H-2-CONTROLLED SUPPRESSION OF T CELL RESPONSE TO LACTATE DEHYDROGENASE B

Characterization of the Lactate Dehydrogenase B Suppressor Pathway*

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Over the past few years, considerable progress has been made in the identification of regulatory T cell pathways and factors that act as mediators between T cell subsets involved in these pathways (1–3). However, at least two important aspects of regulation of the immune response have remained largely unexplored. One of them is the role of antigen presentation and of major histocompatibility complex (MHC)[1] restriction in the activation of suppressor T (Ts) cells. From the paucity of reports on MHC restriction of Ts sets (4–8), one would conclude that MHC-restricted antigen presentation is not essential for the activation of most suppressor pathways. This view gains support from the demonstrated high affinity of some Ts cells for free antigen (9–11). It is, however, equally possible that the uncertainties about MHC restriction in Ts systems reflect the absence of an appropriate experimental model in which the interaction of Ts cells with antigen-presenting cells (APC) could be explored.

The second aspect that appears to be neglected is the mechanism of interaction between Ts effector cells and their targets. The mode of action of suppressor-effector factors is largely unknown (12–14), and, to our knowledge, there is only one report in the literature that bears on the direct interaction between Ts cells and T helper (Th) cells (15).

Our recent work on the proliferative T cell response to lactate dehydrogenase B (LDH_B) has provided a system suitable to study the questions outlined above. We have demonstrated (6–18) that most *H-2* haplotypes respond to LDH_B and that the T cell proliferation in all responder haplotypes is restricted by the molecule controlled by the A_α and A_β loci in the *I-A* region of *H-2* (A [$A_\alpha A_\beta$] molecules). The proliferating cells are most likely Th cells because their genetic control is almost identical with that of antibody production to LDH_B (16, 19). Nonresponder haplotypes to LDH_B include virtually all strains that carry the *k* allele at the E_α and E_β loci of the *H-2* complex. However, nonresponsiveness can be reversed by in vivo or in vitro administration of monoclonal antibodies against the molecule controlled by the E_β locus in the *I-A* and

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[1] *Abbreviations used in this paper:* A molecule, controlled by the A_α and A_β loci in the *I-A* region of *H-2*; APC, antigen-presenting cell; BudR, 5-bromo-2′-deoxyuridine; E molecule, controlled by the E_β locus in the *I-A* and the E_α locus in the *I-E* region of *H-2*; GA, poly($Glu^{60}Ala^{40}$); GAT, poly ($Glu^{60}Ala^{30}Tyr^{10}$); GT, poly ($Glu^{50}Tyr^{50}$); KLH, keyhole limpet hemocyanin; LDH_B, lactate dehydrogenase B; MHC, major histocompatibility complex; SI, stimulation index; SRBC, sheep erythrocytes; Th, T helper cell; Ts, T suppressor cell; Tse, T suppressor effector; Tsi, T suppressor inducer.

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the E_α locus in the *I-E* region of *H-2* (E^k molecule). Furthermore, primed nonresponder T cells depleted of Lyt-2-bearing cells give an A-restricted proliferative response upon challenge with LDH_B in vitro (16–18, 20). The suppression observed in E^k-bearing nonresponder strains is LDH_B specific, and expression of the E^k molecule on the APC is necessary for its manifestation (20). Thus, we have a system where the target of suppression is an A-restricted Th cell; the suppression can be measured as inhibition of Th cell proliferation, and at least one of the Ts cell subsets involved is E^k restricted. The two main participating cell sets also differ in terms of their Lyt phenotypes.

In this communication, we characterize the cells involved in the LDH_B suppressor pathway and describe the interactions between them, with particular emphasis on Ts-Th interaction.

Materials and Methods

Mice. Female and male mice, 8–12 wk of age, were obtained from our colony at the Max Planck Institute for Biology. The strains used and their alleles at *H-2* loci are listed in Tables III and VII.

Antigens and Immunization. Lactate dehydrogenase B4 (Boehringer, Mannheim, Federal Republic of Germany) and poly($Glu^{60}Ala^{40}$) (GA; Miles-Yeda, Rehovot, Israel) were prepared and the immunizations were performed as described previously (16, 21).

Antisera. The culture supernatants of hybridomas 1147 (anti-Lyt-1.1) and 49-31.1 (anti-Lyt-2.1) were a gift from Dr. I. F. C. McKenzie (Department of Pathology, University of Melbourne, Parkville, Victoria, Australia). Ascites fluids containing high-titered monoclonal antibodies were produced using the hybridomas 17/-27.R7 (anti-Ia.m5), 13/18 (anti-Ia.m7), H116-22.R7 (anti-H-2.m1), 19-178 (anti-Lyt-2.2) (22) (the hybridomas were obtained from Dr. G. J. Hämmerling, German Cancer Research Center, Heidelberg, Federal Republic of Germany), and C3PO (anti-Lyt-1.2, produced in this laboratory). Anti-I-J^k alloantiserum was purchased from Cedarline, Hornby, Ontario, Canada.

Cell Preparation. Single cell suspensions prepared from the inguinal and paraortic lymph nodes were passed through nylon wool columns; ~95% of the effluent cells were Thy-1.2^+, as detected by indirect immunofluorescence. Glass-adherent cells from peritoneal washings served as APC (23).

Treatment of Cells with Antibody and Complement. Cells were treated with Lyt-specific antibodies and rabbit complement in a one-stage test, as described previously (24). Anti-I-J^k serum was used according to the producer's instructions.

Preparative, Nonlytic Selection of Lyt Subsets. Cells of the Lyt-1^+2^- subset were selected by incubation of T cells with Lyt-2-specific antibody at 0°C for 1 h, followed by incubation of cells on goat anti-mouse immunoglobulin (Ig) (Medac GmbH, Hamburg, Federal Republic of Germany)-coated plastic dishes at 4°C for 1 h (25). The nonadherent cells were >94% Lyt-1^+2^- (as assessed by indirect immunofluorescence with rat Lyt-specific monoclonal antibodies). The adherent cells were further selected by incubation with Lyt-1-specific antibody, followed by plating on fresh anti-mouse Ig-coated dishes. After this second plating, the adherent cells were ~98% Lyt-1^+2^+.

Cell Cultures. The culture medium was RPMI 1640 supplemented with 5% horse serum, antibiotics, L-glutamine, and 2-mercaptoethanol (26). Priming of T cell subsets in vitro was carried out by culturing 4×10^6 cells/ml of T cells with $1–2 \times 10^6$ cells/ml of syngeneic APC in the presence of 15 μg/ml LDH_B for 3 d, followed by incubation for 4 d without antigen. In some experiments, in vivo primed selected T cell subsets were restimulated in bulk culture with LDH_B and fresh APC.

Short-Term Incubation of Tse with Th or Tsi. Lyt-1^+2^+, J^+ cells that had been primed in vivo (8 d) and restimulated in vitro (3 d) or primed and restimulated in vitro (8 + 3 d) were mixed in culture medium with Lyt-1^+2^-, J^-, or Lyt-1^+2^- cells at ratios of 1:1 or 1:2 and incubated at 37°C for 6 h. After incubation, the cell subset to be assayed was selected by Lyt-2 antibody plus rabbit complement (C) (Lyt-1^+2^-) or by positive selection with Lyt-2 antibody (Lyt-1^+2^+).

Depletion of Alloreactive T Cells and Priming of T Cells with Antigen on Allogeneic APC. These were

performed as described previously (23). Briefly, splenic T cells were cultured with allogeneic peritoneal adherent cells, and, after 3 d, alloreactive cells were removed by treatment with 5-bromo-2′-deoxyuridine (BudR) and light. The surviving T cells were then incubated with LDH_B (15 μg/ml) and fresh allogeneic APC for 3 d and without antigen for a further 4 d.

The T Cell Proliferation Assay. This was performed as described previously (21). Monoclonal antibodies, when present, were included in the same volume (0.2 ml) of culture. Each experimental group was set up in 3 to 15 parallel cultures. The standard deviation rarely exceeded 10%. The results are expressed as Δ cpm, that is cpm in cultures with APC plus LDH_B minus cpm in cultures with APC, and stimulation index (SI), that is, cpm in cultures with APC plus LDH_B divided by cpm in cultures with APC. In experiments involving groups of different cell mixtures, Δ cpm and SI were determined for each individual cell mixture.

Results

The Ts Effector (Tse) Cell Is Lyt-1^+2^+, J^+. We have previously shown (16, 20) that primed T cells from nonresponder mice, when depleted of Lyt-2-bearing cells, can proliferate upon in vitro challenge with LDH_B. Thus, at least one T cell subset involved in the suppression of anti-LDH_B response expresses the Lyt-2 marker. To characterize this subset, we separated different Lyt-subsets from nonresponder (C3H) mice by nonlytic selection and tested their mixtures for proliferation in a secondary response. The results in Table I demonstrate that the proliferating cell has the Lyt-1^+2^-, J^- phenotype. The proliferation is strictly dependent on the presence of both LDH_B and APC and is restricted by the A^k molecule, as shown by the blocking of response with a monoclonal antibody (anti-Ia.m5) specific for this molecule. Addition of primed Lyt-1^+2^+ cells suppresses the response to background level. As was also shown previously (16, 17, 20), blocking of the E^k molecule with Ia.m7-specific antibody abolishes suppression. Thus, the cell that suppresses the proliferation of Lyt-1^+2^- cells belongs to the Lyt-1^+2^+ subset and requires the expression of E^k molecules for its function. The results in Table I also demonstrate that the Lyt-1^+2^+ Tse carries cell-surface J molecules. The Lyt-1^-2^+ subset, no matter whether J^+ or J^-, primed in

TABLE I
The Tse Is an Lyt-1^+2^+, J^+ Cell

Experiment	T cell subsets from nonresponder (suppressed) C3H mice		Secondary proliferative response in the presence of	
	Subset 1	Subset 2		
				Δ *cpm, SI*
1	Lyt-1^+2^-*	None	APC + LDH_B	29.410 (9.7)
	Lyt-1^+2^-	None	LDH_B	272 (1.1)
	Lyt-1^+2^-	None	APC + LDH_B + αIa.m5 (αA)	502 (1.2)
	Lyt-1^+2^-	Lyt-1^+2^+‡	APC + LDH_B	793 (3.3)
	Lyt-1^+2^-	Lyt-1^+2^+	APC + LDH_B + αIa.m7 (αE)	18.273 (53.2)
2	Lyt-1^+2^-, J^-§	None	APC + LDH_B	38.596 (21.2)
	Lyt-1^+2^-, J^-	Lyt-1^+2^+‖	APC + LDH_B	388 (1.2)
	Lyt-1^+2^-, J^-	Lyt-1^+2^+ (αJ^k + C-treated)	APC + LDH_B	43.365 (23.7)
	Lyt-1^+2^-, J^-	Lyt-1^+2^+ (C-treated)	APC + LDH_B	−316 (0.8)

* Primed in vitro after nonlytic selection with anti-Lyt-2.1 antibody.
‡ Primed in vitro after double positive selection with anti-Lyt-2.1 and anti-Lyt-1.1 antibodies.
§ In vivo primed cells selected by anti-Lyt-2.1 antibody and treated with anti-J^k + C before culture.
‖ Obtained by double positive selection of in vivo primed T cells.

vivo or in vitro, did not suppress the response (unpublished data). Thus, these results identify an Lyt-1^+2^+, J^+ cell that suppresses the proliferative anti-LDH_B response of an Lyt-1^+2^-, J^- (presumably Th) cell.

The Lyt-1^+2^+ Tse Is E Restricted in Both the Induction and the Effector Phase. We have repeatedly demonstrated that the coating of the E^k molecule with monoclonal antibodies prevents LDH_B-specific suppression from occurring (16–18, 20, and Table I). We have also shown (20) that priming of B10.A(2R) ($K^kA^kE^kD^b$) T cells with LDH_B presented on APC from the E^k-nonexpressor strain B10.A(4R) ($K^kA^kE^oD^b$) does not lead to suppression (20). Taken together, these data provide strong evidence that recognition of LDH_B in the context of E^k molecules on APC is required for the generation of suppression. It has remained to be established which cells (and at what stage of their development) require antigen presentation in the context of E^k molecules. This question was partially addressed by the experiments in Table I and fully explored by those in Table II. The latter data show that blocking of the E molecule with antibody, either during priming of the Lyt-1^+2^+ cells, or at their effector phase, abolishes suppression. Antibodies against class I (K) or the class II (A) molecules are ineffective. (The A-specific antibody, however, blocks the proliferation of Lyt-1^+2^- cells; see also Table I). Thus, it is the Lyt-1^+2^+ Tse that requires antigen presentation in the context of E^k molecules for both its induction and its effector function.

The Interaction between Tse and Th Cells Is Restricted by the A Region of the H-2 Complex. We found in preliminary experiments that a short-term (6 h) incubation of Th cells with Tse cells (followed by removal of Tse cells) is sufficient to abolish the proliferation of Th cells in a subsequent 3-d assay. We have chosen this technique to investigate the genetics of Th-Tse interaction because it eliminates complicating allogeneic effects. Thus, Tse (Lyt-1^+2^+) cells primed in vivo and restimulated in vitro were incubated for 6 h with Th (Lyt-1^+2^-, J^-) cells from different strains; the Tse cells were then killed with anti-Lyt-2 antibody and C, and the Th cells were tested for

TABLE II

The Lyt-1^+2^+ Tse Is E Restricted at Both the Induction and the Effector Phase

T cell subsets from nonresponder (suppressed) C3H mice		Secondary proliferative response in the presence of	
Subset 1*	Subset 2‡		
			Δ *cpm, SI*
Lyt-1^+2^- primed in vivo	None	APC + LDH_B	18.675 (12.8)
None	Lyt-1^+2^+ primed in vitro	APC + LDH_B	84 (1.1)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro	APC + LDH_B	−134 (0.9)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro	APC + LDH_B + αIa.m7 (αE)	4.094 (4.9)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro in	APC + LDH_B	−184 (0.8)
Lyt-1^+2^- primed in vivo	the presence of αH-2.m1 (αK)	APC + LDH_B + αIa.m7 (αE)	9.331 (10.9)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro in	APC + LDH_B	−7 (1.0)
Lyt-1^+2^- primed in vivo	the presence of αIa.m5 (αA)	APC + LDH_B + αIa.m7 (αE)	14.294 (18.1)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro in	APC + LDH_B	8.000 (20.0)
Lyt-1^+2^- primed in vivo	the presence of αIa.m7 (αE)	APC + LDH_B + αIa.m5 (αA)	1.943 (5.6)

* Obtained by treatment with anti-Lyt-2.1 + C.

‡ Obtained by nonlytic double positive selection with anti-Lyt-2.1 and anti-Lyt-1.1 antibodies.

antigen-specific proliferation. The genetic mapping of Tse-Th cell interaction is summarized in Table III. The data demonstrate the following: first, the non-*H-2* background does not influence the Tse-Th cell interaction because C3H Tse cells suppress C3H, CBA, and B10.BR Th cells equally well. Similarly, B10.AL Tse cells suppress both B10.AL and A.AL Th cells. Second, the experiment with B10.AL Tse and Th cells from different strains excludes the involvement of the *K, C, S,* and *D* regions of *H-2* in the control of the Tse-Th cell interaction. Furthermore, the failure of B10.AL Tse cells to suppress B10.D2 Th cells indicates that sharing of genes in the *A, J,* or *E* regions is necessary for an effective Tse-Th cell interaction to occur. Third, the capability of B10.A(2R) Tse cells to suppress B10.A(4R) Th cells demonstrates that compatibility at the *A* region is sufficient for Tse-Th cell interaction. Collectively, these data map the genes that restrict Tse-Th cell interaction to the *A* region.

The Receptor of the Th Cell Determines the Restriction of Tse-Th interaction. Because the Th cell is A restricted, whereas the Tse cell is E restricted, the A restriction of the Tse-Th cell interaction is probably determined by the receptor of the Th cell. To test this hypothesis experimentally, we investigated the capacity of Tse cells to suppress Th cells that recognize LDH_B in the context of allogeneic MHC molecules. The latter cells can be generated by priming in vitro with antigen on allogeneic APC, after removal of alloreactive T cells with BudR and light treatment. The T cells thus generated are restricted by the allogeneic class II molecules of the APC (23). Using this system, we generated B10.A(2R) Th cells that recognize LDH_B on B10.S APC

TABLE III

The Tse-Th Cell Interaction Is Restricted by the A Region of H-2

Th(Lyt-1$^+$2$^-$, I-J$^-$) cells*								Proliferative response of Th cells after contact with ‡		
Strain	Alleles at *H-2* regions							Primed Lyt-1$^+$2$^+$* (Tse cells)	Unprimed Lyt-1$^+$2$^+$ (control Tse cells)	
	K	*A*	*J*	*E*	*C*	*S*	*D*			
									Δ *cpm, SI*	
								C3H	C3H	
C3H	*k*	*k*	*k*	*k*	*k*	*k*	*k*	22 (1.0)	7.300 (6.8)	6.069 (5.8)
CBA	*k*	*k*	*k*	*k*	*k*	*k*	*k*	332 (1.4)	7.507 (9.1)	9.005 (10.7)
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	1.696 (1.6)	8.776 (4.1)	9.885 (4.3)
								B10.AL	B10.AL	
B10.AL	*k*	*k*	*k*	*k*	*k*	*k*	*d*	−532 (0.8)	10.123 (3.3)	12.067 (4.0)
A.AL	*k*	*k*	*k*	*k*	*k*	*k*	*d*	−2.068 (0.6)	5.993 (2.1)	6.846 (2.2)
B10.A	*k*	*k*	*k*	*k*	*d*	*d*	*d*	1.023 (1.2)	10.698 (3.1)	9.430 (2.9)
A	*k*	*k*	*k*	*k*	*d*	*d*	*d*	−2.258 (0.6)	8.500 (2.5)	7.824 (2.3)
B10.A(2R)	*k*	*k*	*k*	*k*	*d*	*d*	*b*	−3.177 (0.4)	9.111 (2.7)	9.231 (2.7)
B10.TL	*s*	*k*	*k*	*k*	*k*	*k*	*d*	−1.468 (0.7)	9.107 (2.7)	11.766 (3.2)
B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	9.375 (3.9)	8.364 (3.7)	10.132 (3.5)
								B10.A(2R)	B10.A(2R)	
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	842 (1.4)	ND§	10.652 (6.5)

* Cells were primed in vivo with LDH_B and restimulated in vitro with LDH_B on syngeneic APC.

‡ Th cells were incubated with Tse cells for 6 h; Tse cells were then killed with anti-Lyt-2.1(C3H) or anti-Lyt-2.2 [B10.AL, B10.A(2R)] and C, and Th cells were tested for proliferative response to LDH_B on syngeneic APC in a 3-d assay.

§ Not done.

and B10.S Th cells recognizing LDH_B on B10.A(2R) APC (Table IV). Both cell populations were incubated with B10.A(2R) Tse cells (Lyt-1^+2^+) for 6 h, the Tse cells were then removed by anti-Lyt-2.2 and C treatment, and the Th cells were tested for antigen-specific proliferation. The results in Table IV demonstrate that both Th populations respond to LDH_B on the priming allogeneic APC but do not recognize the antigen on syngeneic APC. Furthermore, B10.A(2R) Tse cells suppress the response of B10.S Th cells to LDH_B on B10.A(2R) APC but do not affect the response of syngeneic B10.A(2R) Th cells to LDH_B on B10.S APC. Thus, allogeneic Th cells restricted by the MHC halplotype of Tse are suppressed, whereas the response of Th cells syngeneic to the Tse but restricted by allogeneic MHC are not affected. These results demonstrate that the receptor and not the MHC haplotype of the Th cells determines their capability to interact with Tse cells. The most straightforward (although not the only possible) interpretation of these data is that the Th cells recognize the Tse cells, and not vice versa.

The Lyt-1^+2^+, J^+ Tse Requires a Nonspecific Lyt-1^+2^-, J^+ Ts-inducer (Tsi) Cell. Thus far, the results have demonstrated that the Lyt-1^+2^+ Tse can be primed with LDH_B presented by E^k-expressing APC in the absence of other T cell sets. This cell, therefore, seems to be more autonomous than the Tse of other suppressor systems, which require a series of inductive cell interactions to become activated (3). We have, however, not ruled out the possibility that after antigen priming, the Tse receives an additional signal provided by the Lyt-1^+2^- cells in secondary cultures. To investigate this possibility, we compared the ability of Tse cells to suppress Lyt-1^+2^- and Lyt-1^+2^-, J^- (treated with anti-J^k + C) cells, respectively. As seen in Table V (and also in Table I), in vivo primed Tse are capable of suppressing the response of Lyt-1^+2^- and of Lyt-1^+2^-, J^- cells equally well. In contrast, in vitro primed Lyt-1^+2^+ cells exert their suppressor function only in the presence of Lyt-1^+2^-, J^+ cells (Table V). Thus, to become functional, in vitro primed Tse requires a second signal provided by an Lyt-1^+2^-, J^+ Tsi cell. Because Tse from immunized mice can suppress Th in the absence of Tsi, it is likely that in this case the Tsi-Tse interaction occurred in vivo.

TABLE IV

The Restriction Specificity of Th Cells Determines the Interaction between Th and Tse Cells

In vitro priming of Th (Lyt-1^+2^-) cells*		Incubation of Th with B10.A(2R) Tse (Lyt-1^+2^+) cells‡	Secondary proliferative response of Th cells to LDH_B§		
Th cells from strain	APC from strain		APC from strain	Δ cpm	SI
B10.A(2R)	B10.S	−	B10.S	14.362	3.7
B10.A(2R)	B10.S	−	B10.A(2R)	209	1.0
B10.A(2R)	B10.S	+	B10.S	11.762	3.2
B10.S	B10.A(2R)	−	B10.A(2R)	10.133	5.4
B10.S	B10.A(2R)	−	B10.S	313	1.1
B10.S	B10.A(2R)	+	B10.A(2R)	1.297	1.6
B10.S	B10.A(2R)	+	B10.S	−180	0.9

* Th cells were depleted of alloreactive cells by BudR and light treatment and were then primed with LDH_B on allogeneic APC for 7 d.

‡ Th cells were incubated with in vivo primed irradiated Tse cells for 6 h. The Tse cells were then killed with anti-Lyt-2.2 and C, and Th cells were tested for secondary proliferation.

§ Response of 1×10^5 Th cells to LDH_B on 1×10^5 APC in a 3-d assay. Background cpm values for allogeneic APC were 5.692 (B10.S) and 2.293 B10.A(2R).

TABLE V

Requirement for a Ts-inducer Cell

T cell subsets from nonresponder (suppressed) C3H mice		Secondary proliferative response to APC + LDH_B
Subset 1*	Subset 2‡	
		Δ *cpm, SI*
Lyt-1^+2^- primed in vivo	None	59.105 (20.8)
Lyt-1^+2^-, J^- primed in vivo	None	39.235 (12.5)
None	Lyt-1^+2^+ primed in vivo	−1.218 (0.8)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vivo	1.709 (1.4)
Lyt-1^+2^-, J^- primed in vivo	Lyt-1^+2^+ primed in vivo	6.139 (2.9)
Lyt-1^+2^- primed in vivo	None	10.323 (14.8)
Lyt-1^+2^- primed in vivo	Lyt-1^+2^+ primed in vitro	406 (1.4)
Lyt-1^+2^-, J^- primed in vivo	Lyt-1^+2^+ primed in vitro	8.600 (10.6)

* See footnotes to Table II.

TABLE VI

The Tsi Cells Need Not Be Antigen Specific

T cell subsets from nonresponder (suppressed) B10.A mice*			Secondary proliferative response to LDH_B + APC
Tsi‡	Tse§	Th‡	
			Δ *cpm, SI*
None	None	Lyt-1^+2^-, I-J^-	4594 (8.7)
None	Lyt-1^+2^+ primed in vivo	None	53 (1.1)
None	Lyt-1^+2^+ primed in vivo	Lyt-1^+2^-, I-J^-	626 (2.1)
None	Lyt-1^+2^+ primed in vitro	Lyt-1^+2^-, I-J^-	5027 (9.4)
Lyt-1^+2^- LDH_B-primed	None	Lyt-1^+2^-, I-J^-	5647 (10.4)
Lyt-1^+2^- LDH_B-primed	Lyt-1^+2^+ unprimed	Lyt-1^+2^-, I-J^-	4935 (9.2)
Lyt-1^+2^- GA-primed	Lyt-1^+2^+ unprimed	Lyt-1^+2^-, I-J^-	3497 (6.8)
Lyt-1^+2^- LDH_B-primed	Lyt-1^+2^+ primed in vitro	Lyt-1^+2^-, I-J^-	430 (1.7)
Lyt-1^+2^- GA-primed	Lyt-1^+2^+ primed in vitro	Lyt-1^+2^-, I-J^-	499 (1.8)
Lyt-1^+2^- unprimed	Lyt-1^+2^+ primed in vitro	Lyt-1^+2^-, I-J^-	374 (1.6)

* Tse (obtained by double positive selection) were first induced by incubation with Tsi for 6 h, then positively selected using anti-Lyt-2.2 antibody, and incubated with Th for another 6 h. After the second incubation Tse were killed with anti-Lyt-2.2 + C and Th were tested for proliferation in a 3-d assay.
‡ Primed in vivo with LDH_B, unless indicated otherwise. Obtained by treatment with anti-Lyt-2.2 + C.
§ Primed with LDH_B unless indicated otherwise.

We investigated whether antigen is required for the activation of the Lyt-1^+2^-, J^+ Tsi cells. The results in Table VI show that Lyt-1^+2^- cells from nonimmunized mice or from mice immunized with LDH_B or GA serve equally well as inducers of the LDH_B-specific Tse. Thus, the Tsi cells in the LDH_B system appear to be nonspecific.

Absence of Genetic Restriction in the Interaction between Tsi and Tse Cells. The short-term cell-mixing protocol applied for the studies of Th-Tse interaction (Table III) was also used to establish the genetic requirements for interaction between Tsi and Tse cells (Table VII). Lyt-1^+2^+ Tse cells from B10.AL mice were primed in vitro and incubated for 6 h with Lyt-1^+2^- (Tsi) cells from different strains. The Tse cells were then positively selected with Lyt-2.2 antibody and incubated for a further 6 h with primed syngeneic Th (Lyt-1^+2^-, J^-) cells. After the second incubation, the Tse cells were killed with anti-Lyt-2.2 antibody and C, and the Th cells were tested for antigen-specific proliferation in a 3-d assay. The data in Table VII demonstrate that the Tsi-

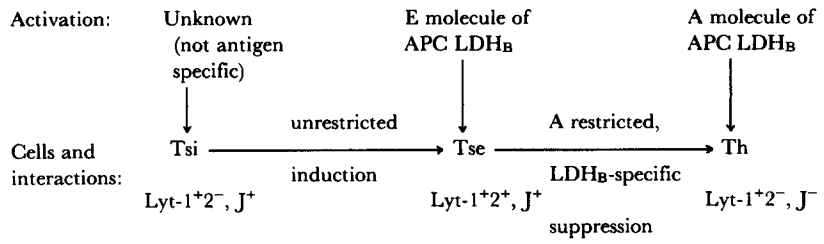

FIG. 1. The LDH_B suppressor pathway.

Tse cell interaction is independent of both the *H-2* complex and the non-*H-2* background; in other words, the interaction is unrestricted.

Discussion

Based on the data in this communication, the LDH_B suppressor pathway can be summarized, as shown in Fig. 1. At first glance, this pathway appears to be different from other suppressor pathways described thus far, first, in its simplicity, second, in that the antigen must be presented to the Tse cell in the context of E molecules, and third, in the involvement of a nonspecific Tsi cell. However, a closer analysis of the known suppressor pathways suggests that these differences may be more seeming than real.

With regard to the first difference, we would like to point out that the suppressor pathways involved in the regulation of antibody responses to SRBC (1), poly($Glu^{60}Ala^{30}Tyr^{10}$) (GAT), poly($Glu^{50}Tyr^{50}$) (GT) (2, 3), and keyhole limpet he-

TABLE VII

Lack of Genetic Restriction in the Interaction between Tsi and Tse Cells

Tsi (Lyt-1+2−) cells*								Secondary proliferation of B10.AL Th cells (Lyt-1+2−, J−) after contract with‡	
Strain	Alleles at *H-2* regions							Primed B10.AL, Lyt-1+2+ cells§	Unprimed B10.AL, Lyt-1+2+ cells
	K	*A*	*J*	*E*	*C*	*S*	*D*	Δ *cpm, SI*	
B10.AL	*k*	*k*	*k*	*k*	*k*	*k*	*d*	−511 (0.4)	13.744 (17.1)
A.AL	*k*	*k*	*k*	*k*	*k*	*k*	*d*	−2 (1.0)	13.342 (24.0)
B10.A	*k*	*k*	*k*	*k*	*d*	*d*	*d*	−267 (0.7)	8.698 (15.1)
A	*k*	*k*	*k*	*k*	*d*	*d*	*d*	158 (1.4)	5.506 (14.1)
B10.TL	*s*	*k*	*k*	*k*	*k*	*k*	*d*	292 (1.4)	10.015 (13.0)
A.TL	*s*	*k*	*k*	*k*	*k*	*k*	*d*	−132 (0.8)	13.958 (21.1)
B10.TFR1	*s*	*k*	*k*	*k*	*k*	*k*	*f*	62 (1.1)	13.541 (13.8)
A.TFR1	*s*	*k*	*k*	*k*	*k*	*k*	*f*	527 (1.9)	12.751 (28.3)
B10.A(2R)	*k*	*k*	*k*	*k*	*d*	*d*	*b*	224 (1.3)	9.492 (12.2)
C3H	*k*	*k*	*k*	*k*	*k*	*k*	*k*	876 (1.8)	12.257 (14.1)
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	316 (1.4)	12.958 (15.0)

* Cells were primed in vivo with LDH_B.

‡ Tse cells were first induced by incubation with Tsi cells for 6 h, then positively selected and incubated with Th for another 6 h. After the second incubation, Tse cells were killed with anti-Lyt-2.2 and C, and Th cells were tested for proliferative response to LDH_B on APC in a 3-d assay.

§ Cells were primed in vitro with LDH_B on APC.

mocyanin (KLH) (26) actually consist, as does the LDH_B-specific pathway, of only two cells. Although in two pathways, the GAT-specific and the SRBC-specific ones, a third Lyt-1^-2^+ cell has been implicated, it has not been determined whether it is a distinct cell, or whether it has arisen from the second, Lyt-1^+2^+ cell, by a phenotype change. Thus, all known regulatory pathways of antibody responses appear to be bicellular, in contrast to the delayed-type hypersensitivity suppressor pathways that seem to involve three cells (2, 3).

The second seemingly unique feature of the LDH_B pathway is the requirement for the antigen to be presented to the Tse cells in the context of E molecules. It should be emphasized, however, that in all pathways, triggering of the second cell in the pathway (Ts_2) requires antigen (1, 26–28) in addition to the signal provided by Ts_1 (Tsi) cells. It is, therefore, conceivable that antigen presentation may play a role at the level of Ts_2 induction, although this aspect has not yet been explored in the other systems. In fact, we found another example for E-restricted suppression in the immune response to IgG_{2a} myeloma proteins (16). Furthermore, it is possible that the mechanism behind the control of anti-GT response by two complementing *Is* genes (29) is E-restricted suppression because the *Is*-gene complementation is very similar to that required for the cell-membrane expression of E molecules (30).

The third difference between the LDH_B pathway and other known suppressor pathways is that the Tsi cell is nonspecific in the former and apparently antigen-specific in the latter. However, a direct comparison of inducer cells in the pathways is hampered by the insufficiency of information on these cells. For example, the Ts_1 cells in the GAT, GT, and KLH systems have not yet been isolated. The antigen specificity of inducer cells in the GAT, GT, and SRBC systems is extrapolated from the specificity of inducer factors extracted from lymphoid cells of immunized mice (28, 31, 32). Control experiments to test the inducer activity of Tsi cells cultured with an unrelated antigen or without antigen have not been reported. These examples illustrate the prematurity of attempts at establishing similarities or differences between different suppressor pathways.

Our data have also provided some information about the mechanisms of interactions between cells of the LDH_B suppressor pathway. The mechanism by which Tsi cells activate Tse cells has not yet been studied in detail. It is, however, likely that this activation occurs via a nonspecific factor, which may be similar to interleukin 2 (33). This assumption is based on the observations that, first, the Tsi cell does not require specific activation by antigen; second, it acts on Tse cells after the latter have been primed; and third, the action of Tsi on Tse cells is unrestricted. The interaction between Tse and Th cells is more difficult to visualize. This interaction is antigen specific (20; and C. Baxevanis, unpublished data) and MHC restricted (Table III). Because the interacting cells themselves are also antigen specific and MHC restricted, the interaction is not likely to be based on the recognition of idiotypic determinants. The key information for understanding the Tse-Th cell interaction is that the A restriction of this interaction is dictated by the receptor (anti-A^k) of the Th cells (Table IV). Thus, in terms of recognition, the active party is the Th and not the Tse cell. Considering also that the interaction is antigen specific and A restricted, its simplest mechanism would be a concomitant recognition of LDH_B and *A* region-controlled molecules by Th cells on the surface of Tse cells. This recognition would trigger a suppressor mechanism, for example, the production of a short-range sup-

pressor factor, that inactivates the Th cell. The mechanism outlined here requires, first, the recognition by Th and Tse of two different epitopes on the LDH_B molecule (that is, formation of an antigen bridge), and second, the expression of A molecules by Tse cells. We are presently investigating whether these two requirements are met.

Summary

We characterized the cell types involved in the *H-2*-controlled suppression of T cell response to lactate dehydrogenase B (LDH_B). The suppressor effector (Tse) was found to be an Lyt-1^+2^+, J^+ cell that recognizes antigen together with E^k molecules of antigen-presenting cells (APC). To become functional, the Tse cell requires a second signal from a nonspecific, Lyt-1^+2^-, J^+ suppressor-inducer (Tsi) cell. The Tsi-Tse interaction is not subject to any genetic restriction. The target cell of suppression is an Lyt-1^+2^-, J^- (most likely T helper [Th]) cell that recognizes LDH_B in the context of A molecules on APC. The suppression is manifested in inhibition of the antigen-specific, A-restricted proliferation of Th cells. The interaction between Tse and Th is restricted by the *A* region of the *H-2* complex. Because this restriction is determined by the receptor of Th cells, the mechanism of Th-Tse interaction most likely involves a concomitant recognition of LDH_B and *A* region-controlled molecules by Th cells on the surface of Tse cells.

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