Brief Definitive Report

SELF RECOGNITION IN ALLOGENEIC THYMIC CHIMERAS

Self Recognition by T Helper Cells from Thymus-engrafted Nude Mice is Restricted to the Thymic H-2 Haplotype

By ALFRED SINGER, KAREN S. HATHCOCK, and RICHARD J. HODES

From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Most T cells recognize conventional antigens only in the context of self determinants encoded within the major histocompatibility complex (MHC). Recently, it was shown (1) that the MHC determinants that T helper (T_H) cells recognize as self structures for responses to conventional antigens were entirely determined by the host environment in which the T_H cells had differentiated, even if the host differentiation environment was fully allogeneic to the T_H cells themselves. Thus, strain A T_H cells from A → B fully allogeneic radiation bone marrow chimeras recognized conventional antigens in the context of host type strain B MHC determinants but did not recognize conventional antigens in the context of syngeneic donor type strain A MHC determinants.

The present study was undertaken to evaluate the possibility that the thymus was the specific host element responsible for the restricted self-recognition repertoire expressed by T_H cells. The T_H cell populations examined in this study were obtained from the spleens of congenitally athymic nude mice that had been engrafted with neonatal thymic lobes that were allogeneic to the nude host. The self-specificity of these T_H cells was determined by their ability to cooperate with either syngeneic (nude host type) or allogeneic (thymus type) B+ accessory cell populations for responses to either trinitrophenyl keyhole limpet hemocyanin (TNP-KLH) or to sheep erythrocytes (SRBC). To avoid alterations in the T_H cell repertoire of thymic chimeras induced by in vivo priming to antigen in association with the presenting cells resident in these animals, all the cell populations used in this study were from unprimed animals.

Materials and Methods

Animals. Pregnant C57BL/6 (B6), A/J, and C3H/HeJ mice as well as normal adult B10, B10.A, B10.D2, B10.BR, and (B6 × DBA/2)F_1 (B6D2F_1) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c *nu/nu* mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass.

Antigens. TNP-KLH was prepared as previously described (2). SRBC were obtained from a single sheep, 1245, National Institutes of Health Animal Center, Poolesville, Md.

Thymus Transplantation. Three thymic lobes from neonatal mice <24 h old were subcutaneously implanted into each BALB/c nude recipient animal. These mice were rested for 8–12 wk after transplantation before use. Spleen cells as well as thymocytes from these mice were typed with strain-specific reagents on the fluorescence-activated cell sorter (FACS), confirming that all cells were indeed of nude host origin (3).

TABLE I

Spleen Cells from Thymus-engrafted Nude Mice are Specifically Tolerant to Both the Nude Host and Thymic H-2 Haplotype

Responder spleen cells		Stimulation index*			
Nude host	Engrafted thymus	B10	B10.A	B10.D2	B10.BR
BALB/c	A/J	3.7 ± 0.1‡	<1	<1	ND§
BALB/c	B6	<1	ND	<1	1.6 ± 0.15‡
BALB/c	C3H	2.2 ± 0.1‡	ND	<1	<1
BALB/c	—	1.0 ± 0.1	<1	1.0 ± 0.1	1.0 ± 0.1
Normal controls					
B10		1.0 ± 0.3	ND	2.6 ± 0.2‡	4.8 ± 1.1‡
B10.A		8.0 ± 0.9‡	<1	4.7 ± 0.5‡	ND
B10.D2		4.2 ± 0.4‡	3.6 ± 0.3‡	<1	8.9 ± 1.3‡
B10.BR		13.6 ± 0.6‡	ND	6.5 ± 0.9‡	<1

* Experimental cpm/medium cpm for the stimulating spleen cell populations indicated.
‡ Significantly >1 ($P < 0.05$) by two-tailed Student's t test.
§ Not determined.

Preparation of Cells and Culture Conditions. T cells were prepared by passage of spleen cells over nylon fiber columns and collection of the nylon nonadherent (NNA) eluate. To eliminate K^k-bearing T cells, T cells were treated with the culture supernatant of hybridoma 11-4.1 (anti-K^k) plus complement (C), as previously described (1). Cells that are designated as B+ accessory (B+Acc) cells were prepared by depleting spleen cells of T cells by pretreatment with a monoclonal anti-Thy-1.2 reagent plus C. All cultures were performed in microtiter plates, as previously described (2). The number of B+Acc cells in culture was the optimum number for that response and was 4×10^5 cells for responses to TNP-KLH and 8×10^5 cells for responses to SRBC. Cultures stimulated with TNP-KLH were assayed for anti-TNP PFC, whereas cultures stimulated with SRBC were assayed for anti-SRBC PFC. Each data point represents the geometric mean of triplicate cultures. 4-d mixed lymphocyte reactions (MLR) contained 4×10^5 responder spleen cells and 4×10^5 2,000 rad irradiated stimulator spleen cells and were pulsed with 1 μCi [^{3}H]thymidine 18 h before harvest.

Results and Discussion

Spleen T Cells from Thymus-engrafted Nude Mice Are Tolerant by MLR to Both Nude Host Type and Thymus Type MHC Determinants. BALB/c (H-2^d) congenitally athymic nude mice were engrafted with thymic lobes from A/J (H-2^a), B6 (H-2^b), or C3H (H-2^k) neonatal mice 8 wk before use. At the time of assay, no lymphocytes from the thymic donor were found by FACS analysis in either the spleens or engrafted thymuses of these mice. Before assessing the self-specificity of the T_H cells in these mice, their reactivity in MLR to both syngeneic and allogeneic MHC determinants was measured. The MLR was specifically selected as the most appropriate measure of tolerance in this study because MLR reactivity, like T_H cell function, is predominantly I-region specific. As can be seen in Table I, spleen T cells from thymus-engrafted mice were competent to react against third-party allogeneic stimulator cells but were specifically tolerant to both syngeneic (nude host type) and allogeneic (thymus type) stimulator cells. In contrast, spleen cells from unengrafted nude mice expressed no T cell activity at all.

T_H Cells from Nude Mice Engrafted with Fully Allogeneic Thymic Lobes Are Competent but Restricted to Cooperating with Thymus Type B+Acc Cells for the Generation of Primary PFC Responses. The self-specificity of unprimed T_H cells present in the spleens of thymus-engrafted nude mice was assayed by their ability to collaborate for the generation of

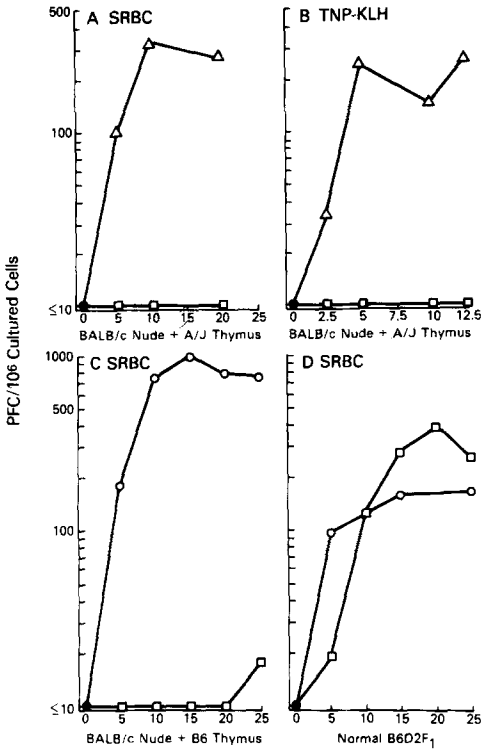

FIG. 1. Number of NNA spleen T cells added per culture × 10^{-4}. T_H cells from the spleens of allogeneic thymic chimeras only cooperate with B+Acc cells of the thymic haplotype. Graded numbers of spleen NNA T_H cells from the indicated allogeneic thymic chimeras were assayed for their ability to cooperate for the generation of primary responses to either SRBC or TNP-KLH with unprimed B+Acc cells of either the nude host or thymic haplotype. B+Acc cells: B10.D2 (□), B10.A (△), and B10 (○). Less than 10 PFC/10^6 cultured cells were obtained in the absence of antigen.

primary PFC responses with unprimed B+Acc cell populations, whose MHC determinants were either syngeneic to the nude host or to the engrafted thymus. As can be seen in Fig. 1, T_H cells from BALB/c nude mice engrafted with A/J thymus tissue only collaborated with B10.A (thymus type) B+Acc cells but not with B10.D2 (nude type) B+Acc cells for responses to either SRBC or TNP-KLH (Fig. 1 A, B). Similarly, T_H cells from BALB/c nude mice engrafted with B6 thymus only collaborated with B10 (thymus type) but not B10.D2 (nude type) B+Acc cells (Fig. 1 c). The failure of T_H cells from the thymic chimeras to collaborate with syngeneic B10.D2 B+Acc cells was not due to any incompetence of the B10.D2 B+Acc cell population because these cells were activated by "unrestricted" T cells from normal $B6D2F_1$ mice (Fig. 1 D). Thus, these results demonstrate that the self-MHC repertoire of unprimed T_H cells resident in the spleens of thymus-engrafted nude mice is specific for the MHC haplotype of the engrafted thymic lobes.

Even though cells of thymic origin were not detected in these thymic chimeras, an experiment was performed to rule out the possibility that the functionally competent and thymus-restricted T_H cells present in these animals were actually of thymus origin. T_H cells from BALB/c nude mice engrafted with C3H thymus cooperated only with $H\text{-}2^k$ (thymus type) B+Acc cells and not with $H\text{-}2^d$ (nude type) B+Acc cells

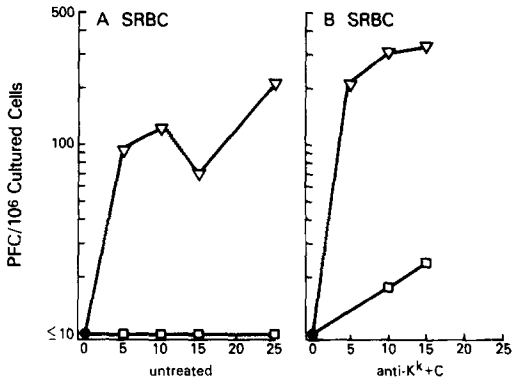

FIG. 2. Number NNA spleen T cells added per culture × 10^{-4}. Thymus-restricted T_H cells from the spleens of allogeneic thymic chimeras are not themselves of thymic origin. Graded numbers of either untreated (A) or anti-K^k + C (B) NNA T_H cells from BALB/c nude mice engrafted with C3H thymus were assayed for their ability to cooperate for primary responses to SRBC with B+Acc cells from either B10.BR (▽) or B10.D2 (□). Less than 10 PFC/10^6 cultured cells were observed in the absence of antigen.

(Fig. 2A). If the functionally competent and thymus-restricted T_H cells in these mice were in fact of thymus origin, they should be killed by pretreatment with monoclonal anti-K^k plus C. Therefore, in the same experiment, the T_H cell population obtained from these mice was also pretreated with monoclonal anti-K^k plus C and assayed for its ability to collaborate with the same B+Acc cell populations. As can be seen in Fig. 2B, pretreatment with anti-K^k plus C altered neither the helper cell function nor the self-specificity of the T cell population. The modest enhancement of immune responsiveness observed in the treated T cell population was observed by treatment with C alone (data not shown).

Thus, these experiments demonstrate that the MHC determinants that nude host-derived T_H cells from thymic chimeras recognize as self-structures are those of the engrafted thymus. These results strongly support the concept that the thymus performs a critical role in the self-specificity expressed by those T cells that differentiate within it (4, 5). Indeed, it can be concluded from these experiments that the self-specificity that T_H cells express is entirely determined by the MHC phenotype of the intrathymic environment in which they had differentiated.

The results of the present study appear to conflict with those of previous studies by Kindred (6) and by Zinkernagel et al. (7) in which the self-specificity of T cells from thymus-engrafted nude mice was also examined. They found that the self-specificity of SRBC-specific T_H cells as well as virus-specific cytotoxic T lymphocytes (CTL) from such mice was apparently restricted to recognition of the nude host MHC haplotype. The reasons for differences with the present results are unclear at this time. However, whereas the present study examined the self-specificity of nylon nonadherent spleen T cells in vitro, both of the older studies involved sensitization of unfractionated chimeric spleen cells in an irradiated and adoptively transferred short term host. In at least one of these older studies (7), the nude mice used appeared not to be entirely inbred and consequently not completely tolerant to the MHC determinants expressed by the adoptively transferred, irradiated second host. Whether these differences can account for the differences in results is currently under investigation.

A comparison of the results of the present study on self-recognition by T_H cells from fully allogeneic thymic chimeras with those from a recent study that examined self-recognition by precursor cytotoxic T lymphocytes (pCTL) from fully allogeneic thymic chimeras (3) is potentially informative. The present study demonstrates that the T_H cells that are resident in the spleens of thymic chimeras are entirely restricted to the self-recognition of the thymic haplotype, implying that there exists only one differentiation pathway for T_H cells and that is intrathymic. In the pCTL study (3), it was observed that whereas nude host-derived pCTL resident within the engrafted allogeneic thymus were absolutely restricted to the self-recognition of allogeneic thymic MHC determinants, the pCTL resident in the spleens of the identical mice were not absolutely restricted to the self-recognition of thymic MHC determinants in that they recognized both thymic MHC determinants as well as nude host type MHC determinants. It was suggested that there exist two distinct differentiation pathways for pCTL, one that is intrathymic and restricted by the MHC phenotype of the intrathymic environment and one which is extrathymic and, although dependent upon humoral factors secreted by the thymus, is not restricted by the thymic haplotype. Because the thymic chimeras used in the present study and those used in the pCTL study were from the same groups of experimental animals, the two sets of observations taken together demonstrate that the T_H cells resident in the spleens of allogeneic thymic chimeras are highly restricted to the thymic MHC haplotype, whereas pCTL resident in the same spleens are only partly restricted to the thymic MHC haplotype. Thus, these results suggest that K/D region-restricted T cells (e.g., pCTL) are capable of differentiating along both an intrathymic and extrathymic differentiation pathway, whereas I region-restricted T cells (e.g., T_H cells) can only differentiate along an intrathymic differentiation pathway.

Summary

To examine the possibility that the thymus determines the I region-restricted self-recognition repertoire expressed by T helper (T_H) cells, thymic chimeras were constructed by transplanting allogeneic neonatal thymic lobes into congenitally athymic nude mice. Spleen T_H cells from the thymic chimeras were themselves of nude host origin but only cooperated with B+ accessory cells of the thymic haplotype for primary in vitro responses to sheep erythrocytes and trinitrophenyl conjugate of keyhole limpet hemocyanin. Thus, these experiments demonstrate that the self-recognition repertoire expressed by T_H cells is determined by the H-2 phenotype of the intrathymic environment in which the T_H cells had differentiated.

We thank Mr. John Williams and Ms. Carolyn Harrison for expert technical assistance.

Received for publication 1 September 1981.

References

1. Singer, A., K. S. Hathcock, and R. J. Hodes. 1981. Self recognition in allogeneic radiation bone marrow chimeras. A radiation-resistant host element dictates the self specificity and immune response gene phenotype of T-helper cells. *J. Exp. Med.* **153**:1286.
2. Hodes, R. J., and A. Singer. 1977. Cellular and genetic control of antibody responses in

vitro. I. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. *Eur. J. Immunol.* **7**:892.

3. Kruisbeek, A. M., S. O. Sharrow, B. J. Mathieson, and A. Singer. 1981. The H-2 phenotype of the thymus dictates the self-specificity expressed by thymic but not splenic cytotoxic T lymphocyte precursors in thymus-engrafted nude mice. *J. Immunol.* **127**:2168.
4. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* **147**:882.
5. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* **148**:766.
6. Kindred, B. 1978. Functional activity of T cells which differentiate from nude mouse precursors in a congeneic or allogeneic thymus graft. *Immunol. Rev.* **42**:60.
7. Zinkernagel, R. M., A. Althage, E. Waterfield, B. Kindred, R. M. Welsch, G. Callahan, and P. Pinceth. 1980. Restriction specificities, alloreactivity, and allotolerance expressed by T cells from nude mice reconstituted with H-2-compatible or -incompatible thymus grafts. *J. Exp. Med.* **151**:376.