Brief Definitive Report

FUNCTIONALLY RESTRICTED, ALLOSPECIFIC, HUMAN HELPER T CELL LINES THAT AMPLIFY EITHER B CELL OR CYTOLYTIC T CELL RESPONSES*

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Helper T cells play a crucial role in the induction and amplification of humoral and T cell-mediated immune responses in man. For example, helper T cells are absolutely required for the polyclonal immunoglobulin response triggered by pokeweed mitogen (PWM) (1) as well as for antigen-specific antibody responses to virus (2), soluble proteins (3), hapten (4), and heterologous erythrocytes (5). Moreover, helper T cells markedly facilitate the in vitro generation of allospecific (6) and altered self-reactive (7) cytotoxic T lymphocytes (CTL) from $OKT8^+$ CTL precursors. Although help for B cells and CTL precursors is mediated, predominantly, by the $T4^+$, $T8^-$ T cell subset, it is not established whether a single helper T cell pool regulates both arms of the immune response, or if functionally distinct human helper T cell subpopulations exist. To address this question we have analyzed the immunoregulatory activities of several HLA-DR-1-specific, alloproliferative, human T cell lines (TCL) recently established in our laboratory (8). Our results demonstrate that helper TCL which trigger polyclonal antibody production by B cells are distinct from those which amplify the generation of allospecific CTL.

Materials and Methods

Isolation and Fractionation of Peripheral Blood Lymphocytes (PBL). Fresh PBL were isolated from healthy volunteers by Ficoll-Hypaque centrifugation. T cells were isolated by E rosette formation, whereas B cell populations consisted of E rosette-negative cells depleted of contaminating T cells by complement (C)-mediated lysis in the presence of OKT3, a monoclonal anti-human T cell antibody (9).

Allospecific TCL. The TCL used in these studies have been described previously (8). Briefly, alloreactive T cells, generated by co-culture of donor A (HLA-DR 2, 3) responder cells and donor B (HLA-DR 1, 7) x-irradiated stimulators, were isolated by limiting dilution culture and propagated by repetitive stimulation with interleukin 2 (IL-2) and x-irradiated feeder cells. Every 7 d, the TCL cells were expanded to a density of 1×10^5/cc of final medium, and supplemented with an equal number of x-irradiated $DR\ 1^+$ allogeneic feeder cells (donor B) and an optimal concentration of IL-2 (8). The final medium consisted of Iscove's modified Dulbecco's medium (Gibco Laboratories, Grand Island, NY), supplemented with 10% fetal calf serum, penicillin-streptomycin, and 5×10^{-5} 2-mercaptoethanol (Gibco Laboratories). Partially cloned TCL derived from cloning plates seeded at 10 cells per microwell or 1 cell per microwell were assigned the prefix 10- or 1-, respectively.

B Cell Helper Assay. 5×10^5 B cells were cultured alone or with 5×10^4 autologous T cells

* Supported in part by grants AI 14969 and AI 18263 from the National Institutes of Health and by the Arthritis Foundation.

or TCL cells in 0.5 cc of final medium. After 6 d of incubation at 37°C in a 95% air, 5% CO_2 humid atmosphere, cultures were assayed for polyclonal B cell activation using the reverse hemolytic plaque assay (10). For each culture, three separate 100-μl aliquots were assayed and the data expressed as plaque-forming cells (PFC) per culture. SEM for all PFC results were <10%.

CTL Helper Assay. Each sensitization culture consisted of 12 replicate microwells (Linbro MR-2; Flow Laboratories, Inc., Hamden, CT) containing 2×10^5 responder T cells and 1×10^5 x-irradiated (1,500 rad) stimulator cells suspended in 0.2 cc of final medium. In all studies, responder T cells were autologous to the allospecific TCL (i.e., derived from donor A). Stimulator populations consisted of fresh autologous (donor A) or DR 1^+ allogeneic (donor B) PBL, or TCL cells that had been cultured with x-irradiated donor B feeder cells for 7 d as described above. As a negative control, x-irradiated donor B PBL, which had been cultured alone for 7 d, were used as stimulators. After 6 d, sensitization cultures were harvested and assayed, in triplicate, at the killer to target (K/T) ratios indicated for cytotoxicity against ^{51}Cr-labeled targets in a standard 4-h ^{51}Cr-release assay. Results are presented as percent lysis ± SEM. The surface phenotype of the cytotoxic effector cells was determined by C-mediated lysis in the presence of ascites, OKT4, or OKT8 as previously described (7).

Results

The allospecific TLC used in these studies have been described previously (8). Each is comprised exclusively of T cells belonging to the $T4^+$, $T8^-$ subset and proliferates specifically to allogeneic stimulator cells bearing the HLA-DRw 1 antigen. In addition, many of these TCL (10-31, 10-36, 10-41, and 1-7) provide major histocompatibility complex (MHC)-restricted help for B cell differentiation. Thus, co-culture of TCL cells with DRw 1^+, but not DRw 1^-, B cells results in a vigorous polyclonal PFC response as measured in the reverse hemolytic plaque assay. Although MHC restricted at the inductive level, helper TCL cells that have been activated by DRw 1^+ stimulators can trigger the differentiation of "bystander" DRw 1^- B cells (8).

In contrast, other DRw 1-specific TCL (e.g., 1-8 and 10-33) did not provide help for B cells, suggesting that they may be involved in other aspects of immunoregulation. We were, therefore, interested to determine if these TCL could provide help for T cell responses and in particular for the generation of cytotoxic T cells. To this end, TCL cells that had been "fed" x-irradiated DRw 1^+ allogenic cells 7 d previously were harvested, washed, x-irradiated, and co-cultured with freshly isolated autologous T cells. After 6 d, the allospecific cytotoxic response generated in these cultures was assayed. This sensitization protocol was chosen for two reasons. First, we have shown that DRw 1^+ feeders provide a powerful antigen-specific stimulus that activates and enhances the growth of the allospecific TCL cells (8). Second, after several days in culture, x-irradiated feeder cells are poorly immunogenic, therefore permitting any helper influence mediated by the TCL cells to be more readily evaluated.

As shown in Table I, precultured donor B allogeneic feeders, either alone or in the presence of 1-7 or 10-31 TCL cells, are poor stimulators of an alloreactive CTL response. In contrast, precultured donor B feeders, in conjunction with 1-8 TCL cells, stimulate a cytotoxic response against donor B targets that is equivalent to that triggered by fresh allogeneic stimulators. The effector CTL generated in these sensitization cultures are $OKT8^+$ (Table II) and specific for Class I MHC (HLA-A 23) determinants expressed on the allogeneic feeder cells (Table III). Moreover, TCL 1-8 cells can enhance the cytotoxic response directed against DRw 1^- cells present in the sensitization culture, suggesting that these TCL may act through the elaboration of nonspecific helper factor(s) (data not shown). In summary, TCL 1-8

TABLE I

Amplification of Allospecific Cytotoxicity by TCL 1-8

Description of culture		Percent lysis ± SEM on targets			
		A	B		
Responder	X-irradiated stimulator	K/T, 40:1	K/T, 40:1	K/T, 20:1	K/T, 10:1
E^+	Donor A	−0.5 ± 0.7	−3.5 ± 1.4	0.1 ± 2.2	NT
E^+	Donor B	−1.1 ± 0.9	23.1 ± 1.8	12.7 ± 1.6	5.7 ± 1.4
E^+	Donor B feeders	−2.3 ± 1.4	4.4 ± 0.5	2.6 ± 1.1	−0.3 ± 1.7
E^+	TCL 1-7 + Donor B feeders	NT	6.1 ± 2.3	−0.4 ± 1.3	−2.4 ± 1.2
E^+	TCL 1-8 + Donor B feeders	−1.2 ± 0.4	19.4 ± 0.4	10.9 ± 2.0	4.9 ± 0.4
E^+	TCL 10-31 + Donor B feeders	NT	1.5 ± 0.2	0.6 ± 0.7	0.1 ± 0.4

Responder T cells, autologous to the TCL, were co-cultured with fresh x-irradiated autologous (donor A) or allogeneic (donor B) PBL, x-irradiated allogeneic PBL feeders precultured alone for 7 d (donor B feeders) or TCL cells that had been precultured for 7 d with x-irradiated B feeders. TCL stimulators were x-irradiated before use as stimulators. After 6 d, cytotoxicity on ^{51}Cr-labeled PBL targets was measured at the K/T ratios indicated. NT, not tested.

TABLE II

Cytotoxic Effector Cells are OKT8$^+$

Treatment of killer cells	Percent lysis ± SEM of B targets			
	K/T ratio			
	40:1	20:1	10:1	5:1
Ascites + C	45.9 ± 4.5	37.1 ± 4.9	22.6 ± 3.2	11.2 ± 1.2
OKT 4 + C	50.4 ± 4.2	44.5 ± 1.4	30.3 ± 0.4	16.9 ± 7.1
OKT 8 + C	7.1 ± 2.2	2.4 ± 1.3	2.6 ± 0.8	0.2 ± 0.8

Responder T cells were co-cultured with x-irradiated 1-8 TCL cells that had been precultured for 7 d with B feeders. After 6 d, the culture was harvested, divided into three groups and treated with a 1:250 dilution of ascites, OKT4, or OKT8 in the presence of C. Each group was washed, counted, and assayed at the K/T ratio indicated for cytotoxicity on ^{51} Cr-labeled B target cells.

TABLE III

CTL Induced by TCL 1-8 are Specific for Class I MHC Determinants

	Target cell donor	Target cell HLA type			Percent lysis ± SEM on targets		
					K/T ratio		
		A	B	DRw	40:1	20:1	10:1
Experiment 1	A	2,3	12,35	2,3	3.1 ± 1.2	NT	0.8 ± 0.3
	B	3,23	12,21	1,7	56.9 ± 6.3	NT	27.1 ± 2.2
	C	1,2	8,w44	3,5	3.9 ± 1.4	NT	2.2 ± 0.5
	D	23,26	14,w44	,7	24.1 ± 3.7	NT	9.3 ± 1.6
	E	3,w33	27,w35	1,5	2.6 ± 0.8	NT	1.4 ± 0.2
Experiment 2	B	3,23	12,21	1,7	56.5 ± 3.5	34.7 ± 2.2	20.0 ± 2.4
	F	26,29	16,35	4,5	9.2 ± 3.1	5.8 ± 1.4	0.6 ± 0.3
	G	23,26	16,21	4,7	49.4 ± 3.7	30.4 ± 1.6	13.5 ± 2.6

Responder T cells were co-cultured with x-irradiated 1-8 TCL cells that had been precultured for 7 d with B feeders. After 6 d, the culture was harvested and assayed at the K/T ratios indicated for cytotoxicity on a panel of ^{51}Cr-labeled target cells of known HLA haplotype. NT, not tested.

cells appear to provide a "second signal" for CTL precursors that have been partially activated by interaction with antigen.

It is striking that TCL 1-8, which amplifies allospecific CTL responses, is minimally active in the B cell helper assay, while TCL 1-7 and 10-31, which provide potent

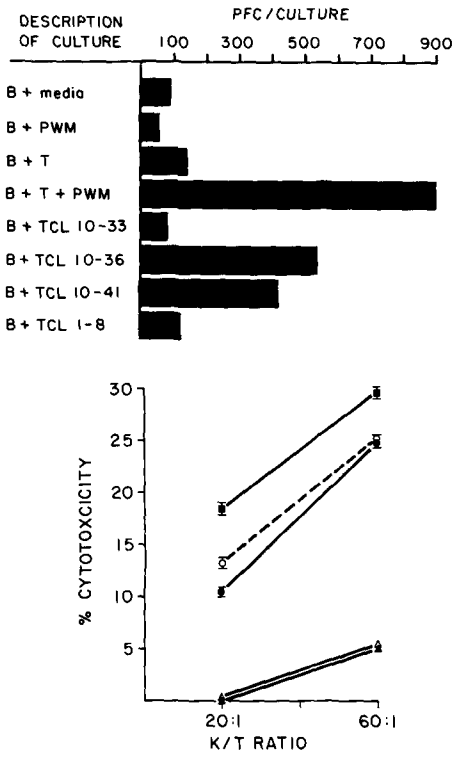

FIG. 1. Reciprocating helper activities of allospecific TCL. Four DRw-1-specific TCL, cultured for 7 d with donor B feeders, were harvested, washed, and assayed for B cell- and CTL-directed helper activity. (Top) 5×10^5 DRw 1^+ B cells (donor B) were cultured alone or with 5×10^4 autologous T cells or the various TCL cells. After 6 d, cultures were assayed for PFC activity. (Bottom) Responder T cells, co-cultured with the following x-irradiated stimulator populations, were assayed for cytotoxicity on ^{51}Cr-labeled donor B targets: fresh donor B stimulators (○); TCL 1-8 (■); 10-33 (●); 10-36 (▲); 10-41 (△).

DRw-restricted help for B cells, do not activate CTL precursors. These results suggest that allospecific human T cells may mediate distinct, reciprocating helper activities. To pursue this point, we extended our studies to include other DRw-1-specific TCL available in our laboratory and assayed simultaneously for B cell- and CTL-directed helper activity. As shown (Fig. 1, top), TCL 10-36 and 10-41, but not 10-33 or 1-8, effectively trigger a polyclonal PFC response by DRw 1^+ B cells. In contrast, TCL 10-33 and 1-8 markedly enhance the generation of allospecific CTL, whereas TCL 10-36 and 10-41 do not provide T cell help (Fig. 1, bottom).

Discussion

This report describes the identification of two types of OKT4$^+$, DRw-1-specific, alloproliferative human TCL that mediate distinct, reciprocating helper functions. One preferentially triggers the differentiation of B cells into PFC, while the other selectively amplifies allospecific CTL responses. It is likely that these TCL differ with respect to the helper molecules which they elaborate. Thus, after antigen activation, the former may produce T cell replacing factor (TRF) while the latter releases killer cell activating factor (KAF). It should be noted that cloned human (11) TCL may

elaborate a wide spectrum of immunoregulatory molecules, including TRF and KAF. One might argue, therefore, that the restricted helper functions mediated by our TCL reflect an artifact of long-term in vitro growth that has led to the selective loss of mediator production. We would point out that the functional restrictions described have been a stable characteristic of these TCL since their inception >1 yr ago (8). Moreover, our findings are consistent with reports describing functional heterogeneity within freshly isolated human helper T cell populations (12, 13). For example, Gatenby et al. (13) observed that both Leu $3^+,8^+$ and Leu $3^+,8^-$ T cells proliferate during the autologous mixed lymphocyte response, but only the Leu $3^+,8^-$ T cells provide help for B cell differentiation. It will be of interest to characterize our TCL with respect to Leu 8 and other monoclonal antibodies that recognize a fraction of the inducer T cell population.

Finally, these findings may be relevant to our understanding of acquired immunodeficiency (AID) syndromes in man. For example, in some patients with common variable hypogammaglobulinemia, T cell-mediated responses are preserved, while in the recently described AID syndrome, patients are markedly deficient with respect to T cell function but have normal or heightened humoral immune responses. Interestingly, both conditions may be characterized by a reduced ratio of T4 to T8 cells in the peripheral blood (14, 15). Conceivably, these diametrically opposed forms of immunodeficiency may result from an acquired defect (e.g., infectious agent) involving one of the helper T cell subsets described in this report, or an immunoregulatory circuit specifically targeted to one of these subsets.

Summary

Using a panel of partially cloned, $OKT4^+$, DRw-1-specific, alloproliferative human T cell lines, we have identified two functionally restricted and reciprocating types of helper T cells. One provides major histocompatibility complex-restricted help for plaque-forming cell responses by DRw 1^+ allogeneic B cells; the other preferentially amplifies the generation of allospecific cytotoxic T lymphocytes (CTL) from CTL precursors that have been suboptimally triggered by alloantigen.

We wish to thank Dr. Nicole Suciu-Foca for performing the HLA typing of the donors used in this study and Dr. Leonard Chess for his encouragement and help throughout all phases of this work.

Received for publication 28 January 1983.

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