CYTOTOXIC T LYMPHOCYTE RESPONSES BY CHIMERIC THYMOCYTES

Self-Recognition Is Determined Early in T Cell Development

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Products of genes encoded within the major histocompatibility complex (MHC)[1] play an important role in antigen recognition by T lymphocytes, in that most antigens are recognized by T cells only in the context of specific MHC determinants (1, 2). Studies utilizing T cells from chimeric mice have demonstrated that the capacity of T cells to recognize foreign antigens in the context of specific H-2 structures seems to be determined by the host environment in which T cells mature, rather than by their own H-2 genotype (2–6). However, both the mechanism and host elements responsible for the determination of the self-recognition repertoire by developing T cells are controversial. It has been suggested that it is specifically those H-2 determinants that are encountered during intrathymic differentiation and maturation that will subsequently be recognized as self, because it has been observed that certain viruses and minor histocompatibility antigens can only be recognized by cytotoxic T cells or their precursors on stimulator and target cells expressing the same H-2 determinants as the thymus in which these T cells have differentiated (7–9). Recent observations that nonlymphoid thymus cells express H-2K and H-2D as well as I-region-encoded MHC determinants support the proposed role of the thymus in dictating T cell recognition specificities (10–13). In contrast other studies have suggested that it is the extrathymic rather than the thymic environment that determines the H-2 restricted self repertoire of T cells (14). In addition, the results of studies involving T cell populations that had been acutely depleted of specific alloreactive specificities (15, 16) as well as studies involving nude mice with transplanted allogeneic thymuses (17) seriously question the importance of the differentiation environment at all in the determination of H-2-restricted self-recognition by T cells.

Because the thymic environment performs a critical role in the differentiation of incompetent precursor cells into functional T cells, determination of the point in T cell ontogeny during which H-2-restricted recognition is first observed would potentially be important to our understanding of the mechanisms by which it occurs. The aim of the present study was to examine the recognition repertoire of cytotoxic T cell (CTL) precursors within the thymi of recently reconstituted chimeric mice in order to determine the influence of the environment on expression of self-recognition and

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[1] *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; FCS, fetal calf serum; Il-2, interleukin-2 (T cell-growth factor); MHC, major histocompatibility complex; TNBS, trinitrobenzene sulfonate; TNP, trinitrophenyl.

allorecognition by T cells at an early stage of differentiation. Because cells of donor origin do not appear in the thymus of radiation bone marrow chimeras until 12–15 d after irradiation and reconstitution (S. O. Sharrow, B. J. Mathieson, and A. Singer. Manuscript in preparation.), and because the thymuses are not fully repopulated with donor cells until 3 wk after irradiation and reconstitution, (S. O. Sharrow et al. Manuscript in preparation.), the recognition pattern of donor CTL precursors obtained from such chimeras could not be assessed earlier than 3–4 wk after reconstitution. In the course of the present studies it was found that at 4 wk after reconstitution, the T cell populations from either the spleens or thymuses of chimeras were incapable of mediating alloreactive or H-2-restricted CTL responses in vitro. However, in the presence of interleukin-2 (Il-2), the putative nonspecifically acting T helper cell product (18–20), both alloreactive and H-2-restricted CTL responses could be generated by thymocytes and spleen cells from such recently reconstituted chimeras, indicating that CTL precursor cells were present at this early time point in their differentiation. The results of the present study demonstrate (*a*) within the thymus of recently reconstituted parent → F_1 (designated A → A × B) chimeras no alloreactive CTL responses against either A or B MHC determinants could be detected and that thymocytes from A → A × B chimeras recognized trinitrophenyl (TNP) in association with both A and B parental haplotypes, and (*b*) in F_1 → parent (A × B → A) chimeras, thymic CTL precursors were restricted to recognition of TNP in association with the recipient's (parent A) H-2 type, even though they were also tolerant to both A and B parental haplotypes. Thus, these data demonstrate that, in chimeras, one of the earliest detectable antigen-specific T cell functions mediated by donor bone marrow-derived cells is already H-2 restricted and specific for those MHC-determinants encountered in the chimeric host environment.

Materials and Methods

Animals. C56BL/10Sn (B10), B10.A, B10.D2, B10.BR, (B10 × B10.A)F_1, (B10 × B10.BR)F_1, and BALB/c male mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Normal spleen cells were obtained from 10- to 16-wk-old mice, whereas normal thymocytes were obtained from 5- to 7-wk-old mice. All chimeras were tested at 3.5–6 wk after bone marrow transplantation.

Chimeras. An extensive description of the production and H-2 typing of chimeras has been given elsewhere (21). Briefly, recipient mice were irradiated with 950 rads x ray and reconstituted 4–6 h later with 1.5×10^7 bone marrow cells, which had been depleted of T cells by pretreatment with a rabbit anti-mouse brain serum and complement. This serum is specifically cytotoxic for T cells and lacks anti-stem cell activity (22). Chimeras are designated as bone marrow donor → irradiated recipient and were tested individually. Typing of thymocytes from chimeras by flow microfluorometry on the fluorescence-activated cell sorter demonstrated that essentially all thymocytes and spleen cells (>98%) were of donor origin by 3 wk after bone marrow transplantation. A detailed report of the results of these typing studies will be presented elsewhere.[2]

Preparation of Il-2. Il-2 was prepared as previously described (23). Briefly, spleen cells from BALB/c mice were cultured for 18–20 h at a density of 10×10^6 cells/ml, 5×10^6/cm^2 with 2.5 μg/ml conconavalin A (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, and extra glucose to a final concentration of 4.5 g/liter. After harvesting and filtering the supernates, these were supplemented with 0.2 M

[2] Sharrow, S. O., B. J. Mathieson, and A. Singer. Cell surface appearance of unexpected host MHC determinants on thymocytes from radiation bone marrow chimeras. Manuscript submitted for publication.

α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, Mo.) to prevent mitogenic effects of the remaining concanavalin A (23, 24). These Il-2 preparations were used at a 50% (vol:vol) concentration and were previously shown not to be mitogenic for thymocytes and spleen cells (23). Further details on the effects of Il-2 on anti-allo and anti-TNP-modified-self CTL responses of normal thymocytes and thymocyte subpopulations are given elsewhere (23). Although different batches of Il-2 were used throughout the present experiments, all had quantitatively and qualitatively similar effects on CTL responses of thymocytes and did not have an effect when no stimulator cells were present.

In Vitro Generation of CTL against Alloantigens and TNP-Self. Mixed lymphocyte cultures of thymocyte responder cells (from either normal mice or chimeras) and splenic stimulator cells (from normal mice) were performed in RPMI-1640, supplemented with 10% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, 1 mM sodium pyruvate, 0.1 M nonessential amino acids, and extra glucose to a final concentration of 4.5 g/liter, as described in detail earlier (25). Both allogeneic and syngeneic stimulator spleen cells were freed of erythrocytes with ammonium chloride. TNP modification was performed with 10 mM trinitrobenzene sulfonate (TNBS) (Pierce Chemical Co., Rockford, Ill.) (26). Both anti-allo and anti-TNP-self CTL responses were generated with 5×10^6 responder thymocytes and 5×10^6 irradiated (1,500 rads) stimulator cells in 1 ml of the above medium and supplemented with 1 ml Il-2, unless stated otherwise. The ^{51}Cr-release assay was performed on day 5 with ^{51}Cr-labeled untreated or 10 mM TNBS-treated concanavalin A-induced splenic blasts as target cells as described (25). Specific ^{51}Cr-release values were calculated as the difference between experimental and spontaneous release counts divided by the difference between maximal release and spontaneous release counts. Values obtained with responder cells cultured with syngeneic unmodified stimulator cells were never different from those obtained in the presence of medium alone and, therefore, are not included in the tables.

Results

In Vitro Generation of CTL Effectors from the Thymus and Spleen of Recently Reconstituted Radiation Chimeras Requires the Presence of Il-2. The aim of the present study was to study CTL specificity patterns of donor-derived T cells in the thymus of radiation bone marrow chimeras at the earliest possible time point after reconstitution. Analysis of thymus repopulation in chimeras indicated that host T cells were still present in the thymus during the initial 15 d after irradiation and reconstitution (S. O. Sharrow et al. Manuscript in preparation.). Such cells might be derived from an intrathymic radioresistant thymocyte precursor, which as was previously reported (27) is capable of partially restoring the thymus of irradiated mice. However, by day 21 after reconstitution only donor T cells were detected in the thymi and spleens from both parent → F_1 and F_1 → parent mice (S. O. Sharrow et al. Manuscript in preparation.). As a result, the earliest point at which the function of cells entirely of donor origin could be assessed was 3–4 wk post-reconstitution. However, neither the cells from the thymus nor the spleen of recently reconstituted chimeras were capable of generating either an allo-specific or TNP-modified self-specific CTL response (Table I). When Il-2 was added to the sensitizing cultures, chimeric thymocytes always expressed the ability to generate CTL responses against both TNP-modified-self and allogeneic stimulator cells (Table I). Similarly, the only CTL responses obtained from the spleens of these early chimeras also required the presence in culture of Il-2 (Table I), though alloreactive responses remained marginal. As expected, both alloreactive and TNP-modified-self CTL responses of normal thymocytes were enhanced in the presence of Il-2 (Table I), in agreement with earlier reports (20, 23, 24, 28, 29), as were the CTL responses of normal spleen cells (Table I). It is of interest to note that whereas CTL responses were always generated by thymocytes from recently reconstituted chimeras

TABLE I

In Vitro CTL Responses of Thymocytes and Spleen Cells from Recently Reconstituted Chimeras Are Generated Only in the Presence of Il-2

Responder cells	Presence of Il-2	Effector: target cell ratio	Percent specific ^{51}Cr release* of stimulator:target					
			B10.A-TNP: B10.A-TNP		B10.A-TNP: B10.A		BALB/c: BALB/c	
			Experiment I‡	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II
B10 × B10.A → B10.A thymocytes§	−	40:1	−1	−3	1	2	4	0
		20:1	−3	−2	2	0	0	−3
		10:1	2	−4	0	−1	−1	−2
	+	40:1	58	55	−3	−2	51	46
		20:1	44	39	−3	0	38	34
		10:1	32	25	1	0	20	20
B10 × B10.A → B10.A spleen cells§	−	40:1	−2	2	0	−1	5	2
		20:1	−4	0	0	0	0	1
		10:1	0	0	0	0	0	−1
	+	40:1	58	60	2	2	14	7
		20:1	43	54	0	0	11	6
		10:1	33	50	−1	−1	9	4
Normal B10 × B10.A thymocytes‖	−	40:1	0	−5	2	−2	15	16
		20:1	1	−3	0	−2	10	12
		10:1	1	−4	0	0	7	2
	+	40:1	69	71	2	0	70	60
		20:1	60	70	2	0	61	52
		10:1	52	64	1	−4	47	47
Normal B10 × B10.A spleen cells‖	−	40:1	53	46	−1	−4	62	59
		20:1	35	37	0	0	50	41
		10:1	26	30	2	−4	38	31
	+	40:1	96	75	0	−3	88	65
		20:1	90	70	0	−5	76	57
		10:1	84	64	3	0	69	57

* Data represent the means of triplicate determinations (SD always <4%) and have been corrected for background ^{51}Cr release values (ranging from 13 to 24%). Maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 3,876 to 4,720 cpm.

‡ Data from two separate experiments are given (thymocytes and spleen cells in each experiment were derived from the same chimeric mice).

§ Chimeric lymphocytes were derived from mice which had been irradiated and reconstituted 4 wk before the assay was performed.

‖ Normal control lymphocytes were derived from 8-wk-old F_1 mice.

in the presence of Il-2, spleen cell populations from these same early chimeras were not always competent to generate CTL responses despite the presence in culture of Il-2 (Table II), suggesting that cytotoxic precursor cells appear first in the thymus and then in the periphery.

Thus, 4 wk after irradiation and reconstitution, when the thymuses of chimeras have just become fully repopulated with donor cells (S. O. Sharrow et al. Manuscript in preparation.). CTL effectors from the thymus of chimeras could be generated

TABLE II

CTL Responses Can be Generated by Thymocytes but Not Always by Spleen Cells from Recently Reconstituted Chimeras, Even in the Presence of Il-2

Responder cell	Presence of Il-2	Stimulator and target cells	Effector: target cell ratio	Percent specific ^{51}Cr release*	
				Effector cells	
				Thymus	Spleen
B10 × B10.A → B10	+	B10-TNP	40:1	32	2
			20:1	15	1
			10:1	17	1
B10 → B10 × B10.A	+	B10-TNP	40:1	28	3
			20:1	13	−5
			10:1	8	−6
B10 × B10.A → B10.A	+	B10.A-TNP	40:1	30	−2
			20:1	26	−1
			10:1	18	−3
B10.A → B10 × B10.A	+	B10.A-TNP	40:1	51	13
			20:1	38	8
			10:1	23	7

* Data represent the means of triplicate determinations (SD always <3%) and have been corrected for background ^{51}Cr-release values (11 and 18% for B10.TNP and B10.A-TNP, respectively). Maximum ^{51}Cr-release values for 5×10^3 target cells were 2,319 cpm (B10-TNP) and 3,178 cpm (B10.A-TNP).

provided a nonspecific helper cell factor (Il-2) was added to the cultures. Therefore, in all subsequent experiments involving CTL responses of thymocytes from recently reconstituted chimeras, Il-2 was added to the cultures.

Il-2 Does Not Alter the Specificity of Anti-Allo or Anti-TNP-Self CTL Responses of Thymocytes or Spleen Cells. Because the presence of Il-2 in the sensitizing cultures was required for the generation of CTL responses from the thymuses and spleens of chimeras early after reconstitution, it was important to test the possibility that the specificity of the responses thus obtained might be influenced by the presence of the factor. Therefore, the specificity of anti-allo and anti-TNP-self responses of thymocytes generated in the presence and absence of Il-2 was compared with the specificity of responses generated by spleen cells from the same mice.

As can be seen in Table III, Il-2 strongly enhanced the low or absent CTL response of normal B10 thymocytes. However, the responses obtained expressed the same pattern of specific lysis and cross-reactive lysis for both anti-allo and anti-TNP-self responses as did responses of normal spleen cells from these mice cultured in the absence of Il-2 (Table III). Specifically, B10 anti-BALB/c CTL cross-reactively lysed TNP-modified B10 targets and anti-B10-TNP CTL cross-reactively lysed TNP-modified allogeneic B10.A targets (Table III) as has been previously observed for CTL from normal spleens (30–34). Thus, even though the ability to generate thymocyte CTL responses in the culture conditions used required the presence of Il-2, the pattern of specific and cross-reactive lysis for anti-allo and anti-TNP-self responses of B10 thymocytes was no different than for B10 spleen cells and consequently could not be attributed to the presence of Il-2 in the thymocyte cultures. Essentially similar results were also obtained with B10.A responding cells (data not shown).

Thymocytes from A → A × B Chimeras are Nonalloreactive to Either A or B MHC

TABLE III

Specificity of CTL Activity Obtained in the Presence and Absence of Il-2

Responder cells	Stimulator cells	Presence of Il-2*	Effector: target cell ratio	Percent specific ^{51}Cr release‡				
				B10-TNP	B10	B10.A-TNP	B10.A	BALB/c
B10 thymocytes	BALB/c	−	40:1	4	−6	10	11	19
			20:1	1	−4	8	12	18
		+	40:1	39	0	68	51	78
			20:1	37	0	70	44	67
B10 spleen cells	BALB/c	−	40:1	20	−1	65	43	73
			20:1	18	−1	53	40	68
B10 thymocytes	B10-TNP	−	40:1	2	−2	2	−4	4
			20:1	0	−3	1	−5	0
		+	40:1	81	3	40	0	0
			20:1	63	0	31	−1	7
B10 spleen cells	B10-TNP	−	40:1	44	−4	21	−6	2
			20:1	36	−6	12	−6	2

* Il-2-containing cultures were supplemented with 50% (vol:vol) Il-2.

‡ Data represent the means of triplicate determinations (SD <3%) and have been corrected for background ^{51}Cr-release values (ranging from 16 to 22%). Maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 3,124 to 4,209 cpm.

Determinants and Recognize TNP in Association with both A and B H-2 Antigens. Thymocytes from B10 → B10 × B10.A and B10.A → B10 × B10.A chimeras were assayed for their abilities to generate alloreactive CTL responses in the presence of Il-2. Both B10 → F_1 and B10.A → F_1 thymocytes generated alloreactive CTL against third-party BALB/c stimulator cells (Table IV), indicating that even at this early point in time after bone marrow reconstitution, alloreactive CTL precursors were present in the thymuses of chimeras. The levels of the responses obtained were comparable with those obtained with normal B10, B10.A, and F_1 mice (Table IV), as was cell survival at the end of the culture period (data not shown). In contrast, B10 → F_1 and B10.A → F_1 thymocytes did not generate an alloreactive CTL response specific for either of the recipient's H-2 haplotypes, because neither B10 → F_1 nor B10.A → F_1 thymocytes lysed unmodified B10 or B10.A target cells when stimulated with either TNP-modified B10 or B10.A stimulator cells (Table IV). It is important to note that TNP modification of allogeneic stimulator cells does not affect their ability to induce an alloreactive CTL response, as evidenced by the fact that normal thymocytes generated an alloreactive response when stimulated with the same TNP-modified allogeneic stimulator cells (Table IV). Thus, thymocytes from A → A × B chimeras are capable of generating an alloreactive response against third-party stimulator cells, but are specifically nonalloreactive to both of the hosts' parental H-2 haplotypes, even at an early point in time (i.e., 4–6 wk) after irradiation and reconstitution.

Because A → A × B chimeric thymocytes did not recognize either A or B H-2 determinants as foreign, their ability to recognize TNP in the context of either A or B could be assessed. Indeed, upon stimulation with either TNP-modified B10 or B10.A stimulator cells, both B10 → F_1 and B10.A → F_1 thymocytes generated specific CTL responses against B10-TNP and B10.A-TNP (Table IV). The results were

TABLE IV

Thymocytes from Parent A → A × B Chimeras are Nonalloreactive against Either Parental Haplotype and Can Recognize TNP in Association with Both Parental Haplotypes

Responder thymocytes	Stimulator cells	Effector: target cell ratio	Percent specific ^{51}Cr release*									
			B10-TNP		B10		B10.A-TNP		B10.A		BALB/c	
			Experiment I‡	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II
B10 → B10 × B10.A	B10-TNP	40:1	58	36	−1	−4	24	18	−3	−2		
		20:1	40	26	−3	1	10	13	0	0		
		10:1	26	16	0	−2	10	4	0	−4		
	B10.A-TNP	40:1	21	14	0	−1	91	59	−3	−1		
		20:1	12	2	0	0	83	40	0	0		
		10:1	4	−5	−1	0	73	24	1	1		
	BALB/c	40:1									32	46
		20:1									18	49
		10:1									12	22
B10.A → B10 × B10.A	B10-TNP	40:1	84	52	2	0	46	15	0	−3		
		20:1	61	40	1	0	23	7	−2	0		
		10:1	41	24	2	−2	14	2	−4	−1		
	B10.A-TNP	40:1	14	1	0	0	69	45	−3	−1		
		20:1	2	0	−1	−3	59	24	−1	−2		
		10:1	2	−2	1	0	40	12	0	0		
	BALB/c	40:1									66	65
		20:1									62	60
		10:1									54	40
Normal B10	B10-TNP	40:1	78	60	−2	−2	32	17	3	−1		
	B10.A-TNP	40:1	15	18	−2	2	83	80	83	75		
	BALB/c	40:1									68	59
Normal B10.A	B10-TNP	40:1	70	59	64	61	23	17	1	0		
	B10.A-TNP	40:1	27	10	−1	1	79	62	0	0		
	BALB/c	40:1									58	55
Normal B10 × B10.A	B10-TNP	40:1	56	56	−4	0	25	18	0	1		
	B10.A-TNP	40:1	21	23	0	0	94	76	−2	1		
	BALB/c	40:1									45	59

* Means of triplicate determinations (SD < 4%) corrected for background ^{51}Cr-release values (ranging from 12 to 24%); maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 2,140 to 3,545 cpm.

‡ Data from two separate experiments are given; values in blocks represent specific lysis of target cells identical with the stimulator cells.

essentially the same for all eight A → A × B chimeras tested (summarized in Table VIII). These data, therefore, demonstrate that either B10 or B10.A CTL precursor T cells differentiating within a (B10 × B10.A)F_1 thymus specifically recognize and react against TNP in association with both B10 and B10.A MHC determinants, whereas they are tolerant to both unmodified B10 and B10.A MHC determinants.

Cross-reactive lysis by both chimeric and normal thymocyte CTL were also observed to a variable degree. For example, B10 → F_1 thymocytes stimulated with B10-TNP cells cross-reactively lysed B10.A-TNP target cells and, when stimulated with B10.A-TNP, cross-reactively lysed B10-TNP target cells (Table IV). Similar patterns of cross-reactive lysis were also observed with both B10.A → F_1 and normal B10 × B10.A thymocytes stimulated with either B10-TNP or B10.A-TNP stimulator cells (Table IV). Essentially, however, the cross-reactive lysis observed was always lower than the specific lysis and was generally lower after stimulation with B10.A-TNP than after stimulation with B10-TNP. Thus, thymocytes from A → A × B chimeras were indistinguishable in their CTL specificity from normal A × B thymocytes.

Thymocytes from A × B → A Chimeras Display Either Restricted or Preferential Recognition

of TNP in Association with A H-2 Determinants. The CTL recognition pattern of thymocytes from A × B → A chimeras was next investigated. First of all, their patterns of allorecognition and allotolerance were determined. The data shown in Table V demonstrate that in (B10 × B10.A)F_1 → B10 and (B10 × B10.A)F_1 → B10.A chimeras alloreactive CTL precursors were present in the thymus, which could react to third-party BALB/c stimulator cells. In contrast, no alloreaction against either parental H-2 was observed as evidenced by the lack of lysis of unmodified target cells of either parental haplotype after stimulation with TNP-modified parental stimulator cells (Table V).

In contrast with the failure of the chimeric host environment to alter the alloreactive potential of A × B → A chimeric thymocytes, the host environment did exert a profound influence on the self-TNP responses of these same A × B → A chimeric thymocytes. In the experiments exemplified in Table V, an absolute restriction of recognition of TNP in association with only the parental recipient's H-2 type was observed, i.e., thymocytes from (B10 × B10.A)F_1 → B10 chimeras were only stimulated by B10-TNP and not B10.A-TNP stimulator cells, whereas thymocytes from (B10 ×

TABLE V

Thymocytes from A × B → A Chimeras are Nonalloreactive against both Parental Haplotypes but are Specifically Stimulated Only by Recognition of TNP in Association with the H-2 Haplotype of Parent A

Responder thymocytes	Stimulator cells	Effector: target cell ratio	Percent specific ^{51}Cr release*									
			B10-TNP		B10		B10.A-TNP		B10.A		BALB/c	
			Experiment I	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II	Experiment I	Experiment II
B10 × B10.A → B10	B10-TNP	40:1	58	40	0	−2	24	16	−1	0		
		20:1	39	31	−1	0	10	12	−3	0		
		10:1	30	24	1	1	5	1	2	1		
	B10.A-TNP	40:1	−1	−2	0	−2	0	0	−1	−3		
		20:1	1	−4	2	−1	−2	−1	0	0		
		10:1	0	−3	−1	0	−2	0	−3	−3		
	BALB/c	40:1									44	40
		20:1									31	30
		10:1									18	13
B10 × B10.A → B10.A	B10-TNP	40:1	−2	−3	0	0	−2	2	0	−1		
		20:1	−2	−1	1	−1	−3	−2	−1	1		
		10:1	−1	−1	2	0	2	0	2	−1		
	B10.A-TNP	40:1	9	3	−1	0	60	58	−1	1		
		20:1	5	1	0	−2	53	43	0	0		
		10:1	4	0	2	2	48	33	1	3		
	BALB/c	40:1									39	45
		20:1									33	34
		10:1									24	20
Normal B10	B10-TNP	40:1	66	53	−4	0	34	19	−1	0		
	B10.A-TNP	40:1	32	29	0	−2	80	76	74	78		
	BALB/c	40:1									52	56
Normal B10.A	B10-TNP	40:1	60	61	58	62	21	24	−2	−1		
	B10.A-TNP	40:1	14	8	−1	0	52	58	0	−3		
	BALB/c	40:1									72	68
Normal B10 × B10.A	B10-TNP	40:1	65	58	0	0	25	25	−4	0		
	B10.A-TNP	40:1	15	14	1	1	45	52	0	0		
	BALB/c	40:1									72	64

* Data represent the mean of triplicate determinations (SD always <5%) and have been corrected for background ^{51}Cr-release values (ranging from 15 to 21%). Maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 3,269 to 4,729 cpm.

‡ Data from two separate experiments are given; values in blocks represent specific lysis on target cells identical with the stimulator cell type.

B10.A)F_1 → B10.A chimeras were only stimulated by B10.A-TNP and not B10-TNP stimulator cells. Such absolute restriction was observed in 10 of 14 F_1 → parent A chimeras tested. In the remaining 4 of 14 F_1 → parent A chimeras tested, preferential rather than absolutely restricted recognition of TNP-modified parent A stimulator cells was observed (one representative experiment is portrayed in Table VI). In these latter A × B → A chimeras, specific lytic activity was stimulated by both A-TNP and B-TNP, although the lysis mediated by CTL stimulated by A-TNP was always greater than that mediated by CTL stimulated by B-TNP. In the experiment displayed in Table VI, one of the B10 × B10.BR → B10 chimeras tested was only stimulated by B10-TNP whereas the other was stimulated by both B10-TNP and B10.BR-TNP. However, in contrast with normal B10 × B10.BR thymocyte CTL, which specifically reacted to B10.BR-TNP stimulator cells to a consistently greater extent than to B10-TNP stimulator cells, B10 × B10.BR → B10 chimeric thymocyte CTL always preferentially reacted to TNP-modified B10 stimulator cells (Table VI). A summary of the data from all the chimeras tested is given in Table VIII.

In view of the extensive cross-reactivities generally observed in anti-TNP-self CTL responses (32–34), it was surprising that such clear-cut restrictions could be observed. Yet, it should be noted that even though F_1 → parent A chimeric thymocytes were only triggered by A-TNP stimulator cells, once triggered they did cross-reactively lyse B-TNP target cells (Tables V and VI). For example, B10 × B10.A → B10 thymocytes,

TABLE VI

Thymocytes from A × B → A Chimeras Occasionally Display Preferential Rather than Restricted Recognition of TNP in Association with Parent A Stimulator Cells

Responder thymocytes	Stimulator cells	Effector: target cells ratio	Percent specific ^{51}Cr release*			
			B10-TNP	B10	B10.BR-TNP	B10.BR
B10 × B10.BR → B10	B10-TNP	40:1	**53**	−2	17	0
		20:1	**44**	−1	7	0
		10:1	**25**	0	6	2
	B10.BR-TNP	40:1	12	−4	**28**	−2
		20:1	10	−3	**23**	−1
		10:1	8	−6	**15**	−3
B10 × B10.BR → B10	B10-TNP	40:1	**41**	0	16	−5
		20:1	**38**	0	9	−1
		10:1	**21**	3	6	0
	B10.BR-TNP	40:1	−2	0	**−4**	−2
		20:1	0	−3	**−1**	−3
		10:1	1	−2	**−3**	−2
Normal B10 × B10.BR	B10-TNP	40:1	**58**	−2	27	−2
		20:1	**47**	0	19	1
		10:1	**31**	−1	14	1
	B10.BR-TNP	40:1	15	−4	**78**	−5
		20:1	12	0	**63**	−3
		10:1	7	−2	**57**	−1

* Data represent the means of triplicate determinations (SD < 3%) and have been corrected for background ^{51}Cr-release values (ranging from 16 to 25%). Maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 1,675 to 3,719 cpm. Values in blocks represent specific lysis of target cells identical with the stimulator cells.

which were only triggered by B10-TNP stimulator cells did cross-reactively lyse B10.A-TNP target cells (Table V). Thus, at least some CTL with specificity for both A-TNP and B-TNP might be present in A × B → A chimeras. However, the possible presence of such cells would not be unique to the chimeric thymus because spleen cells from these same chimeras exhibited precisely the same cross-reactivity (data not shown), as do normal spleen cells (32–34).

The Restricted Response of Anti-TNP CTL from A × B → A Chimeric Thymocytes Is Not Caused by Demonstrable Suppression of CTL Specific for TNP in Association with B. The recognition of TNP predominantly in association with recipient's A H-2 type, can reflect either the absence or low frequency of CTL precursors specific for recognizing TNP in association with B or, alternatively, can reflect the presence of a suppressor mechanism directed against A × B T cells that specifically recognize TNP in association with B. If such a suppressor mechanism existed, mixing of chimeric A × B → A and normal A × B thymocytes would be expected to lead to a suppression of the normal A × B cells' capacity to generate a CTL response specific for B-TNP. To test this hypothesis, such a mixing experiment was performed. CTL precursors from (B10 × B10.BR)F_1 → B10 chimeric thymocytes recognized TNP in association with B10 but not B10.BR MHC determinants (Table VII, group 4), in contrast with normal (B10 × B10.BR)F_1 thymocytes, which generated anti-TNP responses in association with both parental B10 and B10.BR determinants (Table VII, group 1). However, the addition of (B10 × B10.BR)F_1 → B10 thymocytes at various ratios to cultures containing normal (B10 × B10.BR)F_1 thymocytes did not significantly affect the ability of the normal (B10 × B10.BR)F_1 thymocytes to generate an anti-B10.BR-TNP response (Table VII, Compare groups 1–3 and groups 5 and 6). Thus, no evidence was found to support the hypothesis that a suppressor mechanism was responsible for the failure of B10 × B10.BR → B10 thymocytes to generate a CTL response specific for B10.BR-TNP.

Discussion

The mechanism by which the host environment influences the self-recognition repertoire expressed by T cells is unknown. Indeed, the host environment could conceivably affect T cells at any point during their development. The results reported here demonstrate that at the earliest time point TNP-self-reactive CTL effector function by donor-derived cells can be measured in chimeras, it is already restricted to recognition of host MHC determinants. These results have implications for the mechanism by which the host environment influences self-recognition. Theoretically, the following possibilities might be considered: (*a*) the host environment might regulate either by selective expansion or deletion the differentiation of precursor cells so that only those with the capacity for self-recognition of host MHC determinants would be able to differentiate fully and become functionally competent; (*b*) alternatively, there might be no selective expansion or deletion of specific cell populations within the thymus, but rather the thymus might permit only those cells with the capacity for self-recognition of host MHC determinants to migrate out of the thymus to the periphery, a concept consistent with the observation that only a small fraction of thymocytes ever leaves the thymus; and, finally, (*c*) the host environment might affect neither the repertoire of the cell populations within the thymus nor T cell migration out of the thymus but rather might influence the recognition pattern of

TABLE VII

The Restricted Recognition of Thymocytes from A × B → A Chimeras Is Not a Consequence of the Presence of Demonstrable Haplotype Specific Suppression

Group	Number of normal (B10 × B10.BR)F_1 thymocytes	Number of chimeric B10 × B10.BR → B10 thymocytes	Effector: target cell ratio	Percent specific ^{51}Cr release* in stimulator:target			
				B10.BR-TNP: B10.BR-TNP	B10.BR-TNP: B10.BR	B10-TNP: B10-TNP	B10-TNP: B10
1	5×10^6	—	40:1	61	1	58	−5
			20:1	49	0	44	0
			10:1	44	2	17	0
2	5×10^6	5×10^6	40:1	78	1	ND‡	ND
			20:1	55	−1	ND	ND
			10:1	46	0	ND	ND
3	5×10^6	2.5×10^6	40:1	52	0	ND	ND
			20:1	36	0	ND	ND
			10:1	32	0	ND	ND
4	—	5×10^6	40:1	0	−2	42	0
			20:1	−3	−2	31	−2
			10:1	−3	0	17	0
5	2.5×10^6	—	40:1	53	0	ND	ND
			20:1	42	−2	ND	ND
			10:1	39	−2	ND	ND
6	2.5×10^6	2.5×10^6	40:1	44	0	ND	ND
			20:1	31	−2	ND	ND
			10:1	24	−1	ND	ND

* Data represent the means of triplicate determinations (SD < 6%) and have been corrected for background ^{51}Cr-release values (ranging from 14 to 27%). Maximum ^{51}Cr-release values for 5×10^3 target cells ranged from 2,397 to 3,894 cpm.

‡ ND, not determined.

competent T cells in the periphery so that only those T cell populations that possess the capacity for self-recognition of host MHC determinants expand and avoid suppression.

The three models outlined above make distinctly different predictions as to the outcome of the experiments performed in the present study. The first model predicts that only those precursor cells with self-specificity for host MHC determinants would become functional thymocytes so that the CTL generated from chimeric thymuses would be restricted to the recognition of TNP in association with only host MHC determinants, as would the CTL from their spleens. Thus, in A × B → A chimeras, TNP would be recognized by thymocytes only in association with A MHC determinants, whereas in A → A × B chimeras, TNP would be recognized in association with both A and B MHC determinants. In contrast, both of the other models predict that the thymuses of chimeras would contain functional CTL capable of recognizing antigen in the context of different MHC determinants so that chimeric thymocytes

TABLE VIII

Summary of TNP-modified Self-Responses of Thymocytes from A → A × B and A × B → A Chimeras

Number of mice	Responder thymocytes	Percent specific ^{51}Cr release* in stimulator:target			
		B10-TNP: B10-TNP	B10.A-TNP: B10.A-TNP	B10.BR-TNP: B10.BR-TNP	BALB/c‡: BALB/c
5	B10 → B10 × B10.A	53 ± 4	57 ± 7	ND§	48 ± 6
3	B10.A → B10 × B10.A	58 ± 14	51 ± 5	ND	52 ± 10
4	B10 × B10.A → B10	33 ± 4	3 ± 2	ND	46 ± 4
5	B10 × B10.A → B10.A	0 ± 1	47 ± 7	ND	41 ± 4
5	B10 × B10.BR → B10	42 ± 6	ND	14 ± 7	62 ± 4

* Data represent the means ± SE of specific lysis obtained with thymocytes CTL at an effector:target cell ratio of 40:1 for the indicated number of mice tested individually in separate experiments.

‡ Only data for alloreactivity against third-party stimulator cells (i.e., BALB/c) are presented; all mice were tolerant for both parental haplotypes (see Tables IV–VI).

§ ND, not determined.

would not be restricted to recognizing TNP only in association with host MHC determinants, even though the specificity of CTL from the spleen of these same mice would be host restricted. The results of the present study demonstrate that CTL precursors in the thymus of A → A × B chimeras were capable of recognizing TNP in association with the MHC determinants of both parents A and B MHC determinants, whereas thymocytes from A × B → A chimeras were restricted to recognizing TNP in association with the MHC determinants of parent A. Because functional CTL appeared in the thymus before they could be detected in the spleen, it is likely that the functional T cells obtained from the thymuses of recently reconstituted chimeras were thymocytes and not peripheral T cells that had recirculated to the thymus. Consequently, the results of this study strongly support the concept that the chimeric host restricts the self-recognition capacity of T cells by influencing the expansion or elimination of precursor cells such that only those precursor clones with the capacity for self-recognition of host MHC determinants fully differentiate and expand in the thymus into competent and functional CTL.

It is important to emphasize that these restrictions were observed even though the chimeric T cell populations in both the thymus and spleen were not yet competent to generate any CTL responses autonomously in the absence of Il-2. The ability of such nonspecific soluble factors as Il-2 to enhance CTL responses has been thought to be a result of its ability to substitute for a relative lack of T helper cells (18–20, 23, 24, 28, 29). Although no direct information on helper T cell function for CTL generation was obtained in these studies, one might speculate from the present data that, while competent alloreactive and H-2-restricted CTL precursors are present in these chimeras, helper T cell function might be less well developed, and that this deficiency can be bypassed by the addition of Il-2. The necessity of using Il-2 in the present study made it essential to examine the possibility that the specificity of CTL responses generated in the presence of Il-2 was determined or altered by the presence of this factor. The presence in culture of Il-2 did not alter the specificity of responses of normal thymocyte populations, nor did it obscure MHC restrictions expressed by chimeric thymocytes. However, it was also necessary to consider the unlikely possibility

that the restrictions that were observed in the presence of Il-2 were somehow the results of the presence of Il-2. Perhaps the only conceivable way such a nonspecific factor as Il-2 might enhance MHC restrictions that otherwise would not be observed would be by expanding a small population of haplotype-specific suppressor cells present in the thymuses of chimeras. Indeed, if the expansion of specific suppressor cells were responsible for the failure of A × B → A chimeric thymocytes to generate a response against TNP-modified B stimulator cells in the presence of Il-2, the mixing of A × B → A chimeric thymocytes with normal A × B thymocytes in cultures containing Il-2 should have also suppressed the ability of normal A × B cells to generate a response against TNP-modified B stimulator cells. However, chimeric A × B → A thymocytes in the presence of Il-2 failed to suppress the ability of normal A × B thymocytes to generate a specific response against TNP-modified B stimulator cells. Thus, the existence of a suppressor mechanism, possibly enhanced by Il-2, was not observed and is an unlikely explanation for the restricted recognition of A × B → A chimeric thymocytes. This conclusion is consistent with the failure to implicate suppression as the mechanism for the restricted responses of chimeric spleen cells in Il-2-free systems (9, 35–37).

The MHC-restricted self-recognition specificities observed in the present report using early thymocyte populations are precisely parallel with those previously reported for spleen cells from radiation bone marrow chimeras using TNP, viral antigens, minor H antigens, or H-Y antigens as foreign antigens (3–9, 38, 39). In A → A × B chimeras, splenic CTL recognized foreign antigen in association with both parent A as well as parent B H-2 type (3–9, 38, 39), whereas in A × B → A chimeras, either absolutely restricted (7, 8) or preferential (6, 38) recognition of foreign antigen in association with parent A H-2 type was observed. Thus, preferential rather than absolutely restricted recognition of TNP-modified host stimulator cells occasionally observed in the present experiments is a peculiarity neither of thymocyte responses nor of anti-TNP-self responses because preferential rather than absolutely restricted recognition of host MHC determinants has also been observed in spleen anti-minor H (6) and anti-viral (38) CTL responses. Although anti-TNP-self responses are generally highly cross-reactive (32–34), the thymocyte anti-TNP-self responses generated in the present studies were primarily cross-reactive only at the effector stage rather than the sensitization stage in that thymocytes from A × B → A chimeras were only or predominately stimulated by TNP-modified parent A stimulator cells; however, the CTL generated by TNP-modified parent A stimulator cells could cross-reactively lyse TNP-modified target cells of parent B. These findings suggest that the recognition requirements for triggering CTL responses may be more highly restricted than the recognition requirements for lysing target cells.

The observation that thymocyte populations from recently reconstituted A → A × B chimeras do not contain precursor CTL reactive to either parent A or B MHC determinants, but do contain precursor CTL reactive to third-party MHC determinants, demonstrates that the functional T cells present in these thymuses are nonalloreactive to either parent A or B MHC determinants. As such, these data support the concept that the chimeric host environment can influence the differentiation of precursor cells into competent T cells. However, the genotype of the T cells themselves may also have some role in the expression by T cells of an MHC-specific receptor

repertoire because influence of the chimeric host environment does not explain the nonreactivity of A × B → A chimeric thymocytes to parent B MHC determinants.

Whereas the present results do not solve the puzzle of how the chimeric host environment determines T cell self-recognition specificities, these results do effectively exclude mechanisms that postulate that host-specific MHC restrictions result entirely from the regulation by the thymus of T cell migration to the periphery or result entirely from the regulation by the extrathymic host environment of postthymic T cell maturation. Rather, the present results strongly support the concept that host-specific MHC restrictions result from the influence of the chimeric host on precursor cells in the prethymic or intrathymic environment such that the cells which differentiate into competent and functional CTL in the thymus are those with the capacity for self-recognition of host MHC determinants. Studies designed to determine whether precursor T cells are restricted by the host intrathymic or prethymic environment are currently in progress.

Summary

In this study the cytotoxic T lymphocyte (CTL) recognition pattern of thymocytes from recently reconstituted parent → F_1 and F_1 → parent radiation bone marrow chimeras was investigated. Chimeric thymocytes were entirely of donor origin approximately 4 wk after irradiation and reconstitution but were not capable of autonomously generating either alloreactive or trinitrophenyl (TNP)-modified-self-reactive CTL responses. However, in the presence of interleukin-2 (Il-2), the putative T helper cell product, CTL could be generated in vitro by thymocytes from recently reconstituted chimeras. Experiments with thymocytes from A → A × B and A × B → A chimeras revealed the following: (*a*) thymocytes from both types of chimeras were nonreactive to either A or B parental major-histocompatibility complex (MHC) determinants even though they were alloreactive to third-party stimulator cells; and (*b*) thymocytes from these chimeras were restricted to the recognition of TNP in association with MHC determinants syngeneic to the chimeric host. Thus, these experiments demonstrate that even at the earliest time CTL effectors of donor origin from the thymuses of chimeras can be studied, their self-receptor repertoire has already been restricted to recognition of host MHC determinants. These results support the concept that the host environment influences the self-recognition capacity of T cells at the pre- or intrathymic stage of differentiation.

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