

Parasite Antigen-specific Human T Cell Lines and Clones

Major Histocompatibility Complex Restriction and B Cell Helper Function

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Abstract. The development of T lymphocyte lines and clones of defined specificity has become an important method for investigating both T cell recognition of foreign antigens as well as T cell influence on B cells. In the present study, human antigen-specific T cell lines and clones have been derived from a patient with a naturally acquired filarial infection. These T cells are of the helper phenotype (Leu 1+, Leu 2-, Leu 3+) and are independent of exogenous interleukin-2. Furthermore, these T cells have been shown to require both antigen-presenting cells and antigen for optimal proliferation.

Helper function mediated by these T cells as manifested by the in vitro induction of parasite-specific antibody was antigen-dose dependent, requiring much lower antigen concentrations than those necessary to induce blastogenesis. More importantly, there is an absolute requirement of the T cell line for HLA-DR histocompatible antigen-presenting cells; clones derived from this T cell line show a more specific DR-related restriction—to only one of the two parental DR haplotypes in antigen stimulated proliferative responses.

Such parasite antigen specific human helper T cell lines and clones should prove useful in exploring the fine control of the host response to naturally acquired helminth infections. In addition, these long-term T cell lines and clones can provide a potent tool for examining not only the events involved in human T cell responses to parasite antigens, but also into the associated cellular and humoral factors necessary for the B cell responses which follow.

Introduction

The human filariases, estimated to affect ~250 million people (1), are chronic parasitic helminth infections that evoke host immune responses which have been implicated both in resistance to infection (2, 3) and in the pathogenesis of the various clinical manifestations of these diseases (3). Though serological studies reveal that infected individuals develop anti-filarial antibodies of different immunoglobulin isotypes, the manner by which the parasite antigens elicit the production of parasite-specific antibodies is not well characterized. More importantly, it has been difficult to evaluate the fine control of the immune response to parasite antigens at either the cellular or subcellular level because of the lack of a satisfactory in vitro model of antigen-induced T or B lymphocyte function.

Epidemiological studies in areas where filariasis is endemic have revealed differential susceptibilities to infection both within the entire population as well as within families studied (4). Though the cause of this difference in susceptibility has not been studied directly in the filarial diseases, work done with other helminths (5–7) and protozoa (8) has implicated the involvement of the major histocompatibility complex, MHC.¹ Studies done with congenic mouse strains have shown that the H-2 complex in mice may affect differences of antibody titers and delayed type hypersensitivity to the parasite *Schistosoma mansoni* (9). More recently, a strong association between HLA-D specificity and low responsiveness of peripheral human T cells to antigen extracted from *Schistosoma japonicum* worms has been reported (10).

Though the requirement of MHC recognition of antigen by T cells is well established, the events controlling this recognition have only lately begun to be elucidated, primarily because of

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1. Abbreviations used in this paper: AET, 2-aminoethylisothiourea bromide; BMA, filarial antigen derived from *Brugia malayi* adult parasites; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; IL-2, interleukin 2; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin A; PWM, pokeweed mitogen; TCL, T cell line; TT, tetanus toxoid.

the ability to maintain antigen-specific T lymphocytes in vitro (11–18). Initially, much of the work on T cell lines (TCLs) and clones was carried out in the murine system; however, the methods for developing long-term T cell populations specific for particulate and soluble antigens have now been extended to human cells (16, 17, 19–22). TCLs have been developed which require histocompatible antigen-presenting cells at the HLA-DR region in order for antigen-specific proliferation to occur; cloned cells from these and other lines have been developed and shown to recognize soluble and particulate antigens only in a DR-restricted fashion (16, 17, 21–25).

Similarly, when supernatants from these T cell clones have been examined, soluble factors have been shown to enhance immunoglobulin production (26). In addition, TCLs and clones of the helper/inducer phenotype have been shown to be capable of helping B lymphocytes produce specific immunoglobulin (21, 24, 27).

In the present study we have established filarial antigen-specific human T cells by the technique of repeated stimulation with a soluble parasite antigen and subsequent rest in the absence of this antigen. The T cells produced in this manner are independent of exogenous interleukin-2 (IL-2) for their growth. Furthermore, this is the first demonstration that antigen-specific, IL-2-independent TCLs and clones are able to be developed from naturally infected subjects rather than from subjects that were artificially immunized. More importantly, work done with these TCLs show that parasite antigen recognition is class II (DR) restricted; these same cells provide help for B cell antibody production.

Methods

Antigens. *Brugia malayi* adult antigen (BMA) was prepared as a saline extract of adult parasites as described and characterized previously (28); tetanus toxoid was obtained from the Massachusetts Public Health Service (a gift of Dr. Thomas Folks).

Cell separation. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood. Leukocytes were separated by low speed centrifugation (200 g) for 8 min and further purified on a Hypaque-Ficoll gradient in a standard fashion (29). PBMC were cryopreserved in RPMI-1640 (Biofluids, Rockville, MD) supplemented with 10% fetal calf serum (FCS), 25 mm Hepes (MA Bioproducts, Walkersville, MD), 80 µg/ml gentamicin (Schering Corp., Kenilworth, NJ), 7.5% dimethyl sulfoxide in a rate-controlled cell freezer (Cryo Med., Mt. Clemens, MI), and stored in liquid nitrogen until use. T cell-depleted mononuclear cells were obtained by rosetting with 2-aminoethylisothiouranium bromide (AET) as described previously (30).

HLA typing. Tissue typing for HLA-A and HLA-B was performed on PBMC of various donors of antigen-presenting cells by using standard microcytotoxicity assays. B cells were typed for DR antigen in a similar fashion. All typing was performed in the HLA Typing Laboratory of the Blood Bank at the National Institutes of Health.

Culture and cloning procedure. The method for preparing antigen-specific T cell lines is that of Volkman et al. (31), a modification of the cyclic stimulation and rest method first described by Kimoto and Fathman (32, 33). Fresh PBMC were initially cultured at a density of 5×10^6 cells per well in 24-well cluster dishes (3524 Costar, Cambridge,

MA) in RPMI-1640 supplemented with 80 µg/ml gentamicin, 25 mm Hepes, 10% FCS (complete RPMI), and 25 µg/ml of BMA. The cells were incubated at 37°C in a humidified atmosphere in 5% CO₂ for 5 d. Viable cells were separated on a Hypaque-Ficoll gradient and cultured (rested) for 10–12 d with freshly thawed autologous PBMC, which were irradiated with 3,500 rads by exposure to a calibrated ¹³⁷Cs-source (Iso-medix, Parsippany, NJ) at a ratio of 10⁶ viable cells to 4×10^6 irradiated PBMC. After the resting cycle, the procedure was repeated by separating viable cells, as indicated above, and combining them with freshly prepared PBMC as a source of antigen-presenting cells along with fresh BMA (25 µg/ml). Four cycles of 5-d stimulation with BMA and 9–11 d rest culture in the absence of antigen were repeated before cloning.

Cloning was accomplished by limiting dilution. Viable T cells were seeded at 0, 0.3, 1, 3, and 10 cells per well in U-bottomed 96-well microtiter dishes (Linbro, Flow Laboratories, McLean, VA) in 200 µl of medium containing both BMA (25 µg/ml) and exogenous IL-2 (15% final concentration). Each well also contained 5×10^4 autologous irradiated antigen-presenting cells. Positive wells were scored by viewing in an inverted microscope after 12–14 d in culture; positive cells were removed and restarted on repeated stimulation and rest cycles in the absence of exogenous IL-2.

IL-2. Supernatants containing IL-2 were obtained from fresh PBMC of four donors whose cells were pooled and incubated at a density of 2×10^6 cells per ml in RPMI 1640 and 0.5% FCS with 2 µg/ml of purified phytohemagglutinin A (PHA; Burroughs Wellcome Laboratories, Research Triangle Park, NC) for 36 h at 37°C (34). The conditioned supernatant from this culture was filtered through a 0.45-µm filter (Milipore Corp.; Bedford, MA) and stored at –20°C.

Proliferative assays. Unless stated otherwise, assays for antigen-specific responses were performed by culturing 5×10^3 – 1×10^4 T cells with 1 and 5×10^5 irradiated (3,500 rads) antigen-presenting cells and antigen in either round-bottomed (1×10^5 cells) or flat-bottomed (5×10^5 cells) 96-well microtiter dishes. Cultures were incubated for 3 d and then pulsed with 1 µCi of [³H]thymidine for 16 h. Cells were collected on glass filters with a Titertek cell harvester (Flow Laboratories, Inc., Rockville, MD) and incorporation of [³H]thymidine was measured by liquid scintillation spectroscopy. Data are expressed as mean counts per minute of triplicate cultures.

In vitro antibody production. Unfractionated mononuclear cells or a T cell-depleted fraction (AET negative) cells were placed in flat bottomed wells of microtiter plates at a concentration of either 250,000 cells/ml (unfractionated) or 125,000 cells/ml (AET negative) in complete RPMI. To these cells were added varying numbers of T cells derived from the TCL in the presence or absence of varying concentrations of BMA antigen. The volume was normalized at 220 µl, and the cells were incubated at 37°C, 5% CO₂, and 100% humidity for 10 d. The supernatants were harvested and immediately assayed.

Micro-enzyme-linked immunosorbent assay for total and parasite-specific IgG and IgM. IgM and IgG in culture supernatants were measured by using enzyme-linked immunosorbent assays. Flat-bottomed microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 0.1 ml of carbonate buffer, pH 9.6, containing either 10 µg/ml Fab fragment goat anti-human IgG or IgM (Fab fragment, Cappel Laboratories, Inc., Cochranville, PA) or 5 µg/ml of parasite-specific antigen (as described above) and allowed to incubate overnight at 4°C. The plates were then washed in PBS containing Tween 20 (Sigma Chemical Co., St. Louis, MO). Samples were appropriately diluted, added to replicate wells in a volume of 100 µl, and incubated at 37°C for 2 h. The plates were washed as before and 100 µl of a 1/500 dilution of heavy

chain-specific goat anti-IgG or anti-IgM conjugated to horseradish peroxidase (Kirkegaard and Perry, Rockville, MD) was added to each well. The plates were incubated at 37°C for 2 h, washed again and allowed to react with *o*-phenylenediamine in a potassium phosphate buffer, pH 7.0, containing 0.006% H₂O₂. The reaction was stopped with 2 N HCL. Color development was measured by using a multiplate reader. For total IgG and IgM the color development was related to a reference pool of human sera with a known amount of IgG or IgM (Meloy Laboratories, Inc., Springfield, VA). For antigen-specific IgG and IgM, arbitrary units were defined on the basis of a reference serum to which all other samples were compared.

Fluorescence-activated cell sorter (FACS) analysis. FACS analysis was performed on an FACS II cell sorter (Becton-Dickinson, Sunnyvale, CA) in the laboratory of Dr. T. Chused. Direct staining was performed using 10⁶ cells per determination. Mouse monoclonal antibodies to Leu 1, Leu 2, and Leu 3 conjugated to fluorescein isothiocyanate (Becton-Dickinson) were incubated with the cells at 4°C for 30 min. Cells were then washed and placed in the cell sorter. Cells stained with an unrelated, fluoresceinated monoclonal antibody were used as a control. The Leu 1, Leu 2, and Leu 3 monoclonal antibodies recognize the total T cell, cytotoxic/suppressor T cell, and helper/inducer T cell subsets, respectively (35).

Statistical methods. Analysis of precursor frequency in limiting dilution experiments was performed by assuming random and independent distribution of the T cells in microtiter wells and by applying the Poisson analysis (36). The remainder of the statistical analyses were performed by using a two-tailed *t* test.

Donor of the cells used in propagating the TCL. The patient, a 29-year-old white female, was diagnosed as having loiasis in February, 1982, after residing for 3 yr in Gabon, West Africa, and subsequently developing the characteristic angioedematous "calabar swellings" on her extremities along with high grade eosinophilia. She had high levels of anti-filarial IgG antibody, and the clinical diagnosis of loiasis was confirmed when an excisional biopsy of a nodule that appeared on her arm after treatment with diethylcarbamazine revealed the microfilarial form of *Loa loa*. The patient required multiple courses of diethylcarbamazine to prevent recurrent swelling (and presumably to kill all of her parasites). At the time of leukopheresis (when the original cells were obtained) the patient was without clinical manifestations of disease and had a decreasing anti-filarial antibody titer. These findings are illustrated in Fig. 1.

Results

Influence of antigen concentration on T cell proliferation. The response of unfractionated PBMC to both the BMA and tetanus toxoid (TT) was explored initially. As seen in Table I, maximal T cell proliferation was noted with a parasite antigen concentration of 25 µg/ml. This level of BMA thus became the established concentration for further studies. The response to TT is also shown in Table I; again, a dose-dependent responsiveness was seen.

BMA-specific TCL and clone preparation. PBMC from the patient were stimulated with BMA (25 µg/ml) in vitro for 5 d and then rested in the absence of BMA. After four cycles of stimulation and rest, a fraction of the viable cells was removed and cloned by limiting dilution. Fig. 2 shows the limiting dilution analysis of plates seeded at 3, 1, 0.3, and 0 cells per well. A linear plot consistent with an independent Poisson distribution

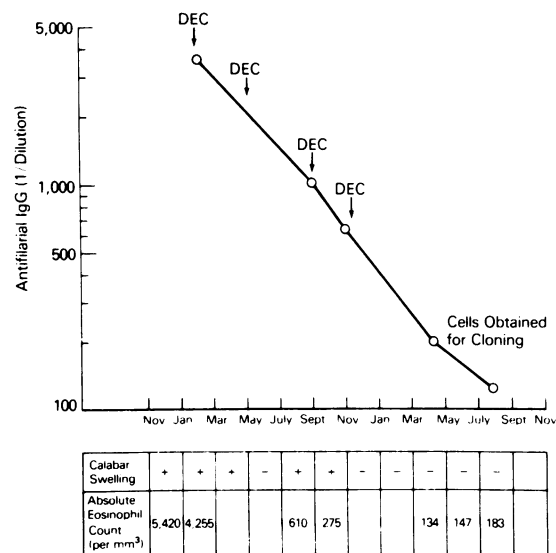


Figure 1. Time course of patient's clinical condition and anti-filarial IgG antibody titers. Arrows indicate points at which the patient received diethylcarbamazine (DEC).

of a single cell type was obtained. We have designated as clones the T cell populations derived from limiting dilution plates seeded at one T cell per well or less (> a 95% probability of true clonality); however given the low clonal efficiency, even

Table I. Proliferative Responses of Unfractionated PBMCs* as a Function of In Vitro Antigen Concentration

Antigen	Antigen concentration‡	Proliferative responses§
		cpm
None	0	226
BMA	0.01	582
	0.1	3,214
	1	6,882
	10	7,094
	25	8,625
	50	5,685
TT	100	6,516
	1/2,000	7,415
	1/5,000	9,281
	1/10,000	9,321
	1/20,000	2,341

* 1 × 10⁵ cells cultured in round-bottomed wells and assayed on day 5.

‡ Micrograms per milliliter of BMA; dilution of stock for TT.

§ Mean of triplicate cultures.

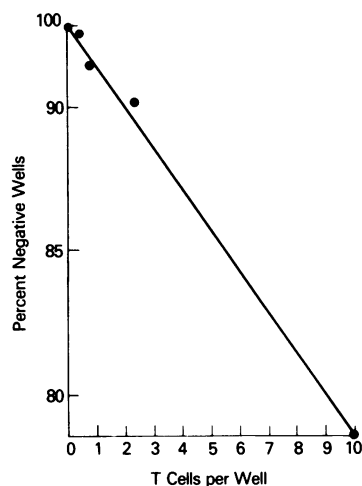


Figure 2. Limiting dilution analysis of BMA-specific T cell clones. T cells were seeded at an average of 3, 1, 0.3, or 0 cells per well and the positive and negative wells were scored after 14 d. The number of negative wells were 48/60, 52/60, 58/60, and 60/60, respectively.

the cells seeded at 3 or 10 cells per well are likely to be true clones. Subsequently, we have been able to improve the clonal efficiency significantly whereby the precursor frequency (obtained by extrapolating the point at which 37% of the wells were negative) of clonable T cells has approached one per 1.5 cells compared with one per 13 cells in the present study (data not shown).

Antigen specificity of the TCL. In order to study the TCL for antigen specificity, a proliferative assay was performed with autologous antigen-presenting cells alone or with the addition of T cells (from the TCL), antigen, or both T cells and antigen (Fig. 3). Proliferation was noted only in the presence of T cells and parasite antigen. Stimulation with an antigen (TT) to which the patient's unfractionated PBMC were capable of responding (Table I) was unable to induce the T cell line derived from this same patient to proliferate.

Cytofluorometric analysis of the TCL. BMA-specific T cells were stained with anti-Leu 1, -Leu 2, and -Leu 3, and examined in the cell sorter. As seen in Fig. 4, after two cycles of stimulation and rest (panel A), 90% of the cells were Leu 1+ and Leu 3+;

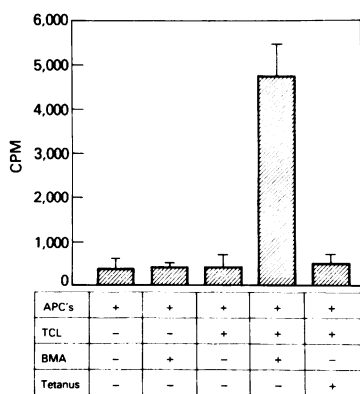


Figure 3. Analysis of antigen-specific proliferative responses of the human T cell line in the presence or absence of specific antigen and antigen-presenting cells (APC). The data are expressed as mean [^3H]thymidine uptake in $\text{cpm} \pm \text{SD}$.

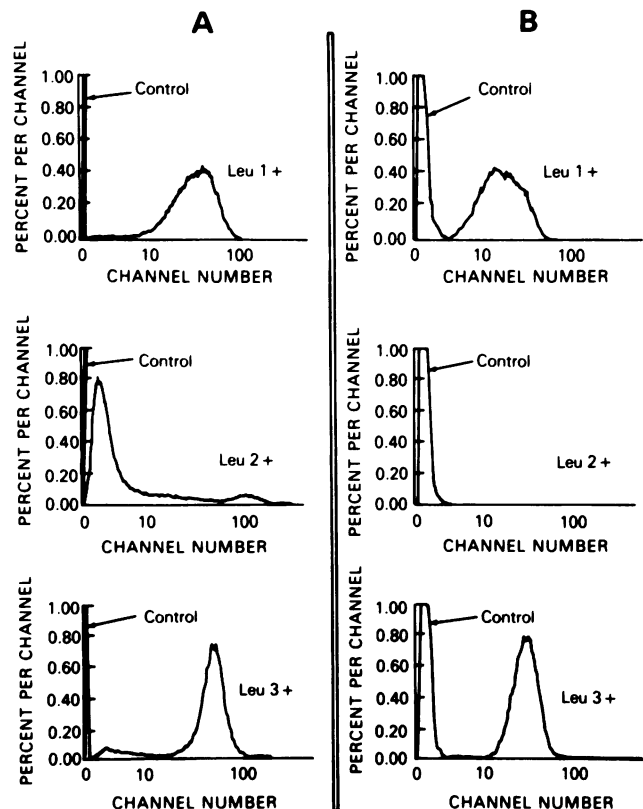


Figure 4. FACS analysis of the parasite-specific T cell line. Viable cells were separated from dead cells by Hypaque-Ficoll gradient sedimentation and stained with the designated fluoresceinated monoclonal antibody (Leu 1, Leu 2, Leu 3) or with a fluoresceinated control monoclonal antibody. Cells were then analyzed on a FACS-II (Beckton-Dickinson). A represents the FACS analysis after two courses of antigen presentation and an intervening rest. B represents the FACS analysis just before cloning.

after the fourth cycle (panel B), all cells in this particular TCL were of the helper/inducer phenotype (35) as determined by FACS analysis. This pattern of Leu reactivity (Leu 1+, Leu 2-, Leu 3+) has remained stable on the cells in culture for >6 mo.

Ability of TCL to stimulate antibody production. When autologous unfractionated PBMC were cultured in the presence of increasing numbers of T cells from the TCL in the absence of antigen, nominal amounts of total and antigen-specific IgG and IgM were produced. When parasite antigen was added, however, the ability to produce both total and parasite specific antibody was markedly enhanced (Fig. 5).

The dose-response curves of the titration of the TCL against the in vitro production of antibody by these unfractionated PBMC demonstrated that as few as 500 T cells could elicit polyclonal helper activity (Fig. 5 A, C), whereas 1,000 T cells were necessary to help in the production of parasite-specific antibody (Fig. 5 B, D). Maximum help, both polyclonal and

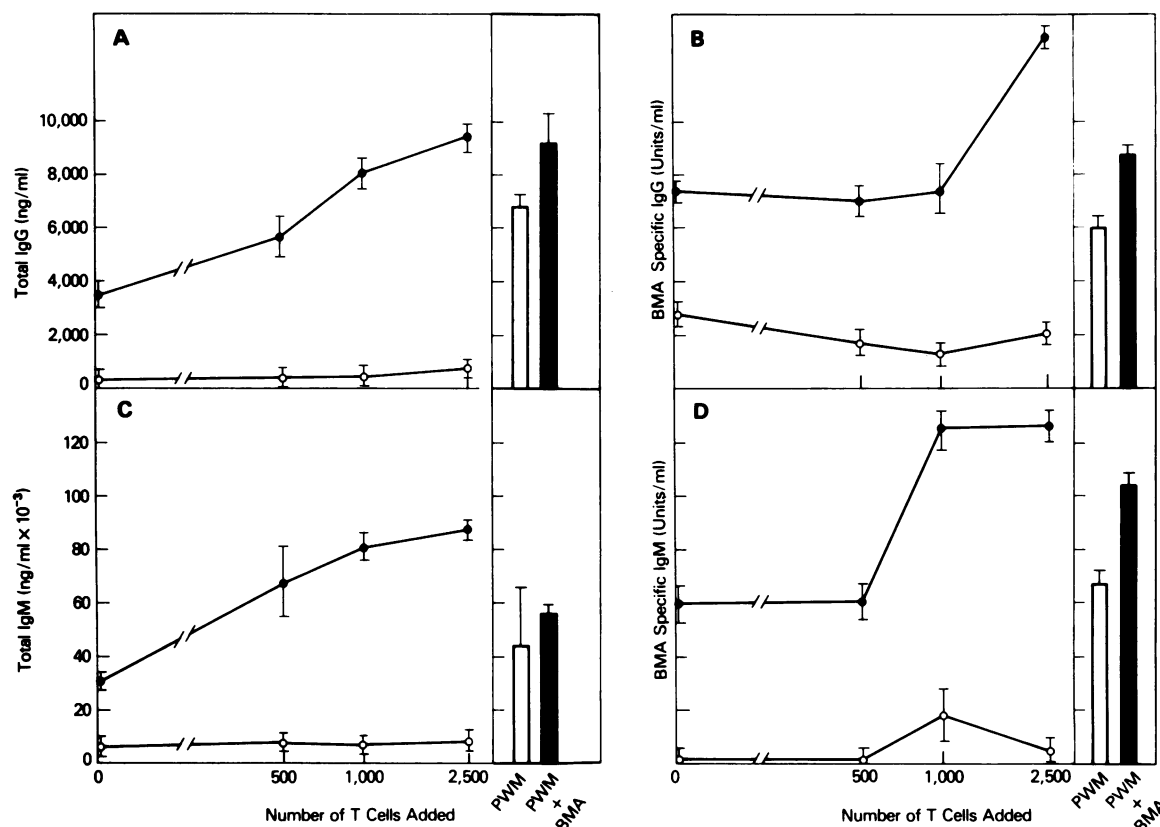


Figure 5. In vitro induction of BMA-specific and total IgG and IgM as a function of increasing numbers of T cells added. Open circles or bars indicate cultures to which no antigen was added. Dark circles or bars indicate cultures to which BMA was added. Data are expressed as mean values \pm SD of triplicate cultures. Responses to PWM are

used as a point of reference. The ordinates of the bar graphs are identical to those of the corresponding line graphs. *A* represents the total IgG responses; *B* represents the BMA-specific IgG response; *C* represents the total IgM responses; and *D* represents the BMA-specific IgM response.

specific, occurred at 2,500 T cells at all concentrations of antigen tested (data not shown). T cells from the TCL added to unfractionated cells stimulated production of at least as much (and usually greater) polyclonal immunoglobulin and antigen-specific immunoglobulin than did the addition of pokeweed mitogen (PWM) or PWM + BMA to unfractionated cells.

The requirements for the antigen-specific help were studied further. When T cells were depleted from the unfractionated PBMC population, thereby removing residual T cells (presumably with both helper and suppressor function) that were capable of responding to the antigen, a more striking in vitro antibody response was seen (Fig. 6). Both antigen-specific and total IgM (panel *A*) and IgG (panel *B*) were measured in culture supernatants after the addition of varying concentrations of BMA to 2,500 T cells and 125,000 AET-negative PBMC. Maximum anti-BMA antibody responses to BMA stimulation were seen at the lowest antigen concentrations used: as the concentration of BMA was increased, a modest suppression of the anti-BMA antibody response was seen. Such was not the case, however,

for total immunoglobulin production, as all concentrations of BMA that resulted in specific antibody responses also resulted in marked increases in the secretion of total IgG and IgM. Thus, BMA added to a BMA-specific TCL in the presence of autologous B cells and monocytes results both in a true polyclonal response (perhaps more marked at high antigen concentrations) and a predominantly antigen-specific response at lower antigen concentrations.

MHC restriction of antigen presentation to the TCL and clones. The capacity of allogeneic antigen-presenting cells to substitute for autologous antigen-presenting cells in the presentation of BMA to these T cells was next explored. The donor of the TCL was typed as HLA-A 1, 3, -B 14, 39, and -DR 7, 8. Antigen-presenting cells were then obtained from five individuals who were heterozygous for the DR8 haplotype, four individuals who were heterozygous for DR7, and six patients who had neither the DR7 nor the DR8 haplotype. As demonstrated in Table II, the TCL was capable of recognizing BMA in association with either DR7 or DR8 allogeneic antigen-pre-

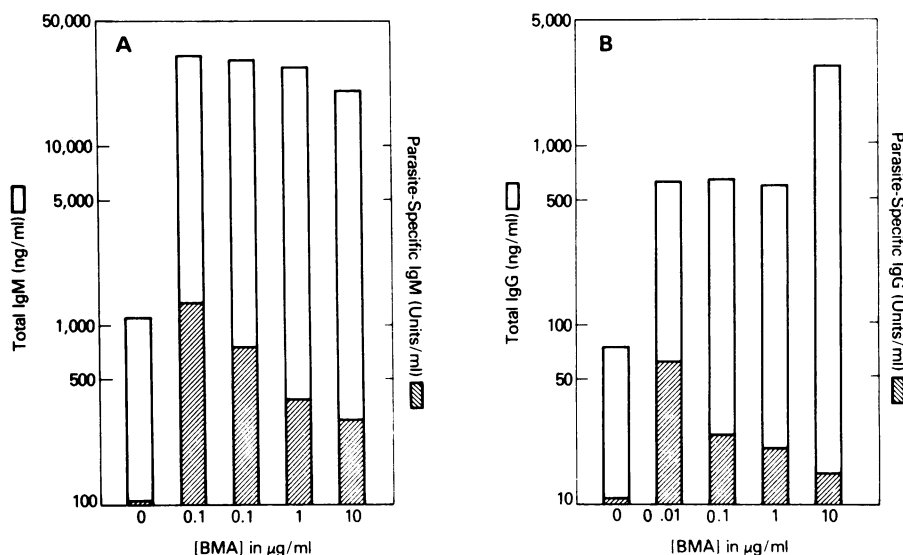


Figure 6. Total and BMA-specific immunoglobulin responses in T cell-depleted mononuclear cells to which were added increasing doses of antigen. *A* represents the IgM responses. *B* represents the IgG responses. Open bars indicated total immunoglobulin and hatched bars indicate antigen-specific responses.

senting cells. Only in the absence of either of these DR types was there no proliferation to the antigen. Furthermore, as demonstrated in Table II, one of the clones derived from the TCL was capable of recognizing BMA in association with DR7 antigen-presenting cells and not DR8 allogeneic antigen-presenting cells. Another clone recognized antigen with only DR8 but not with DR7. Each clone we tested recognized antigen only in association with the restriction element present on DR7 or DR8 cells; no clone recognized antigen with autologous antigen-presenting cells alone; this observation implies that none of the clones was restricted to a DR7, 8 hybrid Ia. In addition, no clone tested proliferated to autologous antigen-presenting cells alone (without antigen present), a finding that suggests the absence of autoreactive clones.

Similarities or differences at the HLA-A or -B locus played no role in antigen presentation to the T cell line.

Discussion

The development of human T cell lines of defined specificity can provide a powerful tool for dissecting the regulatory mechanisms involved in T-T and T-B cell interactions. The present study confirms the earlier observations (31) which demonstrate that antigen-specific human T cell lines and clones can be obtained independent of exogenous growth factors and provides evidence that these TCLs can be derived not only from immunized subjects but also from patients with naturally acquired parasite infections. Furthermore, these T cells exhibit fine MHC restriction to either of the two parental DR haplotypes, and functionally they have the capacity to provide help to B cells for the production of polyclonal immunoglobulin and specific antibody.

The technique of repeated cycles of antigen stimulation and rest selects for a subset of T cells capable of supporting their

own growth. In addition, after four cycles of antigen stimulation, the resulting TCL is comprised almost exclusively of antigen-specific cells so that cloning from this highly antigen-specific line can yield, with reasonable assurance, clones of predefined specificity. These results contrast markedly with the techniques generally used in human T cell cloning in which cloning is performed after one initial *in vitro* antigen stimulation and where specific precursor frequency is often <1%. Furthermore, since these clones and TCLs are independent of exogenous IL-2, they can be assayed for both polyclonal and antigen-specific lymphokine production in the absence of exogenous materials that might be contaminated with other lymphokines. Such assays are in progress at the present time in our laboratory.

Cloned human T cells that mediate helper activity in the formation of both specific and nonspecific antibody have already been described (21, 24, 27). However, since these lines have had an absolute requirement of exogenous IL-2 (usually in the form of PHA supernatants containing many factors including PHA) for growth and maintenance, the helper activity may be related to the action of contaminating exogenous factors on the T cells and not directly to the function of the T cell being studied. Thus, the finding that this helper activity is independent of exogenous IL-2 in human TCLs and clones is of additional importance.

The induction of specific anti-parasite antibody by cloned human T cells was clearly antigen-dose dependent. Stimulation of the T cells with low concentrations of antigen induced optimal antigen-specific antibody production by autologous B cells. In contrast, the concentration for optimal antigen-induced lymphocyte proliferation was consistently 100–1,000-fold higher than that seen for *in vitro* antibody production (Table I). This work supports similar observations reported in keyhole limpet hemocyanin-induced (37, 38) or influenza A-induced (24) specific antibody formation where peak specific antibody production

Table II. Filarial Antigen Presentation Is MHC Restricted

Antigen-presenting cells	Donor	HLA			Ag	Proliferative response*		
		A	B	DR		TCL	Clone A	Clone B
						<i>cpm</i>	<i>cpm</i>	<i>cpm</i>
Autologous	D.Z.	1, 3	14, 39	7, 8	—	6,084	3,174	1,590
					+	20,987‡	22,135‡	3,132‡
DR7	A.B.	28, 32	7, 42	3, 7	—	5,508	NT	NT
					+	11,748‡	NT	NT
	B.A.	23, 23	39, 52	5, 7	—	3,482	NT	NT
					+	10,289‡	NT	NT
	D.W.	1, 26	7, 40	1, 7	—	1,146	330	460
					+	2,900‡	361	3,277‡
	C.T.	29, 30	18, 54	6.3, 7	—	1,044	1,520	175
					+	8,006‡	1,620	2,932‡
DR8	K.S.	2, 3	7, 60	2, 8	—	2,642	NT	NT
					+	13,683‡	NT	NT
	H.T.	2, 32	35, 58	6, 8	—	2,737	NT	NT
					+	6,503‡	NT	NT
	I.R.	33, 33	35, B18	6.3, 8	—	450	971	483
					+	3,521‡	8,207‡	368
	T.B.	28, 32	7, 42	3, 8	—	NT	314	447
					+	NT	7,249‡	475
	A.P.	2, 25	27, 35	3, 8	—	779	600	558
					+	6,044‡	2,295‡	678
Non-DR-related	D.B.	3, 30	45, 57	2, 2	—	1,783	NT	NT
					+	1,758	NT	NT
	J.B.	26, 28	40, 41	5, 6	—	3,708	NT	NT
					+	3,417	NT	NT
	J.J.	1, 23	49, 53	5, 6	—	1,297	215	NT
					+	1,298	194	NT
	J.L.	30, 34	42, 39	1, 3	—	5,055	338	234
					+	4,425	281	329
	J.J.	1, 33	42, 49	3, 6	—	5,234	NT	NT
					+	5,063	NT	NT
	C.D.	28, 30	45, 42	3, 5	—	7,670	NT	453
					+	7,733	NT	296

NT, not tested. * Mean of triplicate culture. ‡ Significant difference ($P < 0.05$) between it and its corresponding control.

occurs in response to antigen concentrations several logs lower than optimal blastogenic antigen concentrations.

The number of helper T cells in our system similarly affects the formation of specific antibody, with high numbers capable of suppressing the specific response. This phenomenon has been seen with cloned mouse cells (39, 40) and antigen-specific human helper T cells (26). In these IL-2-dependent systems, limiting quantities of IL-2 or culture nutrients have been implicated as the cause for the suppression seen at high cell numbers. Alternatively, and more likely in view of the IL-2 independence of

our T cells, an excess of helper factors could turn off immunoglobulin production by acting at the B cell level (41). Also, a clone may be secreting more than one factor, one of which helps while the other suppresses (but only at high concentrations).

The process of parasite antigen recognition by these T cell clones is noteworthy. An absolute requirement for histocompatible antigen-presenting cells is seen. Similar to other human TCL and clones, these filarial parasite-specific T cells recognize antigen only in association with discrete immune response gene products. The present study demonstrates for the first time that

separate clones that are able to recognize BMA do so only in the context of either one or the other parental Ia haplotypes. This finding lends further credence to the concept that the human T cell network contains clones that recognize each of the parental immune response gene products and that an individual T cell recognizes soluble parasite antigen, at least, in the presence of one, but not both, Ia alleles. Our study, therefore, extends to parasite antigens a finding already noted for keyhole limpet hemocyanin recognition by specific human T cell clones (31), namely that hybrid paternal-maternal Ia recognition (reported in the murine model) (32, 42) is not necessary for antigen presentation by human T cells.

Finally, this study clearly indicates the usefulness of exogenous IL-2-independent, antigen-specific TCLs in investigating the nature of T cell recognition of antigen and the B cell responses that occur as a result of this recognition. The ability to extend these observations to the immunology of parasite infection has already provided important insights into the immunoregulatory mechanisms involved. It should also help pinpoint the important antigens necessary for inducing cellular and thereby humoral responses; this then should enable us to begin to manipulate these immune responses in hopes of establishing protective immunity to organisms causing these diseases of worldwide importance.

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