^+$ T cells mediate suppression of OKT4$^+$ T cell-induced Ig production by B cells. The standard culture contained 0.1 $\times$ 10^6 OKT4$^+$ T cells and 2.0 $\times$ 10^6 B cells in addition to 10 μg PWM. To this system was added graded number of either unfractionated T cells (○), OKT4$^+$ (●) or OKT8$^+$ T cells (△). After 5 days, cultures were harvested and assayed for PFC activity. Suppression was calculated as follows:

$$\% \text{ Suppression} = \left(1 - \frac{\text{PFC (Experimental culture)}}{\text{PFC (Standard culture)}}\right) \times 100.$$

TABLE I

Irradiation of OKT8$^+$ cells abrogates suppressor function

Number of OKT8$^+$ Cells per Culture[a]	Irradiation of OKT8$^+$ Cells[b]	PFC/10^6 Cells	% Suppression[c]
0	–	13,550 ± 1,250	0
0.4 × 10^6	–	7,350 ± 520	45
	+	11,750 ± 1,030	14
1.0 × 10^6	–	3,850 ± 85	72
	+	12,500 ± 1,450	8
2.0 × 10^6	–	3,250 ± 101	76
	+	13,000 ± 1,430	4

[a] Graded numbers of OKT8$^+$ T cells were added to standard cultures containing a fixed number of B cells (2.0 × 10^6) and OKT4$^+$ (0.1 × 10^6) cells.

[b] Irradiated T cells are designated by (+).

[c] % Suppression was calculated as before (Fig. 3).

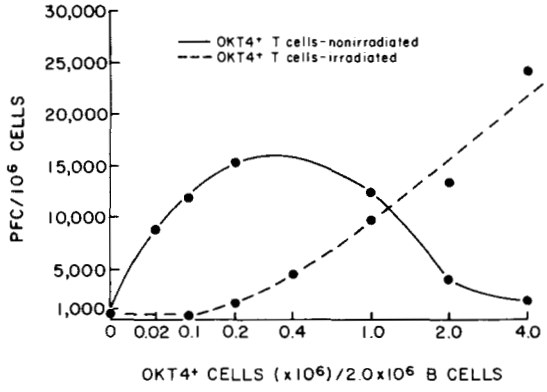

Figure 4. Effect of irradiation on the helper activity of the OKT4$^+$ T cell subset. 2 × 10^6 B cells were cultured in the presence of PWM and graded numbers of untreated OKT4$^+$ T cells (——) or irradiated OKT4$^+$ T cells (-----). Five days later, cultures were harvested and assayed for PFC activity.

munoregulatory functions other than induction of B cell differentiation is presented in the next section.

IV. *Interactions between radiosensitive OKT4$^+$ and OKT8$^+$ populations in the generation of suppression.* In the following experiments we asked whether OKT4$^+$ cells collaborated with OKT8$^+$ cells in mediating suppression of B cell differentiation. Thus, graded numbers of OKT8$^+$ cells were tested for their capacity to suppress the PFC response of B cells cultured either with unirradiated OKT4$^+$ cells (0.1 × 10^6 cells) (Fig. 5*a*) or irradiated OKT4$^+$ cells (1.0 × 10^6 cells) (Fig. 5*b*). In this experiment, the level of helper activity obtained with 0.1 × 10^6 OKT4$^+$ unirradiated cells was similar to the helper activity obtained with 1.0 × 10^6 OKT4$^+$ irradiated cells. As expected and consistent with the results shown in Table I, addition of unirradiated OKT8$^+$ cells suppressed PFC formation whereas irradiation of OKT8$^+$ cells abrogated this suppressor function.

In contrast (Fig. 5*b*), addition of unirradiated OKT8$^+$ cells to B cells cultured with irradiated OKT4$^+$ cells did not suppress PFC formation. Furthermore, addition of small numbers of unirradiated OKT4$^+$ cells to cultures containing irradiated OKT4$^+$ cells and unirradiated OKT8$^+$ cells induced suppression (Table II). This experiment demonstrates that the lack of suppression observed with irradiated OKT4$^+$ cells is not simply the result of an excessively high OKT4/OKT8 ratio. These observations, taken together, provide direct evidence that a radiosensitive subset of OKT4$^+$ T cells is required for suppression of the PFC response. Thus, suppressor cell activity requires collaboration between two distinct populations of radiosensitive cells, one residing in the OKT4$^+$ population and the other in the OKT8$^+$ population.

DISCUSSION

In the present studies we employed a highly sensitive reverse hemolytic plaque assay that detects clones of antibody-forming cells to further define the complex T-T and T-B interactions important in the induction and homeostatic regulation of human B cell differentiation triggered by PWM. Two independent experimental approaches were used to dissect the heterogeneity

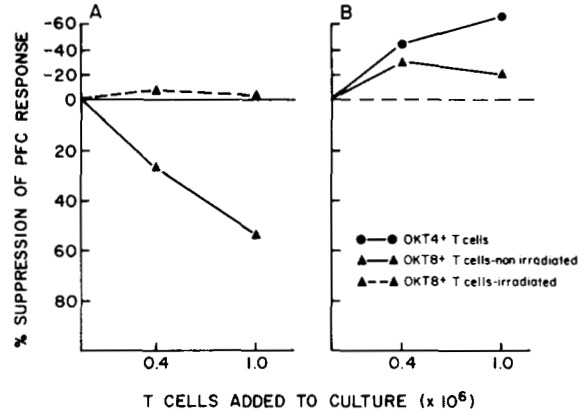

Figure 5. Collaborative interactions between radiosensitive OKT4$^+$ and OKT8$^+$ cells in the generation of suppressor activity. A, the standard cultures contained 0.1 × 10^6 OKT4$^+$ T cells and 2.0 × 10^6 B cells in addition to 10 μg PWM. To this system was added graded numbers of either untreated OKT8$^+$ T cells (△——△) or irradiated OKT8$^+$ T cells (△-----△). B, the standard cultures contained 1 × 10^6 irradiated OKT4$^+$ T cells and 2.0 × 10^6 B cells in addition to 10 μg PWM. To this system was added graded numbers of either OKT4$^+$ T cells (●——●) or OKT8$^+$ T cells (△——△). After 5 days, cultures were harvested and assayed for PFC activity. Suppression was calculated as described in the legend for Figure 3.

TABLE II

Re-expression of suppressor function by addition of unirradiated OKT4⁺ cells

Numbers of OKT4⁺ Cells[a]	OKT8 Cells Added	PFC/10^6 Cells	% Suppression[b]
0	1.0×10^6	1,700 ± 250	0
0.1×10^6 untreated	0	13,550 ± 1,250	0
	0.4×10^6	7,345 ± 650	46
	1.0×10^6	3,850 ± 630	72
1.0×10^6 irradiated	0	4,300 ± 270	0
	0.4×10^6	6,200 ± 890	<0
	1.0×10^6	3,800 ± 620	12
0.1×10^6 untreated	0	15,700 ± 2,050	0
$+1.0 \times 10^6$ irradiated	0.4×10^6	9,500 ± 1,600	40
	1.0×10^6	7,600 ± 650	52

[a] 2×10^6 B cells were cultured during 5 days in the presence of 10 μg of PWM. To these cultures various numbers of OKT4⁺ T cells and OKT8⁺ T cells were added.

[b] % suppression was calculated as before (Fig. 3).

of human T cells. First, the monoclonal antibodies, OKT4 and OKT8, that have been previously shown to identify specific differentiation antigens on reciprocal human T cell subsets were used to isolate functionally distinct immunoregulatory cell populations (30). Second, the differential radiosensitivity of the immunoregulatory functions mediated by these isolated T cell subsets was examined in detail. Several important points emerge from these studies: 1) T cell helper functions are exquisitely radiosensitive over a wide range of T cell concentrations; 2) helper functions of T cells are mediated exclusively by cells identified by the OKT4⁺ monoclonal antibody; 3) the reciprocal T cell population identified by the OKT8⁺ monoclonal antibody are depleted of helper function and enriched in radiosensitive cells involved in the suppression of B cell differentiation; however, 4) the suppression observed with radiosensitive OKT8⁺ cells requires the presence of radiosensitive OKT4⁺ cells.

Based on these observations, it is not surprising that irradiation of unselected T cells before addition to B cells may have complex effects on the net outcome between induction and suppression of subsequent B cell differentiation. Interestingly, previous reports from a number of laboratories have emphasized the relative radiosensitivity of human suppressor T cells and the relative radioresistance of helper cells (14–16). Our studies demonstrate conclusively the presence of a potent and highly radiosensitive helper T cell population (Figs. 1 and 4). Only with large numbers of irradiated T cells or OKT4⁺ cells (T-B ratio >1) are B cells induced to differentiate into PFC. Thus, only at high T-B ratios is the T cell helper activity radioresistant. These studies emphasize the importance of the quantitative analysis of immunoregulatory cell interactions. At the present time, it is unknown whether the radiosensitive and radioresistant helper activities represent functions of distinct T cell subsets within the OKT4⁺ population. It is possible that the radiosensitivity of a single OKT4⁺ helper cell population can be overcome at high cell concentrations, or alternatively a radiosensitive OKT4⁺ cell capable of inhibiting inducer function may be present. Precise analysis of the putative heterogeneity within the OKT4⁺ population may require additional monoclonal antibodies reacting with a fraction of OKT4⁺ cells.

We would emphasize that helper functions were not observed in the reciprocal T cell subset identified by the OKT8 monoclonal antibody. However, the OKT8⁺ population was clearly shown to contain cells important in suppressing the PFC response. The suppressor function mediated by the OKT8⁺ cell population was radiosensitive and, most importantly, required the presence of radiosensitive OKT4⁺ cells. Interactions between distinct T cell sets in the suppression of B cell differentiation in murine systems has been extensively documented (4–8). With respect to human cells, however, the evidence for similar T-T interactions inducing suppression has been limited, in part, by the lack of precise means to isolate functionally distinct T cell sets. It is of interest that in the investigation of patients with T cell leukemias whose blast cells would induce suppression of B cell differentiation, the leukemic cells required radiosensitive normal T cells to induce suppression (31). The data presented here are consistent with these studies and provide direct evidence that two distinct normal human T cell sets, one identified in the OKT4⁺ population and the other in the OKT8⁺ population, collaborate in the suppression of B cell differentiation.

Since the radiosensitive OKT4⁺ population contains cells important in inducing B cell differentiation and also contains cells intimately involved in suppression, the question arises as to whether this functional heterogeneity also reflects distinct T cell subsets within the OKT4⁺ population. Although this point cannot be resolved definitively at the present time, alternative models can be readily envisioned. First, it is possible that the same radiosensitive OKT4⁺ cell that potently induces B cells to differentiate into PFC can also induce OKT8⁺ cells to suppress. Second, it is possible that the target of suppression by OKT8⁺ cells are radiosensitive OKT4⁺ cells. Clearly, these first two models are not mutually exclusive. By analogy with murine T-T interactions, for example, there is ample evidence for the idea that helper cells (largely Lyt1) can induce suppression after interactions with distinct immunoregulatory cells (4–6). In addition, there is also evidence that at least one target for the effector/suppressor cell is the Lyt1 helper cell (4).

Alternatively, a third model can be envisioned in which OKT4⁺ cells contain precursor populations that can be induced to further differentiate into mature helper or suppressor cells. This type of differentiative model would be analogous, for example, to the induction of a mature helper and suppressor cell from Lyt1, 2, 3 murine precursor cells (32). In this regard, we were interested in the possibility that isolated OKT4⁺ cells might be induced to differentiate *in vitro* into OKT8⁺ cells. To date, we have found that the surface phenotype of the OKT4⁺ population is remarkably stable *in vitro*. We have not observed the appearance of OKT8⁺ cells even after triggering of OKT4⁺ cells with antigens, allogeneic cells, or mitogens (unpublished observations). Thus, if mature suppressor cells are generated from precursors within the OKT4⁺ population, they maintain the OKT4⁺ OKT8⁻ phenotype.

The evidence to suggest that the OKT4⁺ population may generate suppressor cells independent of OKT8⁺ cells is that addition of graded numbers of unirradiated OKT4⁺ cells (OKT8 depleted) to B cells does not result in a linear increase in PFC (Fig. 4). A linear increase would, in fact, be predicted if the OKT4⁺ population were exclusively inducer cells (33). Instead, a plateauing effect at relatively low OKT4 concentrations and a subsequent decline of PFC at high concentrations is observed. Although results obtained at high T-B ratios may be complicated, in part, by nonspecific effects of cell culture (nutritional, overgrowth, or contamination with macrophages or other cells), the plateauing effect at low T cell concentrations does support

the possibility that suppressor cells may be generated within the $OKT4^+$ population alone. Simple exhaustion of B cells by highly potent radiosensitive $OKT4^+$ helper populations is excluded by the fact that even greater PFC formation was observed with high numbers of irradiated $OKT4^+$ cells (Fig. 4).

If suppressor/effector cells are generated from the $OKT4^+$ cell population, a fourth model to consider is that a radiosensitive cell within the $OKT4^+$ population may be programmed for either help or suppression. This type of immunoregulatory T cell has been postulated in murine systems in the past, however, no firm evidence to establish its existence has emerged (34). We would emphasize, though, that the presence of such a cell cannot be excluded either from previous analysis of T-T interactions in murine systems or in these current studies of human T cell interactions.

Irrespective of which of these or other models prove to be correct, it is clear that collaboration between distinct human T cell sets is important in the immunoregulation of B cell differentiation. The mechanisms involved in the control of these T-T interactions remain largely unexplored. In addition, it is likely that further heterogeneity within the $OKT4^+$ or $OKT8^+$ populations will be detected. We would anticipate the precise understanding of putative defects that may arise in patients with either immunodeficiency states or autoimmunity will be dependent on further analysis of this T cell heterogeneity as well as on the mechanisms that control the T-T interactions described herein.

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