HOMOLOGIES BETWEEN CELL INTERACTION MOLECULES CONTROLLED BY MAJOR HISTOCOMPATIBILITY COMPLEX- AND Igh-V-LINKED GENES THAT T CELLS USE FOR COMMUNICATION

Tandem "Adaptive" Differentiation of Producer and Acceptor Cells*

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Cells of the immune system often communicate with one another by the use of polymorphic gene products that map to the major histocompatibility complex $(MHC)^1$ and;shor to the immunoglobulin heavy chain complex (Igh). We have described an I-J$^+$ Ly-1 cell that produces an antigen-specific molecule(s) (Ly-1 TsiF) that only induces suppression in cells that share an Igh variable region (Igh-V)-linked polymorphism (1) and described an antigen-specific I-J$^-$ Ly-2 cell that produces a molecule(s) (Ly-2 TsF) that only suppresses cells that express the same MHC polymorphisms as the producer cell (2). These apparent genetic restrictions are not governed by a limited capacity to express "self" recognition units for only those polymorphisms that are encoded within the genome of the cells that make the factors. Rather, if T cell precursors encounter a genetically foreign polymorphism during their differentiation in the thymus, then they can subsequently collaborate perfectly well with cells that express that foreign genetic polymorphism.[2] Thus, the cells that produce the factors described above have the capacity to appropriately interact with any acceptor cell so long as the acceptor cell's relevant genetic polymorphism is present in the thymus where the T cells are differentiating.

Thus, there is an element in the thymus that selects and expands a T cell repertoire

capable of recognizing, as self, polymorphic gene products expressed in the thymus. Consequently, the suppressor-inducer and suppressor-effector molecules derived from parent A into F_1 radiation chimeras and homozygous A nude mice engrafted with F_1 thymus must be different from the suppressor and inducer molecules derived from homozygous A mice, because only in the former cases can the factors react appropriately with cells of the alternate parent in the F_1 cross, even though the molecules retain the genetic markers (i.e., I-J in the case of the Ly-1 TsiF) of the producer cell.

Because the presence of a particular genetic polymorphism during the T cell's differentiation causes the T cell to express a novel repertoire for recognizing self, we wondered whether during its differentiation, the cell on which the molecules acted would see the change in the producer cell's product and consequently "learn" to accept signals from the alternate parent in the F_1 cross. We have found that such is the case; the acceptor cells for both T cell products (Ly-1 TsiF and Ly-2 TsF) undergo tandem "adaptive" differentiation with the producer cells. Thus, not only can genetically determined structures in the thymus select a novel self repertoire from genetically different cells, but additionally, the presence of the expanded repertoire can act to select different acceptor cell specificities.

These findings indicate that during differentiation the cells that are signalled by biologically active T cell products must have the capacity to choose the specificity of the receptors they use to interact with these regulatory molecules (as opposed to antigen per se). In essence, this means that receptors for regulatory molecules not only are seen by those molecules, but that the acceptor cell's receptor sees the inducer molecule as well. Therefore, both the regulatory factors and molecules that act as receptors on the acceptor cells must be composed of constant as well as variable region gene products that can be selected for and expanded during differentiation. Such plasticity of receptors for biologically active cell products may be a unique feature of the immune system, although students of the nervous system may argue this point (3). Certainly the results presented are consistent with the contentions of Katz et al. (4), who have suggested that B cells (which are the acceptor cells in his system) can also learn to recognize genetically different helper T cell products. Thus, the tandem adaptive differentiation of producer cell and its acceptor cell's receptor could be a generalized immunological phenomenon.

Materials and Methods

Animals. Adult BALB/c, B6, BALB/c × C57BL/6 (CB6F$_1$), B6 × A (B6AF$_1$), and AKR mice, as well as B6 impregnated BALB/c mice were obtained from The Jackson Laboratory, Bar Harbor, ME. Adult BALB/c *nu/nu* mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. C.B20 mice were from a breeding colony at Yale University.

Antisera. Congenic anti-Ly-1 and anti-Ly-2 as well as monoclonal anti-Ly-1 and -2 reagents were prepared and used as described previously (1, 2). (We thank F. W. Shen of the Memorial Sloan-Kettering Cancer Center, who made these reagents available to us.) Briefly, 10^7 cells/ml in an appropriately diluted antisera were incubated for 45 min on ice, followed by an incubation with rabbit complement (1:5) for 45 min at 37°C. Rabbit complement used in these experiments was serum from animals selected for low natural cytotoxicity to mouse spleen cells, and was subsequently absorbed with 80 mg of agar/ml of serum. Anti-I-J^b serum (kindly supplied by Dr. D. B. Murphy of Yale University School of Medicine) was prepared by hyperimmunizing B10.A(5R) recipients with a mixture of B10.A(3R) spleen and lymph node cells (antiserum ASM-20). Dr. Murphy also supplied two different anti-H-2D^d monoclones (ASM 29 and ASM 31) and Ethan Lerner (Yale University School of Medicine) kindly supplied us with an anti-

H-2K^b monoclone (Y-3).

Antigens.   Sheep erythrocytes (SRBC) were obtained from Colorado Serum Co., Denver, CO.

Preparation of Lyt-1-derived Suppressor-Inducer (Ly-1 TsiF) and the Lyt-2-derived Suppressor-Effector (Ly-2 TsF) Materials.   Preparation of Ly-1 TsiF and Ly-2 TsF has been previously described (1, 2). Briefly, a suspension of spleen cells from mice hyperimmunized with SRBC was treated with anti-Lyt-2 (for Ly-1 TsiF) or anti-Lyt-1 (for Ly-2 TsF) and rabbit complement, and subsequently cultivated in vitro for 48 h at a concentration of 10^7 cells/ml. After 48 h, supernatant fluids were cleared and passed through millipore filters.

In Vitro Primary Anti-SRBC Response.   Anti-SRBC responses were generated in vitro using a modification (5) of a cell culture technique described initially by Mishell and Dutton. Briefly, spleen cells were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 mM glutamine, 25 mM Hepes, and 5×10^{-5} M 2-mercaptoethanol. Subsequently, 1 ml of the cell suspension containing 10^7 cells was cultured with 0.05 ml of a 1% SRBC suspension in plates (3008; Falcon Labware, Oxnard, CA) in a 5% CO_2-95% air incubator at 37°C for 5 d. The number of plaque-forming cells (PFC) was determined by using the Cunningham modification (6) of the Jerne-Nordin plaque assay as previously described. Results are given as the mean of three individual values of each culture condition. Ly-1 TsiF and Ly-2 TsF were added at a final concentration of 1:10 on day 0 of culture.

Radiation Bone Marrow Chimeras.   Chimeras are designated as bone marrow donor into irradiated recipients. Recipient CB6F$_1$ mice were irradiated with 950 rad cesium gamma irradiation and were reconstituted with 1.5×10^7 bone marrow cells that had been depleted of T cells by pretreatment with rabbit anti-mouse brain serum and complement as previously described (7). Spleen cells from such chimeras were obtained no earlier than 2 mo post-irradiation and bone marrow reconstitution. Immunofluorescent typing of spleen cells from such chimeras with H-2-specific reagents consistently demonstrated that they are of bone marrow donor origin without detectable (<5%) cells of host origin (7).

Thymus-engrafted Nude Mice.   Thymus grafting of BALB/c nude mice was performed by subcutaneously implanting three individual thymic lobes obtained from donor CB6F$_1$ mice <24 h old. Thymocytes and spleen cells from such mice have been extensively typed by flow microfluorometry on a fluorescence-activated cell sorter and consistently demonstrate that each organ is fully repopulated with cells of BALB/c nude host origin with no lymphocytes of F$_1$ thymus origin detected (8).

Results

Cells from Parent into F$_1$ Chimeras Can Serve as Targets for Factors Made by Either Parent in the F$_1$ Cross (Table I).   The results in Table I demonstrate the ability of various T suppressor factors to inhibit the response of cells from parent (BALB/c or B6) into BALB/c × B6 F$_1$ (CB6) chimeras. The cells from the chimeric mice were suppressed by the Ly-1 TsiF and Ly-2 TsF made by the reciprocal parents cells to the same extent as they were suppressed by the factors made by the homologous parent. (Note also that Ly-1 TsiF and Ly-2 TsF from C.B20 mice suppressed both chimeras, indicating that the Igh restriction [C.B20 vs. BALB/c] and the H-2 restriction [C.B20 vs. B6] that the acceptor cells usually display [1–3] can be overcome during differentiation, even when nonparental factors are tested.) The inability of AKR factors, which were highly suppressive when tested on AKR cells, to affect the response of the chimeras spleen cells shows that both the Igh-V and the H-2 restrictions remained for third-party cells.

Cells from BALB/c Nude Mice that Have Undergone Differentiation in an F$_1$ Thymus Can Serve as Targets for Factors Made by Either Parent in the F$_1$ Cross (Table II).   The results in Table II demonstrate that nude mice with a thymus graft from a CB6 F$_1$ mouse can (*a*) be induced to express suppressive activity or (*b*) actually have their response

TABLE I

Target Cells for Suppressive Molecules from Parent into F_1 Chimeras Acquire the Ability to Receive Signals from the Reciprocal Parent in the F_1 Cross

Cellular source of factor		Strain of mice on which the factors were tested		
		B6 into CB6 F1		BALB/c into CB6 F_1*
		Experiment I	Experiment II	
		PFC/culture		
Control	—	2,000	750	650
Ly-1 TsiF	B6 Ly-1 cells	800 (60)‡	150 (80)	200 (69)
	BALB/c Ly-1 cells	300 (85)	300 (60)	200 (69)
	C.B20 Ly-1 cells	ND§	200 (73)	350 (46)
	AKR Ly-1 cells	2,100 (0)	ND	ND
Ly-2 TsF	B6 Ly-2 cells	900 (55)	200 (73)	300 (54)
	BALB/c Ly-2 cells	600 (70)	ND	ND
	C.B20 Ly-2 cells	ND	200 (73)	250 (62)
	AKR Ly-2 cells	2,400 (0)	ND	ND

* For demonstration of restrictions expressed by parental acceptor cells see Tables II and III.
‡ Numbers in parentheses represent percent suppression of PFC response.
§ Not determined.

TABLE II

Target Cells for Suppressive Molecules from Parent into BALB/c Nude Mice with F_1 Thymus Grafts

Cellular source of factor	Strain of mice on which the factors were tested		
	B6	BALB/c	BALB/c *nu/nu* with CB6 F_1 thymus graft
	PFC/culture		
—	1,400	800	3,500
B6 Ly-1	500 (64)*	600 (25)	500 (86)
BALB/c Ly-1	1,800 (0)	50 (94)	1,000 (71)
AKR Ly-1	1,600 (0)	900 (0)	3,200 (9)
B6 Ly-2	500 (64)	700 (12)	200 (94)
BALB/c Ly-2	1,400 (0)	100 (87)	400 (89)
AKR Ly-2	1,200 (14)	800 (0)	3,300 (6)

* See ‡; Table I.

suppressed by B6 Ly-1 TsiF and Ly-2 TsF, respectively, although the only cells that expressed B6 gene products that these mice had seen previously were those present in the F_1 thymus graft. Thus, parent into F_1 bone marrow chimeras and nude mice with F_1 thymus grafts show similar changes in receptor specificity on their acceptor cells for the parental regulatory factors.

The Acquisition of New Receptor Specificities during Adaptive Differentiation Is Not Caused

TABLE III

Acceptor Cells from Chimeric Mice that React with Suppressive Products of Genotypically Different Cells Are Not Affected by Treatment with Antisera to the H-2 Haplotype of the Producer Cell*

| Cellular source of TsiF and TsF | Strain of mice on which factors were assayed | | | |
	B6	BALB/c	B6 into CB6F$_1$	BALB/c nu/nu with CB6F1 thymus graft
			PFC/culture	
None	3,200	900	2,100	2,300
B6 Ly-1	200 (94)	700 (22)	300 (86)	100 (96)
BALB/c Ly-1	2,700 (16)	0 (100)	0 (100)	0 (100)
AKR Ly-1	3,200 (0)	1,000 (0)	1,400 (33)	2,200 (4)
B6 Ly-2	400 (87)	900 (0)	100 (95)	100 (96)
BALB/c Ly-2	2,800 (12)	100 (89)	500 (76)	200 (90)
AKR Ly-2	3,300 (0)	1,000 (0)	1,800 (14)	2,300 (0)

* All cells from BALB/c and BALB/c *nu/nu* mice were treated with an anti-H-2^b antisera, whereas all cells from C57BL/6 into CB6F$_1$ chimeric mice were treated with an anti-H-2^d antiserum. Treatment with these antisera of homozygous cells that expressed these genotypic H-2 determinants resulted in >99% killing of target cells in all cases.

‡ See note to Table I.

by Contamination of the Chimeric Mice with Cells that Express the Parental Haplotype that is Presumed Missing (Table III). Although the procedures used to make the various chimeras under study have been continually monitored to show that >95% of the hematopoietic cells from the radiation chimeras express the bone marrow donor haplotype (7) and that viable T cells from the F$_1$ thymus graft cannot be detected in either the engrafted thymus or spleen of the BALB/c nude host (8), we felt it was important to demonstrate that the loss of receptor specificity in the mice under study was truly occurring in cells that had the wrong genetic haplotype. In previous studies of inducer factors (3), we have been able to show that the I-J marker on the adaptively differentiated Ly-1 TsiF expresses the haplotype of the bone marrow donors or the BALB/c nude mouse.[2] In the present set of experiments dealing with charges in receptor specificity, it is almost impossible to conceive that a small contamination of T cells with the wrong haplotype could account for the potent direct suppression by the Ly-2 TsF. However, a small number of these contaminating cells could, at least in theory, be responsible for the suppression induced by the Ly-1 TsiF. In any case, to rule out contamination, we performed a series of studies where the factors were tested on cells of chimeric mice, in which the T cells had undergone adaptive differentiation, by treating the assay cells with anti-H-2 sera directed to the haplotype of the homozygous mouse that produced the regulatory factors. The results in Table III show that removal of any possible contaminating cell type in the assay population having the same H-2 markers as the mice that made the factors does not affect the activity of the various biologically active molecules. Thus, our results clearly demon-

strate induced changes in the acceptor cell population, and are not caused by artifacts created by contaminating F_1 host cells.

Discussion

The results show that during differentiation of T cells, parallel changes take place in both the parental producer cell and the receptor for its product on its acceptor cell. Thus, not only do producer cells learn to recognize new determinants on the target cells, but the target cells themselves learn to accept otherwise incompatible factors. Because this tandem change in regulatory molecules and their receptor specificities takes place in nude mice with F_1 thymus grafts, it can be presumed that the thymus exerts a key influence that governs their adaptive differentiation.

However, because this type of tandem differentiation probably requires two separate selecting elements, it is not necessary to postulate that both selecting elements are expressed by the thymus or even take place in the thymus. Thus, the thymus might have the relevant structure that selects the self specificity of the producer cells, but it can be the self specificity expressed by the producer cell that in turn influences differentiation and specificity of the acceptor cell. Although both selectional events could occur within the thymus, our findings only show that both selectional events (adaptive differentiation of the producer and acceptor cells) are dependent on the thymus. The second selectional event (adaptive differentiation of the acceptor cells) is likely to be a consequence of adaptive differentiation of the producer cell. Thus, it is possible that adaptive differentiation of the acceptor cell can occur after the cells have left the thymus. In either case, our findings of tandem differentiation of biologically active molecules with the receptors for them on acceptor cells in two separate situations confirm and solidify the notion of adaptive differentiation of "partner cell sets" that has been put forth by Katz and colleagues (4).

Actually, it is possible that the differentiation of immunological partner cells may not be confined to ontogeny, but may also occur during the course of an immune response. It has been shown in at least two instances that interactions between inducer and acceptor cell that express different polymorphisms at Igh-V lead to the development in the acceptor cell population of cells and molecules that will only interact in a functional way with cells expressing Igh-V polymorphisms of the mismatched inducer cell in the circuit (9, 10). They will not work on cells that are genomically identical. Thus, these are examples where inducer cells can signal acceptor cells, but when they do so, the acceptor cells differentiate in a way that confines the continuation of the conversation to cells that express the Igh-V gene product of the cell that started the cellular conversation. This type of sequential differentiation or induction has been called a "pseudorestriction" (11). If it turns out that this pseudorestriction occurs by the same mechanism as we have shown to occur during ontogeny, it might be more appropriate to call this type of event an induced restriction, as it is likely to reflect a physiological event and not be solely caused by experimental manipulation.

At present, we have only studied two pairs of inducer and acceptor cells. However, it would be interesting to find out whether, in immunological regulatory circuits where more than two cells are involved, these selectional events that have changed the receptor on the acceptor cell for product "A" would now produce a selecting molecule (product "B") for the cell on which the acceptor cell exerts its influence. A teleological explanation for such receptor plasticity could be offered. If a mutation

were to occur in somatic regulatory cells that altered the variable region product of those cells, the subsequent change in receptor specificity would help mitigate possible deleterious effects on the mutation. Also, this type of plasticity would give the immune system some learning capabilities that are presently thought to be the sole province of the nervous system (3).

In sum, the original finding that molecules bearing MHC markers can adaptively differentiate in the thymus and as a result recognize new Igh-V-linked controlled polymorphisms was a bit surprising.[2] This work implied that the MHC- and Igh-V-recognizing molecule had a constant region that could be identified with the appropriate anti-I-J serum and a variable region that was selected for, and could be identified by determining what types of cells the factors would work on. The present work is more surprising because it implies that the receptors for biologically active T cell communication molecules also have constant and variable region gene products, and that the receptors that express appropriate variable gene products are selected during differentiation (and perhaps also during the course of an immune response).

Summary

We have asked the question: how do partner cells in immunoregulatory interactions between T cell subsets acquire the ability to recognize and react appropriately with one another? In particular, we have asked whether these communication events are completely determined by the cell's genetic constitution, or whether the recognition events can be learned during ontogeny. We have found that the T cells of parent into F_1 chimeras and homozygous nude mice with F_1 thymus grafts not only learn to react with genetically disparate acceptor cells, but that the receptors on the acceptor cells themselves learn to react with genetically disparate producer cells. This learning process can overcome both major histocompatibility complex- and immunoglobulin heavy chain variable region-linked restricted communication between T cell subsets. We interpret these findings to indicate that thymic elements can start a cascade of differentiational events. The thymic elements, whether endogenous or passively acquired, select from a pool of producer cells those that will react appropriately with the thymic selecting cells, and these cells become expanded. Then, the private markers (idiotype) on the expanded pool of producer cells act as selecting and expanding elements that choose from a pool of acceptor cells those that recognize the producer cells idiotype as self. This second differentiational event, although apparently thymus dependent, need not take place in the thymus itself. There is some circumstantial evidence that this type of acceptor cell differentiation could also take place during the course of an immune response.

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