VACCINIA-SPECIFIC CYTOTOXIC T-CELL RESPONSES IN THE CONTEXT OF H-2 ANTIGENS NOT ENCOUNTERED IN THYMUS MAY REFLECT ABERRANT RECOGNITION OF A VIRUS-H-2 COMPLEX*

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Experiments with radiation chimeras have established that H-$2^{k/b}$ F1 T cells which mature in an H-2^k thymic environment can only respond to vaccinia virus presented in the context of H-2^k and not of H-2^b (1, 2). Does this reflect that the capacity of T cells to interact with virus-infected target cells is completely restricted by the spectrum of H-2 antigens expressed on radiation-resistant thymus epithelium, or is it the case that some form of total nonresponsiveness exists in the context of H-2^b? Can T cells of the H-2^k genotype which develop physiologically in an H-2^k mouse ever respond to virus-infected H-2^b cells if H-2^b has not been encountered during the process of lymphocyte differentiation in thymus?

This problem has been approached using T cells that have first been filtered through irradiated mice to remove alloreactive precursors. Thoracic duct lymphocytes (TDL)[1] drained from 950 rads H-$2^{k/b}$ F1 recipients that were injected up to 42 h previously with H-2^k lymph node cells (negative selection, H-$2^k_{\text{-H-}2^b}$) can be induced to respond specifically to H-2^b-trinitrophenyl(TNP) (3). However, the converse situation was found when these experiments were repeated with vaccinia and influenza viruses. Negatively selected H-2^k lymphocytes did not lyse virus-infected H-2^b targets after stimulation in irradiated H-$2^{k/b}$ F1 recipients (4). Similarly, H-2^d T cells[2] were found not to recognize vaccinia virus in the context of H-$2K^b$.

Further experiments have now shown, however, that negatively selected H-2^d T cells may interact with vaccinia virus presented in association with H-$2K^k$ or H-$2K^s$. The implications of these findings for concepts of T-cell recognition are discussed, and the possibility that this may reflect some form of aberrant recognition is considered.

Materials and Methods

Mice. The H-2 haplotypes of the mouse strains used are given in Table I. Mice were purchased from The Jackson Laboratories, Bar Harbor, Maine; The Institute for Cancer

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[1] *Abbreviations used in this paper:* H-Y, male-specific antigen; TDL, thoracic duct lymphocytes; TNP, trinitrophenyl; V_H gene, encoding the variable region of the immunoglobulin heavy chain.

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TABLE I

H-2 Haplotypes of Mice and Target Cells

	K	A	B	J	E	C	S	D
B10.Br, C3H, CBA/J, L cell	k	k	k	k	k	k	k	k
DBA/2, BALB/c, BALB/cSV	d	d	d	d	d	d	d	d
C57BL/6, (B6)	b	b	b	b	b	b	b	b
A/J, B10.A	k	k	k	k	k	d	d	d
B10.A(2R), 2RSV*	k	k	k	k	k	d	d	b
B10.A(4R)	k	k	b	b	b	b	b	b
B10.A(5R), 5RSV	b	b	b	k	k	d	d	d
B10.HTG, HTGSV	d	d	d	d	d	d	d	b
B10.S, B10.S, SV	s	s	s	s	s	s	s	s
A.TL	s	k	k	k	k	k	k	d

* SV, target cells transformed with SV40 virus.

Research, Fox Chase, Philadelphia; or bred at the Wistar Institute. We are indebted to Dr. D. Götze (Wistar Institute) for supplying the (A.TL × DBA/2)F_1 recipients.

Negative Selection and Stimulation. Mice were first irradiated (950 rad), then injected intravenously 24 h later with 3×10^8 lymph node cells or 5×10^8 spleen cells, and TDL were drained over the subsequent 16–42 h (4, 5). From 1.2 to 1.8 × 10^7 TDL (per mouse) were then injected into groups of two or three recipients (950 rads, 24 h previously), which were dosed with vaccinia virus 3 h later. Spleen populations were assayed for cytotoxic capacity after a further 6 days.

Cytotoxic Assay. The cytotoxic assay was performed as described previously (4), using the target cells identified in Table I. The cell populations were incubated together for 14–15 h at 37°C, and results are expressed as specific ^{51}Cr release relative to the medium and detergent-lysis controls (4).

Antiserum Treatment. Spleen and TDL populations were first incubated for 30 min at 0°C in an antiserum specific for H-2K^k [(C3H.OH × C57BL/6J)F_1 anti-B10.A(4R), supplied by Dr. D. Götze], and then exposed to guinea pig complement for 45 min at 37°C in a conventional cytotoxicity assay.

Results

Normal BALB/c (H-2K^d-D^d) T cells were first filtered through irradiated (C3H × BALB/c)F_1 mice, and then exposed to vaccinia virus for 6 d in 950-rad A/J (H-2K^k-D^d) recipients. As observed and discussed previously,[2] virus-immune cytotoxic T cells were generated at H-2D^d (Table II, 5RSV or BALB/cSV targets) in the absence of I-A to I-E (Table I) region homology between the lymphocytes and the stimulator environment. However, the BALB/c T cells also develop a strong virus-specific response at H-2K^k (Table II, L-cell targets), thus breaking the rule that T cells can only recognize virus in the context of H-2 private specificities encountered previously in thymus.

The experiment was repeated by filtering BALB/c spleen and lymph node populations separately through 950-rad B10.A mice, and then stimulating with vaccinia virus in further sets of B10.A recipients. The result (Table III) was essentially identical to that found previously (Table II). Furthermore, it was evident that the BALB/c T cells do not develop a virus-immune cytotoxic T-cell response at H-2K^d (Table III, HTGSV target), presumably because this allele is not expressed in the stimulator environment. The T cells generated at H-2K^k are thus not cross-reactive for the H-2K^k and H-2K^d alleles.

The possibility was then considered that the response of H-2^d T cells at H-2K^k

TABLE II

Negatively Selected H-2K^d-D^d T Cells may be Stimulated with Vaccinia Virus at H-2K^k

Population stimulated	950R recipients	% Specific ^{51}Cr release (50:1)					
		L cell (kk)		5RSV (bd)		BALB/cSV (dd)	
		Vacc.	N	Vacc.	N	Vacc.	N
BALB/c* −(C3H × BALB/c)F$_1$	A/J	72	22	62	16	48	2
Unirradiated controls:							
BALB/c (dd)		11	8	40	7	31	0
A/J (kd)		74	0	60	3	10	4
B6 (bb)		21	20	67	21	0	0
(C3H × BALB/c)F$_1$ (k/d k/d)		56	6	33	20	11	18

* Serological analysis did not reveal presence of any TDL of (C3H × BALB/c)F$_1$ origin.

TABLE III

Filtration of BALB/c Lymphocytes through B10.A Followed by Stimulation in B10.A

Source of BALB/c cells	% Specific ^{51}Cr release (25:1)					
	2RSV (kb)		5RSV (bd)		HTGSV (db)	
	Vacc.	N	Vacc.	N	Vacc.	N
Spleen	18	3	10	0	9	16
Lymph node	32	5	38	4	3	0
Unirradiated controls:						
B10.Br (kk)	70	5	6	6	11	11
BALB/c (dd)	11	5	27	5	42	12
B6 (bb)	58	24	84	22	70	45
B10.A (kd)	65	6	25	7	15	9

might be mediated by radiation-resistant (950 rads) T cells which originate from the filter mouse and are concentrated by the TDL procedure. This seemed extremely unlikely as (by serological analysis) we have found no evidence for significant numbers (>10%) of host lymphocytes in the TDL population, as reported previously by Sprent (5, 6). The experiment described in Table II was repeated with the modification that the cytotoxic T-cell populations generated were treated with anti-H-2K^k serum and complement before assay (Table IV). No evidence was found to support the idea that cytotoxicity was mediated other than by the BALB/c T cells.

A further experiment was done in which BALB/c T cells were filtered through (ATL × DBA/2)F$_1$ mice, and then stimulated with vaccinia virus in two more of these recipients. A strong virus-specific cytotoxic T-cell response was recognized for H-2K^s (Table V, B10.S SV target).

Does this capacity of H-2^d T cells to respond to vaccinia virus in the context of previously unencountered H-2K alleles depend on there being concurrent generation of cytotoxic T cells at H-2D (Tables II–V)? Spleen and lymph node populations from (BALB/c × C57)F$_1$ mice, which are low responders (7, 8) at H-2D^b (Table VI, 2RSV target), were filtered through (A/J × C57)F$_1$ mice and then stimulated in B10.A(2R)

TABLE IV

*Treatment of Cytotoxic T-Cell Populations with an Antiserum Directed at Both the Filter and Recipient H-2 Haplotypes**

Population stimulated	950 R recipient	Treatment*		% Specific ^{51}Cr release (25:1)							
		Anti-H-2K^k	Guinea pig complement	5RSV (bd)		L cell (kk)		2RSV (kb)		HTGSV (db)	
				Vacc.	N	Vacc.	N	Vacc.	N	Vacc.	N
BALB/c −(C3H × BALB/c)F$_1$	A/J (kd)	+	+	30	3	37	15	30	—	—	—
Unirradiated controls:											
BALB/c (dd)		+	+	32	2	5	0	1	15	34	7
BALB/c		−	+	34	6	6	5	10	9	34	0
BALB/c		−	−	52	11	15	6	13	10	43	7
C3H (kk)		−	−	14	13	44	7	46	14	14	4
B6 (bb)		−	−	70	17	12	0	33	13	29	15

* The transferred TDL could not be shown to contain any T cells expressing the H-2K^k antigenic specificity. The recipient spleen cells were treated with sufficient antibody and complement to lyse at least 20.0 × 10^6 (C3H × BALB/c)F$_1$ spleen cells. This reduced the cell count from 8.0 × 10^6 to 4.0 × 10^6, the decrease reflecting presence of radiation-resistant cells in the A/J spleen.

TABLE V

Stimulation of Negatively Selected H-2K^d-D^d T Cells with Vaccinia Virus in the Context of H-2K^s

Population stimulated	950R recipients	% Specific ^{51}Cr release (40:1)				
		5RSV (bd)		B10.S SV (ss)		L cell (kk)
		Vacc.	N	Vacc.	N	Vacc.
BALB/c −(A.TL × DBA/2)	(A.TL × DBA/2)F1	29	9	60	2	11
Unirradiated controls:						
BALB/c (dd)		45	12	5	1	7
A.TL (sd)		31	6	20	5	6
B10.S (ss)		14	15	73	0	14
B10.A (kd)		61	14	16	10	51
B10.A(4R) (kb)		20	15	4	5	59

TABLE VI

Stimulation of Negatively Selected H-2K$^{d/b}$-D$^{d/b}$ T Cells at H-2K^k is Independent of a Concurrent Response at H-2D^b

Population stimulated	950R recipient	% Specific ^{51}Cr release (25:1)					
		L cell (kk)		2RSV (kb)		HTGSV (db)	
		Vacc.	N	Vacc.	N	Vacc.	N
(BALB/c × C57)F1 −(A/J × C57)F1	B10.A(2R)	36	2	33	0	11	15
Unirradiated controls:							
BALB/c (dd)		12	5	9	8	51	3
(BALB/c × C57)F1		18	12	15	13	39	16
A/J (kd)		64	8	41	3	12	7
(A/J × C57)F1		66	10	49	7	22	8
B6 (bb)		15	12	24	17	49	20

environment. Virus-immune cytotoxic T cells were again generated at H-2K^k (Table VI, L-cell target), in the absence of any response at H-2D^b (Table VI, HTGSV target).

Discussion

The present situation is that T cells developing in an H-2^d mouse may be induced to recognize vaccinia virus in the context of H-2K^k or H-2K^s, but not[2] of H-2K^b, whereas H-2^k T cells can be stimulated by H-2^b-TNP but not by H-2^b-vaccinia or influenza (3, 4). Much more information is obviously necessary, but these experiments are difficult and not all H-2 haplotypes filter reciprocally (6). Even at this stage, however, the results have considerable implications for the problem of T-cell recognition.

It now seems obvious that the spectrum of H-2 antigens encountered on radiation-resistant thymic epithelium plays a major role in dictating subsequent patterns of T-cell recognition (1, 2). The T-cell repertoire cannot be determined solely by genes mapping in the non-H-2 genetic background. If this were so, B10.D2 (K^d-D^d) lymphocytes which are first filtered to remove alloreactive potential and then stimulated with vaccinia virus in a B10.A(5R) (K^b-D^d) environment should respond to virus presented in association with H-2K^b. However this is not the case,[2] though the B10.A(5R) is a responder at H-2K^b.

Possible modes of action of the thymus may be summarized as follows:

(*a*) The thymus selects positively for T cells with two anatomically distinct receptor units, one of which is specific for self H-2 whereas the other is capable of interacting with virus. The virus-specific receptor may be encoded by a V_H gene (9, 10), or generated by a process of somatic mutation in thymus (11). The present results make these versions of the dual recognition model seem rather unattractive.

(*b*) The two receptor units described in (*a*) are brought together to make a complex receptor with a single, large binding site (9). This might then recognize some complex of virus and H-2 antigen (12–14), as argued consistently for variations of the altered-self concept (15–17).

(*c*) The T cell may express multiple, identical receptors (as in *b*) which fulfill a dual function (18). Interaction with virus would focus the T cell onto the target (or stimulator) cell, thus allowing recognition of H-2 by a second, identical receptor of low affinity.

(*d*) The binding site of the T-cell receptor is encoded by a single V_H gene (10), and operates as described in (*c*). The BALB/c mouse strain apparently possesses at least 100 different B-cell clones specific for a particular influenza virus hemagglutinin antigen (19). It does not seem inconceivable that at least one V_H-binding site which interacts with virus may also exhibit specificity for a small set (as few as three, reference 20) of amino acids expressed on the H-2 private specificity. Nonresponder situations, as seen for H-2K^b influenza virus or H-2D^k vaccinia virus (7, 8), would occur when the V regions which are specific for virus have no concurrent capacity to interact with the H-2 molecules in question. This idea can be argued on the basis of either germ-line (as above) or somatic mutation models (16, 21). In both cases, positive selection of clones with low affinity for self H-2 must occur in thymus.

(*e*) A single recognition unit of the type described in (*c*) may form a high affinity interaction with an altered-self complex.

How may these models be accommodated with the present finding that negatively selected T cells differentiating in the context of one set of H-2 alleles interact with H-2-incompatible virus-infected target cells? Different clones of vaccinia-immune precursor T cells developing in an H-2^d mouse may have multiple, identical receptors specific for H-$2K^d$-vaccinia virus or for H-$2D^d$-vaccinia virus (models *b-e*). We know that the capacity of H-2^d T cells to recognize H-$2K^k$ vaccinia virus is not due to cross-reactivity of vaccinia-specific clones operating at H-$2K^d$ or H-$2D^d$. The interaction of H-2^d T cells with H-$2K^k$ or H-$2K^s$ vaccinia virus may thus reflect aberrant recognition mediated via a binding site with specificity for something else. The set of amino acids (20) recognized on, for example, H-$2K^d$-virus X or H-$2D^d$-minor histocompatibility antigen Z, may be identical to that seen for H-$2K^k$-vaccinia virus.

Aberrant recognition can only be argued along the lines of the altered-self (15–17) concept (models *b* and *e*). We must consider that the segment of the receptor that sees foreign H-2 is, at least in part, located in the region of the binding site that would normally (the self-H-2 situation) interact with neoantigen. Conversely, recognition of virus would also involve abnormal usage of the self-specific component. Thus if we postulate dual recognition by repeated, identical receptors (models *c* and *d*) we must now accept that the receptor for, say, H-$2K^d$-virus × can see: (*a*) vaccinia virus, (*b*) H-$2K^d$, and (*c*) H-$2K^k$, so that lysis of targets expressing H-$2K^k$-vaccinia virus may occur. However the net result must be that the same lymphocytes would also recognize vaccinia-infected H-$2K^d$ cells, which is contrary to the present findings.

Obviously, the larger the binding site and the smaller the number of amino acids that need to be seen on the target antigen, the greater will be the potential for aberrant recognition. The idea of a complex binding site (model *b*) is thus more attractive than that attributing specificity to a single V_H gene (model *e*). Also, the latter idea implies that the T-cell repertoire is selected from a subset of the V_H genes available to B cells. Although this offers a satisfactory explanation for nonresponsiveness, it also places very severe constraints on the size of the T-cell repertoire. Nonresponsiveness may also be explained by failure to form a complex binding site (model *b*) with resultant absence of selection in thymus. Alloreactivity may be attributed in part to aberrant recognition, though much is probably germ-line (9, 10, 21).

Certain general constraints seem to apply. Apparently binding to H-2 alone may not occur, except in the case of alloreactivity. Also, signalling for stimulation and lysis would need to operate via the H-2 private specificities encountered on the stimulator or target cell. Furthermore, to accommodate the chimera results (1, 2), there must be some form of absolute nonresponsiveness to antigen presented in the context of H-2 molecules encountered in thymus other than on radiation-resistant epithelium. Thus, both positive and negative selection may be considered to occur in thymus.

The probability that selection must operate in thymus (1, 2) is further emphasized by the fact that T cells from bone marrow chimeras (22) cannot be induced to lyse TNP-modified cells of the tolerated H-2 type (expressed on other than radiation-resistant thymus), whereas such interactions are possible with some neonatal chimeras (23). In the first situation, long-term tolerance presumably results from events occurring in thymus whereas in the second, induction of tolerance may be extrathymic, being established by injecting baby parental mice with adult F_1 lymphocytes. The rather different results obtained for the negative selection and bone marrow

chimera situations may thus reflect the role of the thymus in determining the T-cell repertoire, rather than representing a contrast between acute (deletion) and persistent forms of tolerance.

Summary

BALB/c (H-2K^d-D^d) spleen and lymph node populations were specifically depleted of alloreactive potential by filtration through H-2 different, irradiated recipients. These negatively selected T cells were then stimulated with vaccinia virus in mice expressing the foreign H-2 determinants encountered previously in the filter environment. Strong virus-immune cytotoxic T-cell responses were seen in the context of H-2K^k and H-2K^s, but not[2] H-2K^b. The T cells generated were not cross-reactive for the H-2K^k and H-2K^d alleles, and responsiveness was independent of concurrent presence of effector populations operating at H-2D. These findings are consistent with the idea that recognition is mediated via a complex receptor, part of which is specific for virus and part for self H-2. The capacity to interact with allogeneic, virus-infected cells may then reflect aberrant recognition of a virus-H-2-antigen complex by this single, large binding site. For instance, the T cell which would normally recognize H-2K^d-virus x, or H-2D^d-minor histocompatibility antigen Z, may now show specificity for H-2K^k-vaccinia virus. Implications for both the selective role of the thymus and for mechanisms of tolerance are discussed.

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References

1. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. Klein, and J. Klein. 1978. On the thymus in differentiation of "H-2 self-recognition" by T cells. Evidence for dual recognition? *J. Exp. Med.* **147**:882.
2. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus-plus-self-specific cytotoxic T cells: evidence for T help. *J. Exp. Med.* **147**:897.
3. Wilson, D. B., K. Fischer-Lindahl, D. H. Wilson, and J. Sprent. 1977. The generation of killer cells to trinitrophenyl-modified allogenic targets by lymphocyte populations negatively selected to strong alloantigens. *J. Exp. Med.* **146**:361.
4. Bennink, J. R., and P. C. Doherty. 1978. T-cell populations specifically depleted of alloreactive potential cannot be induced to lyse H-2 different virus-infected target cells. *J. Exp. Med.* **148**:128.
5. Sprent, J., and H. von Boehmer. 1976. Helper function of T cells depleted of alloreactive lymphocytes by filtration through irradiated F1 hybrid recipients. 1. Failure to collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured *in vivo*. *J. Exp. Med.* **144**:617.
6. Sprent, J. 1977. Recirculating lymphocytes. *In* The Lymphocyte: Structure Function. J. J. Marchalonis, editor. Marcel Decker, Inc. New York.
7. Doherty, P. C., W. E. Biddison, J. R. Bennink, and B. B. Knowles. 1978. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. *J. Exp. Med.* **148**:534.
8. Zinkernagel, R. M., A. Althage, S. Cooper, G. Kreeb, P. A. Klein, B. Sefton, L. Flaherty, J. Stimpfling, D. Shreffler, and J. Klein. 1978. Ir-genes in H-2 regulate generation of anti-

viral cytotoxic T cells: mapping to K or D dominance of unresponsiveness. *J. Exp. Med.* **148:**592.

9. Krammer, P. H., and K. Eichmann. 1977. T-cell receptor idiotypes are controlled by genes in the heavy chain linkage group and the major histocompatibility complex. *Nature (Lond.).* **270:**733.
10. Binz, H., H. Frischkneht, C. Mercoli, and H. Wigzell. 1978. Partial characterization of cell surface idiotypes of alloantigen-activated T lymphocytes. *Scand. J. Immunol.* **7:**481.
11. von Boehmer, H., W. Haas, and N. K. Jerne. 1978. Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice. *Proc. Natl. Acad. Sci. U.S.A.* **75:**2439.
12. Schrader, J. W., B. A. Cunningham, and G. M. Edelman. 1975. Functional interactions of viral and histocompatibility antigens at tumor cell surfaces. *Proc. Natl. Acad. Sci. U.S.A.* **72:**5066.
13. Bubbers, J. E., and F. Lilly. 1977. Selective incorporation of H-2 antigenic determinants into Friend virus particles. *Nature (Lond.).* **266:**458.
14. Bourguignon, L. Y. W., R. Hyman, R. Trowbridge, and S. J. Singer. 1978. Participation of histocompatibility antigens in capping of moleculary independent cell surface components by their specific antibody. *Proc. Natl. Acad. Sci. U.S.A.* **75:**2406.
15. Zinkernagel, R. M., and P. C. Doherty. 1974. Immunological surveillance against altered self components by sensitized T lymphocytes in lymphocytic choriomeningitis. *Nature (Lond.).* **251:**547.
16. Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-immune effector T cells for H-2K or H-2D compatible interactions. Implications for H antigen diversity. *Transplant. Rev.* **29:**89.
17. Doherty, P. C., D. Götze, G. Trinchieri, and R. M. Zinkernagel. 1976. Models for recognition of virally-modified cells by immune thymus derived lymphocytes. *Immunogenetics.* **3:**517.
18. Shearer, G. M., and A. M. Schmitt-Verhulst. 1977. Major histocompatibility complex restricted cell-mediated immunity. *Adv. Immunol.* **25:**55.
19. Cancro, M. P., W. Gerhard, and N. R. Klinman. 1978. The diversity of the influenza-specific primary B-cell repertoire in BALB/c mice. *J. Exp. Med.* **147:**776.
20. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific genes in macrophages and B lymphocytes. *J. Immunol.* **120:**1809.
21. Jerne, N. K. 1971. The somatic generation of immune recognition. *Eur. J. Immunol.* **1:**1.
22. Zinkernagel, R. M., G. N. Callahan, J. Klein, and G. Dennert. 1978. Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus. *Nature (Lond.).* **271:**251.
23. Forman, J., J. Klein, and J. W. Streilein. 1977. Spleen cells from animals neonatally tolerant to H-2K^k antigens recognize trinitrophenyl-modified H-2K^k spleen cells. *Immunogenetics.* **5:**561.