CHARACTERIZATION OF TWO DISTINCT PRIMARY T CELL POPULATIONS THAT SECRETE INTERLEUKIN 2 UPON RECOGNITION OF CLASS I OR CLASS II MAJOR HISTOCOMPATIBILITY ANTIGENS

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The most potent of all antigen-specific immune responses is provoked by cell surface antigens encoded by the MHC. Yet relatively little is known about the nature of the Th cells involved in initiating immune responses against membrane-bound antigens (1–4). Recently, we showed that there exist two phenotypically distinct Th cell populations responsive to major histocompatibility antigens and that each is capable of initiating primary CTL responses (5). Activation of each of the two Th cell subpopulations was MHC-specific and accessory cell–dependent, but distinct in that the L3T4$^+$ Th cell subpopulation was restricted to the recognition of class II accessory cell MHC determinants, whereas the Lyt-2$^+$ Th cell subpopulation was restricted to the recognition of class I accessory cell MHC determinants. Upon activation, both Th cell subpopulations mediated their helper function, at least in part, by secreting soluble IL-2 that was necessary for the triggering and expansion of antigen-activated CTL precursors (pCTL).[1] The goal of the present study was to further characterize these two Th cell subpopulations, especially the poorly understood Lyt-2$^+$ Th cell subset.

The further characterization of anti-MHC Th cells by using a CTL response system was difficult because of the complexities involved in studying two Th cell subpopulations, while simultaneously fulfilling the MHC activation requirements of the indicator pCTL population. T cell proliferation assays have been used to study MHC-specific Th cell populations, but they do not clearly detect class I restricted Lyt-2$^+$ T cells and they measure a parameter (i.e., proliferation) that is not directly related to the helper function of the T cells being studied (6–8). Since we previously showed that secretion of soluble IL-2 is one of the functions

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[1] *Abbreviations used in this paper:* pCTL, cytotoxic T lymphocyte precursors; SN, culture supernatant; tk, thymidine kinase.

TABLE I
MHC Alleles of the Mouse Strain Used in This Study

Strain	MHC alleles		
	K	I	D
C57BL/10 (B10)	*b*	*b*	*b*
B6.C-H-2^{bm1} (bm1)	*bm1*	*b*	*b*
B6.C-H-2^{bm12} (bm12)	*b*	*bm12*	*b*
B10.BR	*k*	*k*	*k*
B10.A	*k*	*k*	*d*
B10.AQR	*q*	*k*	*d*
B10.A(4R)	*k*	*k*	*b*
B10.T(6R)	*q*	*q*	*d*
B10.Q	*q*	*q*	*q*
B10.QBR	*b*	*b*	*q*
(B10 × B10.BR)F_1	*b/k*	*b/k*	*b/k*

performed by each MHC-specific Th cell population (5), the present report examines the activation requirements of MHC-specific primary Th cells by directly assaying their production of IL-2. To do so accurately, we found it necessary to inhibit the consumption of IL-2 during the response period by the addition to culture of an mAb specific for the IL-2 receptor that is expressed by activated murine lymphocytes (9). Using this novel modification of the MLR, we herein document the specificity, phenotype, activation requirements, and relative precursor frequencies of two distinct MHC-specific, IL-2-secreting, primary T cell subpopulations.

Materials and Methods

Animals. The MHC alleles of the mouse strains used in this study are shown in Table I. Mice were purchased from The Jackson Laboratory, Bar Harbor, ME, or were bred in our own animal colony.

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras are designated as bone marrow donor → irradiated recipient. An extensive description of the production and typing of such chimeras has been reported previously (10). Briefly, recipient mice were irradiated with 950 rad from a ^{137}Cs source, and were reconstituted 2–6 h later with 1.5×10^7 bone marrow cells that had been depleted of T cells by pretreatment with a rabbit anti-mouse brain serum, a reagent specifically cytotoxic for all T cells (11), plus guinea pig complement. Spleen cells were obtained from each chimera no earlier than 2 mo after irradiation and bone marrow reconstitution, at which time they were >98% of donor bone marrow origin as assessed by indirect immunofluorescence.

Monoclonal Antibodies. Anti-I-A^b mAb was a culture supernatant of the hybridoma cell line M5/114.15.2 (12). Anti-I-A^k mAb was affinity-purified from a culture supernatant of the hybridoma cell line 10.2.16 (13). Anti-K^k mAbs were a mixture of equal volumes of mouse ascites from the hybridoma cell lines 11.4.1, 3.83, and 36.7.5 (13–15). Anti-Lyt-2.2 mAb was a culture supernatant of the hybridoma cell line 83-12-5, kindly provided by Dr. J. Bluestone, National Institutes of Health, Bethesda, MD. Anti-L3T4 mAb was a culture supernatant of the hybridoma cell line GK1.5 generously supplied by Dr. F. Fitch, University of Chicago, Chicago, IL (16). Rat anti-murine IL-2-R mAb was a mouse ascites of the hybridoma cell line 7D4 (9).

Monoclonal Accessory Cells. JT1.1 is an H-2^k, Ia^- L cell line transfected with the HSV thymidine kinase (tk) gene, which was generously provided by Dr. D. Margulies, National Institutes of Health, Bethesda, MD.

Preparation of Induced P388D.1 Culture Supernatant (P388D.1 SN). P388D.1 SN was produced according to the method of Mizel et al (17). Briefly, the P388D.1 cell line was incubated with 1 μg/ml of PMA in medium containing 1% FCS. At 5 d the supernatant was harvested and incubated twice with activated charcoal which removed >90% of the PMA. The supernatant was then concentrated sevenfold by vacuum dialysis.

Culture Conditions. Responder, stimulator, and accessory cell populations were all obtained from unprimed mice. Spleen stimulator cells were irradiated with 2,000 rad and, where indicated, were modified with TNP as previously described (18). Spleen accessory cells were spleen cells first depleted of T cells by pretreatment with rabbit anti-mouse brain antiserum plus complement and then irradiated with 2,000 rad. Where indicated, responder and stimulator populations were depleted of adherent accessory cells by passage over Sephadex G-10 columns as previously described (19).

Responder cells (4×10^5), stimulator cells (4×10^5), and, where indicated, accessory cells (4×10^5) were cultured together for 4 d in 0.2 ml of culture medium at 37°C in 7.5% CO_2. Culture medium consisted of RPMI 1640 supplemented with 10% FCS, penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 2-ME, and L-glutamine. Unless otherwise indicated, 0.01% (vol/vol) of anti-IL-2-R mAb was added to each culture to inhibit the consumption of IL-2 during the culture period.

Assay for IL-2 Activity. On day 4 of culture, 0.1 ml of supernatant was obtained from each well and assayed for IL-2 activity. Control experiments showed that the IL-2 content of the superantants from these response cultures reached a plateau on day 4. To each supernatant aliquot was added 4×10^3 indicator HT-2 cells, an IL-2-dependent T cell line developed by Dr. J. Watson (20) in 0.1 ml of culture medium. After 24 h of incubation at 37°C in 5% CO_2, and HT-2 cell cultures were pulsed with 1 μCi of [^{3}H]thymidine, incubated for an additional 12–18 h, then harvested. Background counts of HT-2 cells pulsed with medim alone varied between 400 and 1,000 cpm; maximum counts of HT-2 cells pulsed with an excess of IL-2 varied between 60,000 and 100,000 cpm. The concentration of IL-2 present in the culture supernatants was always limiting, in that the HT-2 response they stimulated always declined in a log-linear fashion as the culture supernatants were diluted. Data are expressed as the arithmetic mean counts per minute of triplicate or quadruplicate cultures. Data as presented are the actual counts obtained and have not been corrected for HT-2 background counts. Standard errors were generally <5% of the mean and so have not been included in the Tables.

Limiting Dilution Assay for IL-2-producing Th Cells. 24–48 replicate cultures containing varying numbers of responder cells and 5×10^5 stimulator cells in 0.2 ml of culture medium containing 0.01% anti-IL-2-R mAb were set up in 96-well round-bottom microculture wells. The cultures were incubated for 4–6 d at 37°C in 7.5% CO_2, and IL-2 activity of the supernatant from each well was determined by the method described above. Individual cultures were considered positive if their supernatants stimulated HT-2 responses that were more than three standard deviations above that stimulated by medium alone. Minimal estimates of Th frequencies were calculated by analysis of the Poisson distribution relationship between the percentage of negative cultures per group and the number of responding cells per culture (21). The coefficient of correlation in each experiment was >0.97.

Results

Detection of Primary IL-2-secreting T Cells Specific for Class I and Class II MHC Determinants Is Augmented by Anti-IL-2-R mAb. To begin assessing the MHC specificity of primary IL-2-secreting T cells, we cultured unprimed responder B10.AQR spleen cells (designated by their K, I, D alleles as *q*, *k*, *d*) with spleen stimulator cells that expressed either foreign class I or foreign class II MHC determinants (Table II, Exp. 1). Supernatants from primary cultures stimulated by class II alloantigens contained significant amounts of IL-2, whereas primary cultures stimulated by class I alloantigens did not. However, the amount of IL-2

TABLE II

Effect of Anti-IL-2-R mAb on the IL-2 Content of Primary Culture Supernatants

Exp.	Responder*	Stimulator‡	Added accessory cells§	Stimulator cell antigen	[^{3}H]TdR incorporation (cpm × 10^{-3}) by HT-2 cultured with mAb:		
					None	Anti-IL-2-R‖	Anti-I-A^{b}¶ + anti-IL-2-R
1	q, k, d	q, k, d	—	—	2.9	15.8	—
	q, k, d	k, k, d	—	K^k	4.6	26.4	—
	q, k, d	q, q, d	—	I^q	62.3	80.6	—
2	q, q, d	q, q, q	—	D^q	2.3	64.3	—
	G-10	G-10	—	D^q	0.4	0.7	—
	G-10	G-10	q, q, q	D^q	16.0	56.6	—
3	b, b, b	b, b, b	—	—		9.8	0.3
	b, b, b	bml, b, b	—	K^{bml}		23.9	16.5

* B10.AQR; B10.T(6R); B10
‡ B10.AQR; B10.A; B10.T(6R); B10.Q; B10; bml
§ B10.Q
‖ 7D4 (0.01% of ascites)
¶ M5/114 (10% of culture supernatant)

present in these 4-d culture supernatants would not be expected to accurately reflect the amount of IL-2 produced during culture, since some IL-2 would also have been consumed during the culture period. Consequently, to assess more accurately the amount of IL-2 that was actually produced in these response cultures, we attempted to inhibit the consumption of IL-2 during the culture period by the addition of anti-IL-2-R mAb, reasoning that anti-IL-2-R mAb should interfere with the activation and clonal expansion of IL-2-consuming cells (Table II, Exp. 1). Control experiments demonstrated that the amount of anti-IL-2-R mAb added to the response cultures was insufficient to affect the bioassay used for detecting IL-2 in the culture supernatants, since the mAb neither stimulated HT-2 indicator cells nor interfered with the stimulation of HT-2 cells by IL-2, because of the large numbers of IL-2-R HT-2 cells express (data not shown). Indeed, the addition to culture of anti-IL-2-R mAb permitted us to detect significant quantities of IL-2 in the supernatants of cultures containing either syngeneic or class I disparate stimulator cells, as well as increasing the amount of IL-2 detectable in supernatants of cultures containing class II disparate stimulator cells (Table II, Exp. 1).

It was possible that the increased IL-2 activity present in these culture supernatants resulted from the direct stimulation of IL-2-secreting T cells by the rat anti-IL-2-R mAb. To examine this possibility, anti-IL-2-R mAb was added to response cultures that had been fully depleted of adherent accessory cells (Table II, Exp. 2). Inconsistent with direct stimulation of T cells by the rat anti-IL-2-R mAb, no IL-2 was detected in response cultures devoid of accessory cells (Table II, Exp. 2). The G-10–passed responder cell population was capable of secreting IL-2 since they did so upon addition back to culture of D^q-bearing accessory cells. Thus, even in the presence anti-IL-2-R mAb, activation of IL-2-secreting

T cells is accessory cell–dependent, indicating that anti–IL-2-R mAb does not directly trigger IL-2-secreting T cells.

Next, it was necessary to determine if these responses remained MHC-specific in the presence of anti–IL-2-R mAb. In Table II (Exp. 3) B10 responder cells were cultured with either syngeneic or K^{bm1} disparate stimulator cells in the presence of anti–IL-2-R mAb. As in Exp. 1, detectable quantities of IL-2 were produced in response to syngeneic as well as class I-disparate stimulator cells. However, the addition of anti-I-A^b mAb to the response cultures completely blocked the response to syngeneic stimulator cells, whereas the same mAb left intact a substantial response to class I–disparate stimulator cells, even though both stimulator populations expressed identical I-A^b determinants (Table II, Exp. 3). These results indicate that in the presence of anti–IL-2-R mAb the IL-2 produced in response to syngeneic stimulator cells results from responder T cell recognition of self-Ia determinants, whereas the IL-2 produced in response to class I–disparate stimulator cells results, at least in part, from responder T cell recognition of allogeneic class I determinants. Thus, even in the presence of anti–IL-2-R mAb, IL-2 production results from the activation of MHC-specific responder T cells. To characterize further the T cell populations that secrete IL-2 upon recognition of MHC alloantigens, all subsequent experiments were performed in the presence of anti–IL-2-R mAb.

IL-2 Response to Class I and Class II MHC Alloantigens are Mediated by Phenotypically Distinct T Cell Subpopulations. We chose to phenotype the T cells triggered to secrete IL-2 in response to either class I or class II MHC alloantigens under two theoretically different circumstances: first, under experimental conditions in which self-Ia restricted T cells could not be activated; and, second, under experimental conditions in which self-Ia restricted T cells could be activated and could conceivably participate in responses to both class I and class II MHC alloantigens.

To phenotype alloresponsive T cells under circumstances in which self-Ia–restricted T cells were not triggered, we used fully allogeneic (A → B) radiation bone marrow chimeras as responders because T helper cells from such mice are functionally blind to donor MHC determinants in that they fail to recognize syngeneic donor Ia determinants as either self or foreign (4, 10, 22). Indeed, unlike B10 spleen cells from normal mice (Table II), B10 spleen cells from B10 → B10.BR chimeras failed to produce IL-2 in response to B10 stimulator cells, consistent with their being unresponsive to donor Ia determinants (Table III, group 1). However, the chimeric B10 → B10.BR spleen cells did respond to either D^q or I-A^{bm12} alloantigens (Table III, groups 2, 3), indicating that IL-2-secreting T cells can respond to either class I or class II MHC alloantigens under conditions that do not involve recognition of self-Ia determinants. To phenotype the chimeric T cells responsive to class I and class II MHC alloantigens, responder B10 → B10.BR spleen cells were cytotoxically depleted of either $L3T4^+$ T cells (Table III, groups 4–6) or $Lyt\text{-}2^+$ T cells (groups 7–9). Under these conditions, in which self-Ia–restricted T cells could not be triggered, $L3T4^-$ T cells responded only to class I alloantigen stimulation and $Lyt\text{-}2^-$ T cells responded only to class II alloantigen stimulation (Table III, groups 4–9).

TABLE III

Phenotype of Class I and Class II Responsive Th Cells in the Absence of Self-Ia Recognition

Group	Responder*		Stimulator‡	Stimulator cell antigen	[^{3}H]TdR incorporation by HT-2
	Strain§	Treatment			
					cpm × 10^{-3}
1	b, b, b → k, k, k	C	b, b, b	—	0.3
2			b, b, q	D^q	20.2
3			b, bm12, b	I-A^{bm12}	32.7
4		Anti-L3T4 + C	b, b, b	—	0.3
5			b, b, q	D^q	13.8
6			b, bm12, b	I-A^{bm12}	1.4
7		Anti-Lyt-2 + C	b, b, b	—	0.2
8			b, b, q	D^q	2.0
9			b, bm12, b	I-A^{bm12}	28.4

* All response cultures contained anti-IL-2-R mAb (0.01% of 7D4 ascites) to inhibit the consumption of IL-2 during the response period.
‡ B10; B10.QBR; bm12
§ B10 → B10.BR

Next, to phenotype alloresponsive T cells under conditions in which self-Ia–restricted T cells could participate, normal B10 responder cells were separated into L3T4$^-$,Lyt-2$^-$, and unfractionated cell populations which were then cultured with syngeneic, class II–disparate, or class I–disparate stimulator cells (Fig. 1). L3T4$^-$ B10 cells failed to respond to either syngeneic B10 or class II–disparate bm12 stimulators, but did respond vigorously to class I–disparate bm1 stimulators (Fig. 1, rows *1A*, *4A*, *7A*). The L3T4$^-$ K^{bm1}-responsive cells were phenotyped as being Lyt-2$^+$ since their activation was blocked by anti-Lyt-2 but not anti-L3T4 mAb (Fig. 1, rows *7A–9A*). Thus, as observed in Table III, L3T4$^-$,Lyt-2$^+$ T cells were only activated to secrete IL-2 in response to class I MHC alloantigens.

In contrast, Lyt-2$^-$ B10 cells responded significantly to all three stimulator cell populations, but responded most vigorously to class II–disparate bm12 stimulators (Fig. 1, rows *1B*, *4B*, *7B*). The Lyt-2$^-$ cells responsive to each of the three stimulators were phenotyped as being L3T4$^+$ since, in each case, their activation was blocked by anti-L3T4 but not anti-Lyt-2 mAb (Fig. 1, *B*). Thus, as already observed in Table III, L3T4$^+$,Lyt-2$^-$ T cells were activated to secrete IL-2 in response to class II MHC alloantigens. Unlike Table III, in which responses were obtained under conditions that avoided the activation of self-Ia restricted T cells, normal L3T4$^+$ T cells were also triggered to secrete IL-2 in response to self class II MHC determinants (Fig. 1, rows *1B–3B*). Furthermore, normal L3T4$^+$ cells appeared to also respond against class I MHC alloantigens as indicated by their significantly greater response to bm1 than to B10 stimulators (Fig. 1, compare rows *7B* and *1B*).

The IL-2 response of unfractionated B10 responder cells to the three stimulator cell populations (Fig. 1, *C*) appeared to be a composite of the individual responses of L3T4$^-$,Lyt-2$^+$ (*A*) and L3T4$^+$,Lyt-2$^-$ T cells (*B*). Anti-L3T4 mAb

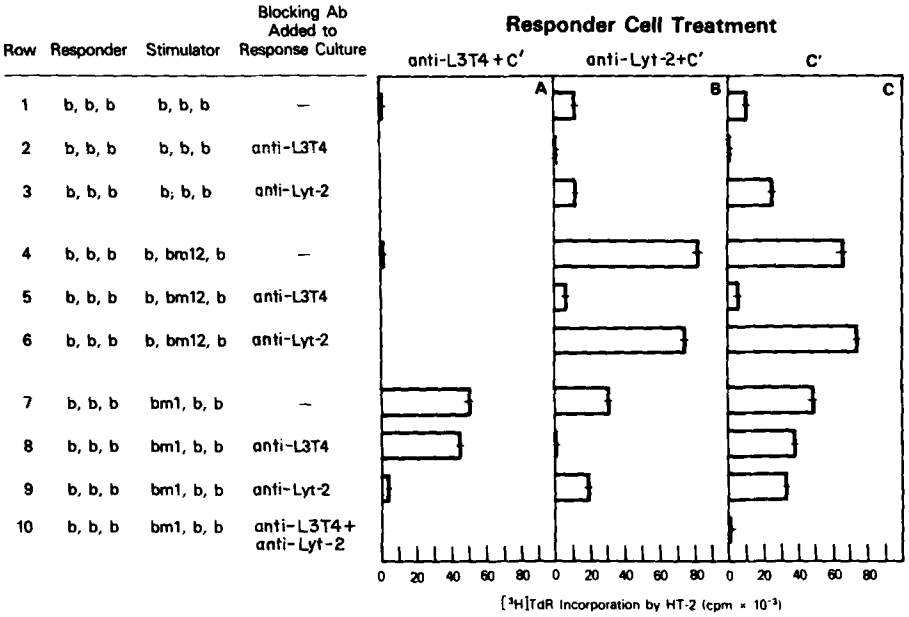

FIGURE 1. Responsiveness of different primary T cell subpopulations to class I and class II MHC alloantigens. Unprimed B10 (b, b, b) responder cells were treated either with anti-L3T4 + C (*A*), anti-Lyt-2 + C (*B*), or C alone (*C*). Each of the treated responder cell populations was cultured with syngeneic B10 (b, b, b), class II–disparate bm12 (b, bm12, b), or class I–disparate bm1 (bm1, b, b) stimulator cells. Soluble anti-L3T4 (25% vol/vol) or anti-Lyt-2 (2.5%, vol/vol) mAb was added to some of the response cultures as indicated. Each response culture also contained anti–IL-2-R mAb (0.01% of 7D4 ascites) to inhibit IL-2 consumption during the culture period. On day 4, the supernatant from each culture was assayed for its IL-2 content on HT-2 cells as described in Materials and Methods.

blocked completely the responses of unfractionated responder cells to both syngeneic and class II–disparate stimulators, whereas anti-Lyt-2 mAb did not inhibit either response (Fig. 1, rows *1C–6C*), demonstrating that $L3T4^+$,$Lyt\text{-}2^-$ cells were the only primary T cells that secreted IL-2 in response to class II MHC determinants. In addition, the anti-bm1 response of unfractionated responders was only blocked completely when the cultures contained both anti-L3T4 and anti-Lyt-2 mAbs (Fig. 1, rows *7C–10C*), indicating that both $L3T4^+$ and $Lyt\text{-}2^+$ primary T cells were involved in the anti-bm1 response.

Since $L3T4^+$,$Lyt\text{-}2^-$ T cells only responded to class I alloantigens under conditions in which self-Ia–restricted T cells could be activated, we examined the possibility that such T cells are self-Ia restricted (Fig. 2). Normal B10 responder cells were first depleted of autologous accessory cells by Sephadex G-10 passage to minimize the activation of self-Ia–reactive T cells, and were then separated into $L3T4^-$,$Lyt\text{-}2^-$, or unfractionated T cell subpopulations (Fig. 2). Both $L3T4^-$ and $Lyt\text{-}2^-$ B10 responder cell populations appeared to recognize the K^{bm1} class I alloantigen, since they responded significantly better to bm1 stimulators than to B10 stimulators (Fig. 2, compare rows *1* and *2*). However, anti-I-A^b mAb completely inhibited the anti-K^{bm1} response of $Lyt\text{-}2^-$ B10 T cells, but did not significantly inhibit the anti-K^{bm1} response of $L3T4^-$ B10 T cells (Fig. 2). Thus, $L3T4^+$,$Lyt\text{-}2^-$ T cells that respond to class I MHC alloantigens

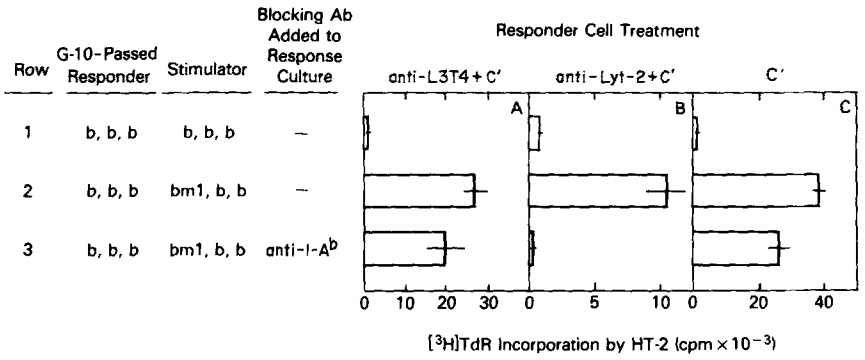

FIGURE 2. L3T4$^+$Lyt-2$^-$ T cells that respond to class I alloantigens are self-Ia–restricted. Unprimed B10 (b, b, b) responder cells were depleted of accessory cells by Sephadex G-10 column passage, and then treated either with anti-L3T4 + C (*A*), anti-Lyt-2 + C (*B*), or C alone (*C*). Each of the treated responder cell populations was cultured with syngeneic B10 (b, b, b) or class I–disparate bm1 (bm1, b, b) stimulator cells. Anti-I-A^b mAb (10% vol/vol) was added to response cultures as indicated. Each response culture also contained anti–IL-2-R mAb (0.01% of 7D4 ascites) to inhibit IL-2 consumption during the culture period. On day 4, the supernatant from each culture was assayed for its IL-2 content on HT-2 cells.

are self-Ia–restricted, presumably recognizing a composite determinant composed of class I plus class II MHC determinants.

In summary, primary T cell populations consist of two distinct IL-2 secreting subpopulations with different MHC recognition specificities, an L3T4$^-$,Lyt-2$^+$ T cell subpopulation responsive only to class I MHC alloantigens and an L3T4$^+$,Lyt-2$^-$ T cell subpopulation restricted to class II MHC determinants.

Effect of Anti–class I and Anti–class II mAbs on the Activation of Lyt-2$^+$ and L3T4$^+$ T Cells. To confirm that Lyt-2$^+$ and L3T4$^+$ IL-2-secreting T cells express distinct MHC recognition specificities, we attempted to block their activation with either anti–class I or anti–class II MHC mAbs. Either intact or fractionated B10 responder cells were cultured with B10.A(4R) stimulators to activate both K^k- and I-A^k-responsive T cells (Fig. 3). The anti-B10.A(4R) response of L3T4$^-$ B10 T cells was inhibited completely by anti-K^k mAbs but was not inhibited by anti-I-A^k mAb (Fig. 3*A*). Reciprocally, the anti-B10.A(4R) response of Lyt-2$^-$ B10 T cells was dramatically inhibited by anti-I-A^k mAb but was only minimally affected by anti-K^k mAbs (Fig. 3*B*). Thus, activation of L3T4$^-$,Lyt-2$^+$ T cells is blocked by anti–class I but not anti–class II mAbs, whereas activation of L3T4$^+$,Lyt-2$^-$ T cells is blocked by anti-class II but not anti-class I mAbs.

Unlike Activation of L3T4$^+$ T Cells, Activation of Lyt-2$^+$ IL-2-secreting T Cells Does Not Require Stimulator Cell Ia Determinants. To document the Ia independence of IL-2-secreting Lyt-2$^+$ T cells, we used an Ia$^-$ monoclonal cell line as the stimulator population. Responder B10 cells that had been depleted of autologous accessory cells were cultured with H-2^k JT1.1 L cells that are Ia$^-$ but possess accessory function since they are able to trigger conventional class II–restricted Th cells upon transfection with class II MHC genes (Fig. 4) (23). In cultures in which the activation of L3T4$^+$ T cells was blocked by anti-L3T4 mAb (Fig. 4*A*), Lyt-2$^+$ H-2^b spleen cells responded vigorously to JT1.1 cells (Fig. 4, row *3A*) and this response was H-2^k-specific since the same JT1.1 cells failed to stimulate Lyt-

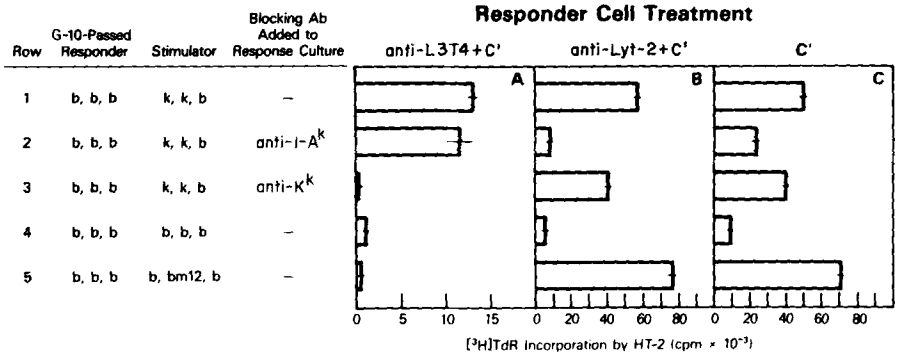

FIGURE 3. L3T4⁻ and Lyt-2⁻ IL-2-secreting T cells express distinct MHC recognition specificities. Unprimed B10 (b, b, b) responder cells were depleted of accessory cells by Sephadex G-10 column passage, and then treated either with anti-L3T4 + C (*A*), anti-Lyt-2 + C (*B*), or C alone (*C*). Each of the treated responder cell populations was then cultured with B10.A(4R) (k, k, b) stimulator cells. Where indicated, anti-I-A^k mAb (10.2.16, 2 μg/ml affinity-purified culture supernatant) or anti-K^k mAb (equal mixture of 11.4.1, 3.83, 36.7.5 mouse ascites, 1% vol/vol final concentration) was added to culture. B10 (b, b, b) and bm12 (b, bm12, b) stimulator cells were used in order to confirm the efficacy of the T cell fractionation procedures (rows 4, 5). Each response culture also contained anti–IL-2-R mAb (0.01% of 7D4 ascites) to inhibit IL-2 consumption during the culture period. On day 4, the supernatant from each culture was assayed for its IL-2 content on HT-2 cells.

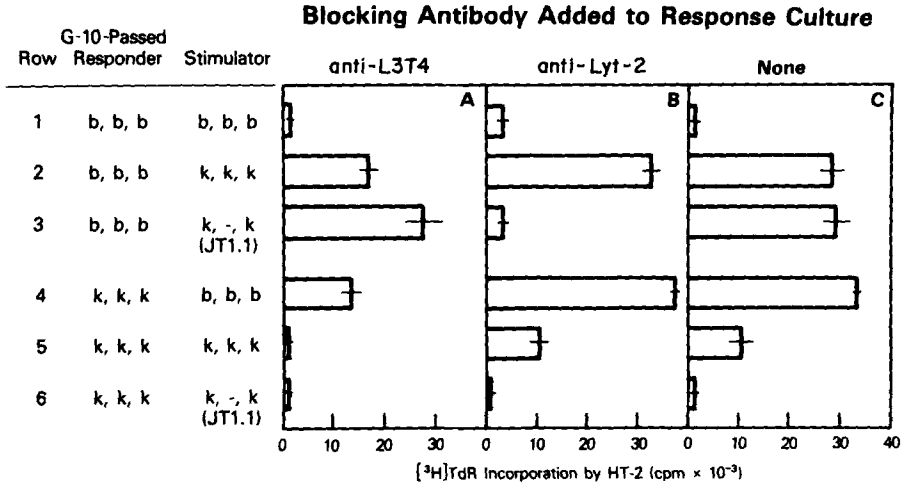

FIGURE 4. Activation of Lyt-2^+ IL-2-secreting T cells does not require stimulator cell Ia determinants. Unprimed B10 (b, b, b) or B10.BR (k, k, k) responder cells were depleted of accessory cells by Sephadex G-10 column passage and then cultured either with normal B10 or B10.BR spleen cells or the JT1.1 cell line which is H-2^k but Ia^-. Anti-L3T4 mAb (*A*), anti-Lyt-2 mAb (*B*), or medium (*C*) was added to the response cultures to selectively block either L3T4$^+$ or Lyt-2^+ responder T cells. Each response culture also contained anti–IL-2-R mAb (0.01% of 7D4 ascites) to inhibit IL-2 consumption during the culture period. On day 4, the supernatant from each culture wss assayed for its IL-2 content on HT-2 cells.

2^+ H-2^k responder cells (Fig. 4, row *6A*). In contrast, in cultures in which the activation of Lyt-2^+ T cells was blocked by anti-Lyt-2 mAb, L3T4$^+$ H-2^b spleen cells failed to respond to JT1.1 cells, consistent with the class II restriction specificity of L3T4$^+$ T cells. Thus, this experiment documents that the activation

TABLE IV
Second Signal Requirements of L3T4⁻,Lyt2⁺ Th Cells

Group	G-10–passed responder cells*		Stimulator cells‡		P388D.1 SN§	[³H]TdR incorporation (cpm × 10^{-3}) by HT-2 cells when blocking antibody is added to response culture	
	Strain‖	Treatment	Strain	Treatment		None	Anti-Lyt-2¶
1	b, b, b	Anti-L3T4 + C	—	—	−	0.4	0.3
2			b, b, b	—	−	0.5	0.3
3			bm1, b, b	—	−	28.5	2.4
4			b, bm12, b	—	−	0.2	0.2
5			b, b, b	G-10	−	0.2	0.2
6			bm1, b, b	G-10	−	0.1	0.2
7			b, bm12, b	G-10	−	0.2	0.1
8			—	—	+	2.9	4.2
9			b, b, b	G-10	+	2.3	2.6
10			bm1, b, b	G-10	+	16.8	1.9
11			b, bm12, b	G-10	+	1.6	2.9

* See Table III.
‡ B10; bm1; bm12
§ 1% (vol/vol)
‖ B10
¶ 2.5% (vol/vol).

of L3T4⁻,Lyt-2⁺ T cells to secrete IL-2 does not require stimulator cell expression of class II MHC determinants.

Second Signal Requirements for the Activation of Lyt-2⁺ T Cells. Since stimulator cell expression of Ia determinants was not required to trigger Lyt-2⁺ T cells to secrete IL-2, it was conceivable that any cell bearing class I MHC alloantigens could trigger class I allospecific Lyt-2⁺ IL-2-secreting T cells. However, such a possibility would conflict with our previous observation (Table II) that a specialized subpopulation of G-10 adherent accessory cells was required for the activation of both class I and class II allospecific IL-2-secreting T cells. To clarify this point, G-10–passed L3T4⁻,Lyt-2⁺ B10 responder cells were cultured with various allogeneic stimulator cell populations (Table IV). As can be seen, this responder cell population responded to class I–disparate (bm1) stimulators, but did not respond to either syngeneic (B10) or class II–disparate (bm12) stimulators, and their anti-K^{bm1} response was blocked by anti-Lyt-2 mAb (Table IV, groups 2–4). However, G-10 passage of the bm1 stimulators abrogated their stimulatory capacity (group 6), which was restored by the addition to culture of P388D.1 SN as an exogenous source of soluble second signals (group 10). The anti-K^{bm1} response obtained in the presence of P388D.1 SN was still blocked by anti-Lyt-2 mAb (group 10). Thus, these results indicate that: (*a*) the activation to IL-2 secretion of Lyt-2⁺ allospecific T cells requires a source of second signals that is presumably provided normally by accessory cells bearing the stimulatory alloan-

TABLE V

Relative Precursor Frequencies of Primary Class I- and Class II-Specific IL-2-secreting Th Cell Subpopulations

Exp.	G-10-passed responder*		Stimulator	T cell recognition specificity	Reciprocal of splenic precursor frequency‡
	Strain	Treatment			
1	B10	—	B10	Self-I^b	88,900
			bm12	I-A^{bm12}	19,800
			bm1	K^{bm1} / Self-I^b / Self-I^b + K^{bm1}	25,100
2	B10	Anti-L3T4 + C	B10	—	$>10^6$
			bm12	—	$>10^6$
			bm1	K^{bm1}	34,100
3	B10 → B10.BR	—	B10	—	$>10^6$
			bm12	I-A^{bm12}	15,900
			bm1	K^{bm1}	21,800

* See Table III.

‡ Limiting dilution analysis was conducted as described in Materials and Methods. The correlation coefficients of the regression lines generated were >0.97 in all experiments. The Poisson statistics were used to calculate the frequencies.

tigen, and (*b*) even in the presence of exogenously added second signals, Lyt-2^+ IL-2-secreting T cells respond only to class I but not to class II MHC alloantigens.

Relative Precursor Frequency of Primary Lyt-2^+ Class I Allospecific IL-2-secreting T Cells. We next determined the relative precursor frequencies of IL-2-secreting MHC-specific T cells in normal unprimed B10 spleens (Table V, Exp. 1). The frequency of cells responsive to class I–disparate bm1 stimulators (1 per 25,100 unprimed spleen cells) was only minimally lower than that of cells responsive to class II–disparate bm12 stimulators (1 per 19,800 unprimed spleen cells). However, as shown in Fig. 1, T cells with three different recognition specificities and different phenotypes could have contributed to the relatively high anti-bm1 frequency observed, i.e., self-I-A^b–specific L3T4^+ T cells, I-A^b + K^{bm1}–specific L3T4^+ T cells, and K^{bm1}-specific Lyt-2^+ T cells. To determine the frequency of L3T4^-,Lyt-2^+ class I allospecific T cells, we next depleted the B10 responder cell population of L3T4^+ T cells (Table V, Exp. 2). The frequency of L3T4^-,Lyt-2^+ T cells responsive to K^{bm1} class I allodeterminants was 1 in 34,100, and was approximately as high as had been observed in Exp. 1, even though the responder population was now devoid of L3T4^+ class II-restricted T cells. Finally, to determine the relative frequencies of IL-2-secreting L3T4^+ class II allospecific T cells and Lyt-2^+ class I allospecific T cells in the same B10 responder cell population, we used B10 → B10.BR fully allogeneic radiation bone marrow chimeras as the responder population (Table V, Exp. 3). The chimeric responder population contained no detectable (< 1 in 10^6) T cells responsive to donor type B10 stimulator cells, consistent with their being functionally blind to donor I-A^b determinants. More importantly, the frequency of K^{bm1} class I alloresponsive T cells (1 per 21,800 unprimed spleen cells) was only minimally lower than the

TABLE VI

Failure of Lyt-2$^+$ T Cells to Respond to TNP-modified Self-MHC Determinants

G-10–passed responder*		Stimulator	Stimulator cell antigen	[^{3}H]TdR incorporation (cpm × 10^{-3}) by HT-2
Strain	Treatment			
(B10 × B10.BR)F_1	Untreated	None	—	0.9
		(B10 × B10.BR)F_1	Self	8.2
		TNP-F_1	TNP-self	20.8
		bm1	K^{bm1}	27.7
		bm12	I-A^{bm12}	28.1
	Anti-L3T4 + C	None	—	0.7
		(B10 × B10.BR)F_1	Self	0.8
		TNP-F_1	TNP-self	1.6
		bm1	K^{bm1}	24.4
		bm12	I-A^{bm12}	1.5

* See Table III.

frequency of I-A^{bm12} class II alloresponsive T cells (1 per 15,900 unprimed spleen cells) (Table V, Exp. 3).

Failure of IL-2-secreting Lyt-2$^+$ T Cells to Respond to TNP-modified Self-MHC Determinants. Because a high proportion of allospecific CTL crossreactively recognize TNP-self and vice versa (24), TNP-modified self class I MHC determinants are thought to structurally resemble allogeneic class I MHC determinants. Thus, it was of interest to examine the ability of IL-2-secreting Lyt-2$^+$ T cells to recognize and respond to TNP-modified self class I MHC determinants. It can be seen in Table VI that L3T4$^-$ H-$2^{b/k}$ T cells responded strongly to class I–disparate bm1 stimulator cells, but failed to respond to syngeneic F_1(bxk) stimulator cells, even when modified with TNP. It should be noted that the TNP-F_1 stimulator cells used in this experiment were competent to stimulate IL-2-secreting T cells present in the unseparated responder cell population (Table VI) as well as to stimulate the generation of anti-TNP CTL (data not shown). Thus, in contrast to their ability to respond to class I MHC alloantigens, primary IL-2-secreting Lyt-2$^+$ T cells fail to respond to TNP-modified self-MHC determinants.

Discussion

The purpose of the present study was to identify and characterize the primary T cell populations that secrete IL-2 in response to recognition of MHC alloantigens. It was found that primary T cell populations contain two distinct MHC-specific, IL-2-secreting T cell subsets that express reciprocal phenotypes and reciprocal MHC restriction specificities: an L3T4$^-$,Lyt-2$^+$ subset specific for class I MHC determinants, and an L3T4$^+$,Lyt-2$^-$ subset restricted by class II MHC determinants. These two reciprocal T cells subsets differed significantly in the diversity of MHC antigens to which they would respond. The Lyt-2$^+$ class I restricted T cell subset displayed a narrow response repertoire, responding exclusively to class I but not class II MHC alloantigens, and failing to respond to

either unmodified or TNP-modified self class I MHC determinants. In contrast, the L3T4$^+$ class II–restricted T cell subset displayed a broad response repertoire, responding to nearly all stimulator cell populations whether they expressed self, foreign class I, or foreign class II MHC determinants. The cellular activation requirements of the two T cell subsets were similar in that both required Sephadex G-10 adherent accessory cells that could be replaced by soluble second signals such as those present in PMA-stimulated P388D.1 SN. The cellular activation requirements of the two T cell subsets differed in that only the Lyt-2$^+$ T cell subset could be triggered by Ia$^-$ accessory cells. Finally, the frequency in primary T cell populations of these two IL-2-secreting T cell subsets was found to be similar.

The L3T4$^+$ IL-2-secreting T cell subset present in primary T cell populations was entirely class II restricted as indicated both by genetic mapping experiments using L3T4$^+$ responder cells from chimeric mice and by anti-Ia mAb blocking experiments using L3T4$^+$ responder cells from normal mice. Thus, syngeneic stimulator cells triggered self-Ia specific L3T4$^+$ cells to secrete IL-2, presumably in analogy to the autologous mixed lymphocyte proliferative response (25); stimulator cells expresssing foreign class II MHC determinants triggered class II allospecific L3T4$^+$ cells to secrete IL-2; and stimulator cells expressing foreign class I MHC determinants triggered L3T4$^+$ cells that were probably specific for composite MHC determinants consisting of allogeneic class I determinants presented in the context of self-Ia determinants (8, 26). The evidence that class I allospecific L3T4$^+$ cells are restricted by self-Ia determinants is that: (*a*) unlike Lyt-2$^+$ T cells, L3T4$^+$ T cells only responded to class I alloantigens presented by Ia$^+$ stimulator cells; and (*b*) unlike Lyt-2$^+$ T cells, L3T4$^+$ T cells only responded to class I alloantigens under conditions in which self-Ia–restricted T cells could be triggered, and never responded to class I alloantigens under conditions in which the activation of self-Ia–restricted T cells was avoided or prevented.

In contrast to the broad response spectrum expressed by the L3T4$^+$ IL-2-secreting T cell subset, the Lyt-2$^+$ IL-2-secreting T cells subset responded only to stimulator cell populations expressing allogeneic class I MHC determinants. Unlike their L3T4$^+$ counterparts, there were no IL-2-secreting cells within the Lyt-2$^+$ T cell subset that were responsive to syngeneic stimulator cells, indicating an absence of Lyt-2$^+$ cells responsive to unmodified self–class I MHC determinants, nor were there IL-2-secreting cells within the Lyt-2$^+$ T cell subset that were responsive to class II–disparate stimulators, indicating an absence of Lyt-2$^+$ cells specific for composite MHC determinants consisting of class II alloantigens presented in the context of self class I MHC determinants. The narrow response spectrum of the Lyt-2$^+$ IL-2-secreting T cell subset was further dramatized by its rather remarkable failure to respond to TNP-modified self determinants, an extremely potent antigen for Lyt-2$^+$ CTL. The ability of primary Lyt-2$^+$ to secrete IL-2 only in response to class I MHC alloantigens did not obviously appear to derive from unusually stringent activation requirements for this T cell subset, since they were readily activated by normal adherent accessory cell populations as well as by Ia$^-$ variants of functional accessory cell populations. The inability of nonadherent cells to activate primary Lyt-2$^+$ T cells

to secrete IL-2 could be reversed by the addition of P388D.1 SN containing so-called second signals such as IL-1 (17), similar to what has been reported for class II–restricted T cells (27). However, the limited specificity of primary Lyt-2^+ T cells for class I alloantigens was unaffected by the presence of exogenously added second signals. Thus, their narrow response spectrum raises the possibility that the clonotypic receptors Lyt-2^+ T cells express have insufficient affinity for antigens other than class I alloantigens to trigger IL-2 secretion (4, 5).

In this report, primary T cell populations that secrete IL-2 in response to MHC alloantigens have been referred to as Th cells, implying that these T cells express classical Th function in anti-MHC immune responses. In fact, we have recently characterized the Th cell populations that do function in anti-MHC CTL responses (4, 5) and we found that the initiation of allospecific CTL responses requires the activation of at least one of two distinct Th cell populations that showed synergy with pCTL when assayed in classical helper cell–effector cell collaboration experiments (5). These two primary Th cell populations were identical to the two primary IL-2-secreting T cell populations characterized in the present report with regard to their: (*a*) phenotypes, (*b*) MHC restriction specificities, (*c*) functional response specificities, and (*d*) cellular activation requirements. Indeed, the two Th cell populations were shown to mediate their helper function in allospecific CTL responses, at least in part, by secreting IL-2 (5). Thus, the evidence is compelling that the primary T cell populations characterized by IL-2 secretion in the present study are identical to the primary T cell populations that function as Th cells in initiating allospecific CTL responses. However, since their ability to also differentiate into CTL effectors was not assessed, it is conceivable that Lyt-2^+ IL-2-secreting T cells may be dual function cells, and so function as Th cells for the activation of help-dependent pCTL in allospecific CTL responses as well as differentiate into allospecific cytolytic effector cells themselves (28, 29). At the present time, we are unable to rule out the possibility that all Lyt-2^+ IL-2-secreting T cells have cytolytic potential. However, the failure of Lyt-2^+ IL-2-secreting T cells to respond to TNP-self determinants indicates that Lyt-2^+ T cells can be stimulated to become cytolytic without being stimulated to also secrete IL-2. Detailed analysis of the repertoire differences involved in stimulating distinct Lyt-2^+ T cell functions will be the subject of future reports.

Since Th cells for most immune responses are L3T4^+ and class II restricted, we were quite surprised to find that the frequency of class I (K^{bm1}) allospecific Lyt-2^+ Th cells approached that of class II (I-A^{bm12}) allospecific L3T4^+ Th cells in primary T cell populations. While the K^{bm1} allodeterminants might be unusually potent stimulators of the primary Lyt-2^+ T cell subset, IL-2-secreting Lyt-2^+ T cells specific for other class I allodeterminants have been reported previously (30, 31), and comparably high frequencies of class I allospecific T cells have been observed by Swain and coworkers with an entirely different assay system (32). However, the results of the present study do conflict with a recent report by Weinberger et al, who reported that K^{bm1} allospecific Th cells were entirely class II restricted and Lyt-2^- (33). We presume it was their highly limiting response conditions that caused these investigators to overlook the participation of Lyt-2^+ Th cells in K^{bm1}-specific responses.

Experiments examining the in vivo function of Lyt-2^+ Th cells are currently in progress.

Summary

This study has characterized the primary T cell subpopulations that secrete IL-2 in response to recognition of either class I or class II MHC encoded determinants. The addition to culture of anti–IL-2-R mAb inhibited the consumption of IL-2 by activated lymphocytes during the response period, permitting a much more accurate assessment of the amount of IL-2 produced in the response cultures. Using this response system, we found that primary T cell populations contain two IL-2-secreting T cell subsets that express reciprocal phenotypes and different MHC recognition specificities: an L3T4^+,Lyt-2^- T cell subset responsive to both class I and class II MHC alloantigens, and an L3T4^-Lyt-2^+ T cell subset responsive only to class I MHC alloantigens.

The L3T4^+ T cell subset expressed a broad functional response repertoire in that L3T4^+ T cells were triggered to secrete IL-2 upon recognition of unmodifed self-Ia determinants, allogeneic Ia determinants, and class I alloantigens presented by self-Ia determinants. The activation of L3T4^+ IL-2-secreting T cells, even those responsive to class I MHC alloantigens, could be blocked completely by anti-Ia mAbs, confirming that the L3T4^+ T cell subset was in fact class II restricted. In contrast, the Lyt-2^+ T cell subset expressed a narrow functional response repertoire in that they were triggered to secrete IL-2 only in response to allogeneic class I MHC determinants, and were not triggered to secrete IL-2 even in response to TNP-modified self-MHC determinants. The specificity of Lyt-2^+ IL-2-secreting T cells for class I MHC allodeterminants was confirmed by the observations that: (*a*) their activation could be blocked completely by anti–class I mAbs, (*b*) they could be triggered by Ia^- cell lines which expressed class I MHC alloantigens and possessed accessory function, and (*c*) they responded to class I MHC alloantigens but failed to respond to class II MHC alloantigens, even in the presence of exogenously added second signals that circumvented the requirement for alloantigen-bearing accessory cells. Finally, the frequency of primary Lyt-2^+ T cells that secreted IL-2 in response to class I (K^{bm1}) MHC alloantigens was shown to be only minimally lower than that of L3T4^+ T cells that secreted IL-2 in response to class II (I-A^{bm12}) MHC alloantigens. Thus, this study characterizes an Lyt-2^+ T cell subset that is present in unprimed T cell populations in unexpectedly high frequency, but functions predominantly, if not exclusively, in immune responses against class I MHC alloantigens.

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