Brief Definitive Report

TRANSPLANTATION BEHAVIOR OF A.TH AND A.TL T-CELL LYMPHOMAS IN CONGENIC RESISTANT AND HYBRID STRAINS*

By JAMES W. GODING and NOEL L. WARNER

(From The Walter and Eliza Hall Institute of Medical Research, P. O., Royal Melbourne Hospital, Victoria 3050, Australia)

In many species, genes controlling specific immune responses are linked to the major histocompatibility complex (MHC) (1). The bulk of available evidence indicates that these genes are expressed in T lymphocytes (1), although some data suggest expression at the B-cell level (2, 3). It has been postulated (4) that the MHC-linked immune response (*Ir*) genes code for the T-cell receptor for antigen, although other models of *Ir*-gene function have been proposed which do not invoke antigen recognition properties of these gene products (5).

In the mouse, the MHC-linked *Ir* genes have been mapped to the *I* chromosomal segment, which lies between the *K* and *S* regions (6). It has recently been shown that the *I* region codes for surface antigens on both T and B lymphocytes (7–10). These *I*-region-associated (Ia) antigens might represent products of the *Ir* genes. The Ia antigens have been studied by complement (C)-mediated cytotoxicity (7), stimulation in the mixed lymphocyte reaction (MLR) (11), autoradiography (10), and immunofluorescence (12).

Yet another approach to the study of cell surface antigens involves the transplantation behavior of neoplastic cell populations. In this report, we describe the results of transplanting a series of seven T-cell lymphomas, and one mammary adenocarcinoma of congenic resistant strain origin.

Materials and Methods

Mice. A.TH, A.TL, B10.A(2R), and B10.A(4R) mice were progeny of breeding pairs obtained from Dr. D. C. Shreffler, University of Michigan, Ann Arbor, Mich. Other mouse strains were from The Walter and Eliza Hall Institute stocks. Mice of either sex were used at an age of approximately 8 wk. The *H-2* and Ia specificities have been previously described (6).

Tumors. All tumors described in this paper arose spontaneously, and were generally detected when a mouse was noted to have a visible subcutaneous lump (mammary adenocarcinoma), or appeared ill and had an enlarged spleen, or was killed for other purposes. In all cases, specimens were processed for histology. Age and sex of tumor origin are given in Table II.

Characterization of Tumors. Cells from tumor masses or tumor-infiltrated organs were tested for membrane immunoglobulin by direct immunofluorescence and for Thy 1.2 antigen by indirect immunofluorescence using anti-Thy 1.2 serum followed by fluorescein-labeled rabbit antimouse gamma globulin. Cells from some lines were also tested for the presence of receptors for the Fc portion of IgG by a rosetting technique using antibody-coated sheep erythrocytes (13).

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Tumor Maintenance. Primary tumors were established and maintained in serial transplantation by subcutaneous or intravenous inoculation of dispersed cell suspensions (approximately 10^7 cells) prepared from tumor involved tissues. Tumor lines are designated by the prefix WEHI and the tumor line number (205, 211, 213, 215, 216, 222, 223, and 228). Experiments described in this paper involve transplant generations from 1–10.

Experimental Design. Mice were injected with known numbers of viable tumor cells (eosin exclusion), either subcutaneously in the flank or intravenously. They were then observed every 2–3 days for signs of tumor growth. At varying periods, depending on the rate of growth, the mice were killed and the spleens and/or local subcutaneous tumor mass excised and weighed.

Results and Discussion

In the course of routine studies of Ia antigens, approximately 500 A.TH and 300 A.TL mice were killed over an 18-mo period. During this time we have found 15 mice with lymphomas, of which 8 have so far proven transplantable, and 7 are reported here. In addition, one A.TL mouse was found to have a mammary adenocarcinoma. We are unable to give true tumor incidence figures, because occasional animals in our colonies died without an autopsy being performed. However, the figures given would represent a lower limit. The tumors occurred in relatively young mice (6–42 wk) of either sex (Table II).

Macroscopically, all the lymphoma-bearing mice had very large spleens (typically 3–4 g in weight) and enlarged lymph nodes in all areas, and most had enlarged livers and obviously infiltrated kidneys. The thymus was macroscopically enlarged in approximately 50% of cases. The neoplastic cells had large, irregular, lightly staining nuclei and scanty pale cytoplasm. Mitotic figures were frequent. Histologically, there was very marked infiltration of lymph nodes, spleen and liver, and patchy perivascular infiltration of kidney and lungs. All the lymphomas were strongly positive for Thy 1.2 antigen and negative for high density surface immunoglobulin, and are hence of T-cell origin. WEHI-211 and WEHI-222 were tested for presence of Fc receptors and found to be negative. Studies on Ia antigens as detected by anti-Ia serum are currently in progress.

The mammary tumor (WEHI-205), which arose in a pregnant mouse approximately 30 wk of age, consisted of a solitary cystic subcutaneous mass with no evidence of metastasis. Histology showed moderate differentiation, with large secretory follicles filled with eosinophilic material.

Upon transplantation into syngeneic recipients, marked differences in growth patterns were observed (Tables I and II). Tumors WEHI-205, 211, 213, and 223 grew subcutaneously, while the remainder grew exclusively by metastasis, notably in the spleen. In general, there appeared to be an inverse correlation between subcutaneous and splenic growth (Table I). The reason for such differences is not known, but within each tumor line the observed differences in growth patterns were quite consistent from one experiment to another.

When transplanted subcutaneously into congenic partner strains (Table II), the tumors were consistently rejected. On the other hand, when given intravenously it was sometimes possible to overcome rejection. Whether this is merely a dosage effect, or is due to some other mechanism, is unclear.

There could be several reasons for the failure of growth in congenic partner strains. Firstly, the neoplastic cells could possess strong tumor-specific antigens, for which the partner strains happen to be responders and the strain of origin

TABLE I

Growth of Subcutaneous Inocula of T Lymphomas in Syngeneic Mice

Tumor line	Strain (donor and recipient)	Tumor growth*	
		Local subcutaneous‡	Spleen§
WEHI-216	A.TL	No local growth (0/9)	9.1 ± 2.2
WEHI-215	A.TH	150 ± 50 (0/16)	5.5 ± 0.8
WEHI-213	A.TH	911 ± 190 (17/17)	2.5 ± 0.4
WEHI-223	A.TH	1400 ± 340 (4/4)	1.4 ± 0.1

* All recipients given subcutaneous injection of 5×10^6 tumor cells (213, 215, and 223) or 15×10^6 (216) and examined at 26 days.

‡ Mean weights of excised tumor (milligrams ± SEM.). Values in parentheses show number of mice/total with increasing tumor growth.

§ Splenic tumor growth expressed as mean ± SEM. of ratio of spleen weights (milligrams per gram body weight) of tumor injected/control mice.

TABLE II

Summary of T Lymphoma Growth in Congenic Resistant Inbred Strains

Tumor line	Origin	Incidence of successful tumor growth‡					
		Subcutaneous inoculation				Intravenous inoculation	
		A.TH local	A.TL local	A.TH spleen	A.TL spleen	A.TH spleen	A.TL spleen
WEHI-211	A.TH(M. 32 wk)	10/10	0/10	0/4	0/4	6/6	0/8
WEHI-213	A.TH(M. 15 wk)	17/17	0/6	8/17	0/6	4/5	NT
WEHI-215	A.TH(M. 11 wk)	0/20	0/16	18/19	0/10	10/12	7/9
WEHI-223	A.TH(F. 42 wk)	4/4	0/4	0/4	0/4	3/3	0/3
WEHI-216	A.TL(F. 36 wk)	0/9	0/9	0/9	9/9	6/6	18/18
WEHI-222	A.TL(M. 34 wk)	0/10	0/7	1/10	6/7	4/12	7/7
WEHI-228	A.TL(M. 6 wk)	0/4	0/5	0/4	0/5	1/7	6/6

* Sex and age of mouse at time of primary tumor detection given in parentheses.

‡ Donor cell inoculum varies from $5\text{–}20 \times 10^6$ and assay time from 8–20 days (both constant within a given experiment). Successful growth scored as subcutaneous tumor nodule of >10 mm diameter or splenic tumor growth of greater than twofold spleen weight increase (based on milligrams per gram body weight). Values show number of mice with successful tumor takes/number inoculated. Underlined values indicate clear strain specificity of tumor takes.

nonresponders. However, the *H-2*-linked *Ir* genes are dominant (1) and thus the F_1 hybrids between responders and nonresponders will be responders. In the cases where tumors were transplanted into A.TH × A.TL F_1 hybrids, they grew as rapidly as in syngeneic hosts (Table III), indicating that *Ir*-gene effects relating to tumor-specific transplantation antigens are unlikely to be the explanation. Furthermore, this explanation would require the rather unlikely presence of an A.TH tumor antigen to which A.TL mice were responders and a different A.TL tumor antigen to which A.TH were responsive.

Secondly, the observed lack of growth could be due to differences at the *TL* locus, at which A.TH and A.TL differ (14). This possibility is unlikely as there are no Tla specificities on A.TL cells which are not shared by A.TH cells (14) and yet A.TL tumors are rejected by A.TH mice. However, the possibility of rejection due to closely linked but distinct histocompatibility antigens controlled by the TL region is not ruled out. In order to eliminate this possibility, A.TL cells

TABLE III
Transplantation in F_1 Hybrids

Tumor	Recipient	Growth	Spleen weight/ body weight (mg/g; 21 days)	
WEHI-205* (mammary adenocarcinoma) (A.TL)	A.TL	20/20	—	
	A.TH	0/30	—	
	A.TH × A.TL	7/7	—	
WEHI-216‡ (A.TL)	A.TL	5/5	66.6 ± 10.1	
	A.TH	0/4	5.3 ± 0.4	
	A.TH × B10.A(2R)	4/5	49.0 ± 17.2	
	A.TH × B10.A(4R)	0/4	7.7 ± 1.9	
	A.TH × CBA	6/10	42.1 ± 13.5	
	A.TH × B10.D2	0/5	4.8 ± 0.2	
WEHI-222‡ (A.TL)	A.TH × B10.D2	0/5	4.1 ± 0.1	
			Subcutaneous tumor growth (mm ± SE)	
			day 11	day 23
WEHI-211§ (A.TH)	A.TH	3/3	4.3 ± 0.9	21.0 ± 1.0
	A.TL	0/3	0	0
	A.TH × A.TL	3/3	7.7 ± 0.9	20.0 ± 1.2

* Tumor mass from one A.TL mouse (approximately 2 g tissue) was dispersed by sieving into 10 ml HEPES-buffered Eagle's medium, and clumps allowed to settle for 1 min. The supernate was decanted and mixed thoroughly. Each animal received 0.5 ml subcutaneously. Animals were observed for 25 days. In positive individuals, subcutaneous lumps approximately 2 cm in size were found, while in negative animals there was no macroscopic growth.

‡ T lymphomas. 20×10^6 viable cells were injected subcutaneously.

§ T lymphoma. 8×10^6 viable cells injected subcutaneously. Similar growth patterns were seen with 2×10^6, 0.4×10^6, 0.1×10^6, and 0.025×10^6 cells.

(WEHI-216 and WEHI-222) were transplanted into A.TH × B10.D2 F_1 hybrid mice (Table III). B10.D2 mice are Tla^c and, as far as is known, are identical to A.TL at *H-2D* and TL (14). Their clear rejection in these hybrids is thus unlikely to be due to TL-region incompatibility. Although sex matching of tumor and recipient was not possible in all experiments, no differences ascribable to the H-Y antigen were detected.

The final possibility, which we favor, is that the tumors are rejected because they possess Ia antigens. A.TH and A.TL mice differ at the *I* region and indeed high titer antibodies against *I*-region products may be raised in both directions. These antisera react strongly with B cells and weakly with T cells (9, 10). It has recently been shown that the *I* region is associated with strong histocompatibility effects as assessed by skin graft rejection (15). However, the presence of certain antigens on skin cells is no guarantee that they will be expressed on other tissues.

In an attempt to more precisely map the *I* region responsible for rejection, WEHI-216 (A.TL) tumor cells were transplanted into A.TH × B10.A(2R), A.TH × B10.A(4R), and A.TH × CBA F_1 hybrids (Table III). The results in the A.TH × CBA recipients were erratic, with about half the mice having very large spleens and the remaining half showing no evidence of tumor growth. There was rapid growth in A.TH × B10.A(2R) but not in A.TH × B10.A(4R) recipients. From present information on *I*-subregion assignment of Ia specificities (6), the rejection in A.TH × B10.A(4R) F_1 mice could only be due to recognition of *IC* locus determinants (specificity 7). An alternative possibility is the presence of as yet

serologically undetected specificities determined by either the *IB* or *IC* locus.

The data imply that antigens controlled by the *I* region are present on T cells. However, we cannot exclude the possibility that the cells are derepressed and express Ia antigens that are not present on normal T cells. The results of experiments involving transplantation of WEHI-205 (mammary adenocarcinoma) suggest that Ia antigens are also present on these cells. At least some Ia antigens may be relatively widespread in their cellular distribution (16). It also seems likely that antigens controlled by different *I* subregions may have differing distribution and function. Clear differences in susceptibility to viral-induced oncogenesis have been shown to be linked to *H-2* in the mouse, and are probably due to *Ir* genes (1). Similarly, the association of certain viral and neoplastic diseases with particular HL-A types may point to *Ir*-gene effects in humans. The tumors may be useful in furthering the understanding of these problems, and in the study of the possible relationship between the Ia antigens and the receptor for antigen on T lymphocytes.

Summary

Seven spontaneously arising T-cell lymphomas originating in A.TH or A.TL mice, which are congenic for the immune response gene (*I*) chromosomal segment were described. When transplanted into partner strains which were incompatible at the *I* region, the tumors were rapidly rejected. Rejection was proposed to be due to the presence of antigens controlled by *I*-region genes.

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References

1. Benacerraf, B., and D. H. Katz. 1974. The histocompatibility-linked immune response genes. *Adv. Cancer Res.* In press.
2. Shearer, G. M., E. Mozes, and M. Sela. 1972. Contribution of different cell types to the genetic control of immune responses as a function of the chemical nature of the polymeric side chains (poly-L-prolyl and poly-DL-alanyl) of synthetic immunogens. *J. Exp. Med.* **135**:1009.
3. Taussig, M. J., E. Mozes, and R. Isac. 1974. Antigen-specific thymus cell factors in the genetic control of the immune response to poly-(tyrosyl, glutamyl)-poly-D, L-alanyl--poly-lysyl. *J. Exp. Med.* **140**:301.
4. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.
5. Marchalonis, J. J., P. J. Morris, and A. W. Harris. 1974. Speculations on the function of immune response genes. *J. Immunogenetics.* **1**:63.
6. Shreffler, D. C., and C. S. David. 1974. The H-2 major histocompatibility complex and the I immune response region: genetic variation, function and organisation. *Adv. Immunol.* In press.
7. Sachs, D. H., and J. L. Cone. 1973. A mouse B-cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* **138**:1289.
8. Hämmerling, G. J., B. D. Deak, G. Mauve, U. Hämmerling, and H. O. McDevitt. 1974. B lymphocyte alloantigens controlled by the I region of the major histocompatibility complex in mice. *Immunogenetics.* **1**:68.
9. Frelinger, J. A., J. E. Niederhuber, C. S. David, and D. C. Shreffler. 1974. Evidence

for the expression of Ia (*H*-2-associated) antigens on thymus-derived lymphocytes. *J. Exp. Med.* **140**:1273.

10. Goding, J. W., G. J. V. Nossal, D. C. Shreffler, and J. J. Marchalonis. 1975. Ia antigens on murine lymphoid cells: distribution, surface movement and partial characterisation. *J. Immunogenetics.* **2**:41.
11. Meo, T., J. Vives, V. Miggiano, and D. Shreffler. 1973. A major role for the Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction. *Transplant. Proc.* **5**:377.
12. Unanue, E. R., M. E. Dorf, C. S. David, and B. Benacerraf. 1974. The presence of I region-associated antigens on B cells in molecules distinct from immunoglobulin and H-2K and H-2D. *Proc. Natl. Acad. Sci. U. S. A.* **71**:5014.
13. Cline, M. J., J. Sprent, N. L. Warner, and A. W. Harris. 1972. Immunoglobulin receptors on B lymphocytes and a plasma cell tumor line. *J. Immunol.* **108**:1126.
14. Frelinger, J. A., D. B. Murphy, and J. F. McCormick. 1974. T1a types of H-2 congenic and recombinant mice. *Transplantation (Baltimore).* **18**:292.
15. Klein, J., M. Hauptfeld, and V. Hauptfeld. 1974. Evidence for a third, Ir-associated histocompatibility region in the H-2 complex of the mouse. *Immunogenetics.* **1**:45.
16. Hämmerling, G. J., G. Mauve, E. Goldberg, and H. O. McDevitt. 1975. Tissue distribution of Ia antigens: Ia on spermatozoa, macrophages and epidermal cells. *Immunogenetics.* **1**:428.