MURINE LEUKAEMIA VIRUS EXPRESSION IN THE AKR FOLLOWING THYMECTOMY

M. TUFFREY, P. CREWE, L. DAWSON, J. HOLLIDAY AND R. D. BARNES

From the Clinical Research Centre, Harrow, Middlesex

Received 2 May 1975. Accepted 20 June 1975

Summary.—Thymectomy effectively prevents the development of spontaneous lymphoma in the AKR but how this effect is achieved remains to be determined. One possible mechanism, namely suppression of genomic expression of the oncogenic murine leukaemia virus now seems unlikely since levels of the group specific MuLV antigen were in comparison with their sham operated controls unaltered in both neonatally and adult thymectomized AKR.

ALTHOUGH quite clearly involved, the exact role of the thymus in the development of the lymphoma which characterizes the AKR (Furth, Seibold and Rathbone, 1933) still remains to be determined (Miller, 1961). One possible effect of thymectomy may be to suppress the titres of murine leukaemia virus group specific antigens and this has been examined here in both neonatally and adult thymectomized AKR.

MATERIALS AND METHODS

Mice.—The derivation of the AKR/CRC used here has been described previously (Barnes and Tuffrey, 1974). Initially AKR/J they were then maintained at the Laboratory Animal Centre, Carshalton before being transferred first to the National Institute of Medical Research at Mill Hill and then to the Clinical Research Centre. At all times the colony was maintained by brother-sister mating. As noted earlier, the AKR/CRC subline have a high incidence of lymphomata reaching 100% by 56 weeks of age (Barnes, Tuffrey and Ford, 1973).

Surgery.—The mice were either thymectomized or sham operated. In both cases the procedure either took place during the first 24 h of life or at varying intervals from the age of 4 weeks. Details of the technique of

thymectomy have been described previously (Tuffrey, Kingman and Barnes, 1973). In the case of the mice operated on during the first 24 h of life, hypothermia was employed. Anaesthesia in adult groups aged between 6 and 9 weeks was achieved using bromethol (Avertin), 0.01 ml/g body weight i.p. of 2.5% solution).

Investigation.—Animals were killed at various intervals following thymectomy. Success of thymectomy was confirmed by histological examination of serial sections prepared from the mediastinum.

Tissues and serum were obtained at sacrifice and stored at -20°C until investigated. The indirect immunofluorescent absorption technique of Hilgers (Hilgers *et al.*, 1974) was performed to determine the gs-antigen titres, but here against fixed AKR-A lymphoma cells. The AKR-A lymphoma cell line was originally derived by Woods *et al.* (1970) and maintained here at the Clinical Research Centre by Dr Jennifer Harvey. In essence, the gs-antigen test is in 2 stages: first the titration of the specific anti-MuLV-gs sera against the target AKR-A lymphoma cells, followed by a second titration after absorption with soluble antigens obtained in the case of solid tissues after ultrasonic disintegration. The gs-antigen titre is expressed as the reciprocal of the reduction in antibody titre following absorption (I.F.A.). In all cases coded

samples were examined and the results were then subsequently related to each animal and to the success of surgery. Some untreated controls were also included. These were 3 old AKR with lymphomata and some young CBA/H-T6.

RESULTS

The results of the gs-antigen titres in the adult thymectomized and sham operated AKR are shown in Table I. Thymectomized mice in which a microscopic remnant of lymphoid tissue (probably lymph node) was subsequently found on histological examination were maintained as a separate subgroup.

The results of the gs-antigen titres from the neonatally thymectomized and sham thymectomized AKR are shown in Table II. The distribution of MuLV-gs antigen appears in good agreement with the titres described by Hilgers *et al.* (1974). Although we were unable to detect any MuLV-gs antigen in any of our serum samples, we were able to detect MuLV-gs antigen in the spleen, liver and kidney from one week of age. Highest titres were usually detected in the spleen samples, apart from those recorded from control AKR mice with thymoma (Table III). This is also in accord with the infectivity titres recorded by Rowe and Pincus (1972). It should be noted that the titres in the AKR are somewhat lower than those described by previous workers (Hilgers *et al.*, 1974). This difference is due solely to technical variation in determination of the "end point".

It is quite obvious from these results that thymectomy, whether neonatal or between 4 and 9 weeks of age, has no effect upon MuLV-gs titres.

DISCUSSION

The involvement of the oncogenic virus in the disease of the AKR strain was clearly established by Gross (1951, 1957). Although the role of the thymus in the disease process of the AKR remains speculative, thymectomy at any stage before the development of a lymphoma effectively prevents the disease (McEndy, Boon and Furth, 1944; Law and Miller, 1950). This advantage might act in one of several ways, one being that thymectomy might suppress viral gene expression and its neoplastic sequelae. From the findings here, this view seems unlikely since comparable amounts of MuLV-gs antigen were found in both sham operated and thymectomized AKR. These results can be considered to support Miller's (1960) work in which he showed that his "leukaemic agent" was able to multiply when transferred through a series of thymectomized hosts. Nakakuki, Shisa and Nishizuka (1967) suggested that the viral concentration needed to reach a critical level to enable initiation of neoplastic transformation, however it would appear that the protective effect of thymectomy is not in reducing the MuLV-gs titre and therefore possibly the level of infectious virus.

The most obvious interpretation would

TABLE III.—*MuLV-gs Titres on Tissues and Serum of Leukaemic AKR/CRC and normal. CBA/H-T6 Mice*

	Controls (age days)	No. of animals	Spleen	Kidney	Liver	Thymus	Serum	B/M	L/N
AKR*	1		8	2	16	16	4	8	16
	2		4	1	8	16	8	8	4
	3		8	2	16	16	8	4	nt
CBA	1 (6) days	7	0	0	0	0	0	nt	nt
	2 (9) days	4	0	0	0	0	0	nt	nt
	3 (+ 100)	3	0	0	0	0	0	nt	nt

Results expressed as reciprocal of I.F.A. titres.

* Leukaemic.

TABLE I.—*MuLV-gs Titres on Tissues and Serum of Sham and Thymectomized AKR/CRC Mice (Adults) (Results Expressed as Reciprocal of I.F.A. Titre)*

	Complete thymectomy group (microscopic lymphoid remnant)						Sham thymectomized group					
	Age at thymectomy (weeks)	Spleen	Bone marrow	Lymph nodes	Kidney	Serum	Age at thymectomy (weeks)	Spleen	Bone marrow	Lymph nodes	Kidney	Serum
Group A												
Mice killed	9	2	2	2	1	0	5	16	0	4	0	0
one week following operation	6	8	1	4	0	0	4	2	0	0	1	0
	6	8	1	2	0	0	4	2	1	2	0	0
	5	4	0	2	0	0	4	8	0	2	0	0
	4	4	—	2	1	0	4	4	0	1	0	0
	(7)	(4)	(0)	(1)	(0)	(0)						
	(6)	(2)	(1)	(1)	(0)	(0)						
	(4)	(4)	(1)	(0)	(0)	(0)						
	(4)	(4)	(1)	(0)	(0)	(0)						
	(4)	(2)	(0)	(1)	(0)	(0)						
Group B												
Mice killed	7	8	1	1	0	0	4	4	0	0	0	0
2 weeks following operation	6	4	0	1	0	0	4	2	0	0	0	0
	5	4	1	1	0	0	4	4	1	2	1	0
	(9)	(4)	(2)	(2)	(0)	(0)	4	2	1	0	0	0
	(6)	(8)	(0)	(4)	(0)	(0)	4	2	0	0	0	0
	(5)	(4)	(0)	(1)	(0)	(0)						
	(4)	(2)	(1)	(2)	(0)	(0)						
	(4)	(2)	(0)	(2)	(0)	(0)						
	(4)	(2)	(0)	(2)	(0)	(0)						
	6	8	0	2	0	0	4	4	1	0	0	0
	6	4	1	0	0	0	4	1	0	1	0	0

TABLE I.—*continued.*

	Complete thymectomy group (microscopic lymphoid remnant)						Sham thymectomized group					
	Age at thymectomy (weeks)						Age at thymectomy (weeks)					
	Spleen	Bone marrow	Lymph nodes	Kidney	Serum		Spleen	Bone marrow	Lymph nodes	Kidney	Serum	
Group C												
Mice killed	5	2	0	1	0	0	4	0	0	0	0	0
one month	4	2	0	2	—	—	4	0	0	0	0	0
following	4	2	0	1	0	0						
operation	4	2	1	0	0	0						
	(9)	(8)	(2)	(2)	(1)	(0)						
	(5)	(4)	(0)	(1)	(0)	(0)						
	(4)	(2)	(1)	(0)	(0)	(0)						
	6	8	1	2	1	0	9	2	0	2	0	0
Group D												
Mice killed	5	4	0	2	0	0	4	2	1	2	0	0
2 months	5	4	1	2	0	0	4	1	1	4	0	0
following	4	4	1	2	0	0	4	4	0	2	0	0
operation	(5)	(2)	(0)	(2)	(0)	(0)	4	2	1	2	0	0
	(5)	(2)	(1)	(2)	(0)	(0)						
	6	4	1	2	0	0	9	4	0	1	0	0
	6	2	1	0	0	0	4	8	—	1	0	0
Group E												
Mice killed	5	2	0	0	0	0	4	4	0	2	0	0
3 months	5	2	1	1	0	0	4	4	0	1	0	0
following	4	2	1	2	0	0	4	4	0	2	0	0
operation	4	2	0	2	0	0	4	2	0	2	0	0
	4	1	0	1	0	0						
	4	1	1	1	1	0						
	4	2	0	2	1	0						
	(5)	(4)	(0)	(1)	(0)	(0)						
	(4)	(4)	(—)	(4)	(0)	(0)						
	(4)	(4)	(0)	(1)	(0)	(0)						

TABLE II.—*MuLV-gs Antigen Titres of Tissues and Serum from Neonatally and Sham Thymectomized AKR/CRC Mice (Results Expressed as Reciprocal of I.F.A. Titre)*

Time of sacrifice after thymectomy	Complete thymectomy group (microscopic lymphoid remnant)						Sham thymectomized group					
	Spleen	Bone marrow	Lymph node	Kidney	Liver	Serum	Spleen	Bone marrow	Lymph node	Kidney	Liver	Serum
Group A 1 week	4 0 4	— — —	— — —	0 0 0	1 0 0	— 0 0	2* 2* —	— — —	— — —	1 0 2	1 0 4	0 0 0
Group B 2 weeks	2 1 (4)	0 0 (1)	— — —	0 1 (0)	1 0 (0)	0 0 (0)	0* 1* 1*	— — —	— — —	1* 1* 0*	1* 1* 0*	0* 0* 0*
Group C 1 month	2 1 1 2 1 (2) (2) (2) (2) (2)	1* 1* — 1* 1* (1*) (1*) (1*) (1*) (1*)	— — — — — (-) (-) (-) (-) (-)	0 0 0 1 16 (0) (0) (0) (0) (0)	0 0 0 0 0 (0) (1) (1) (1) (1)	0 0 0 0 0 (0) (0) (0) (0) (0)	4 2 2 4 2* 2* 4* 4*	— — — — — — — —	— — — — — — — —	1 0 1 1 2 2 2 2	0 0 0 0 1 1 1 1	0 0 0 0 0* 0* 0*
Group D 2 months	4 4 4 (4) (2) (2)	2* 2* — (-) (2*) (2*)	4* — — (4*) — —	2 1 0 (0) (0) (0)	2 1 0 (0) (0) (1)	0 0 0 (0) (0) (0)	2 1 2 2 2 2	2* 2 1*	2* — —	1 0 0 0 0 0	2 0 1 0 0 0	0 0 0 0 0 0
Group E 3 months	2 2 2 (2)	2* 1 2* (2*)	1 1 2* (2*)	0 0 0 (0)	0 1 2 (0)	0 0 1 (0)	2 1 2 2 2 4	— — — — — —	— — — — — —	0 1 1 1 1 0	2 0 0 0 1 0	0 0 0 0 0 0

* Pools represent 2 or 3 animals

be that the target cells for neoplastic conversion are removed. However, Miller (1961) clearly demonstrated that by grafting CBA/H-T6 and AKR thymuses into thymectomized (AKR $\times$ T6) F1, the resulting leukaemic cells were of host origin which suggested that the "thymic influence responsible for leukaemic change was a non-cellular indirect one".

It is now established that AKR mice are not permanently tolerant to MuLV since anti-MuLV gs antibodies are formed and can be demonstrated as antibody-antigen complexes in the kidneys from about 3 months of age (Oldstone *et al.*, 1972). Since MuLV-gs antigen levels are unchanged in non-leukaemic thymectomized AKR mice, we are at present looking for antibodies in these animals to see whether tolerance to the virus has been maintained and whether this is in fact related to tumour development—a view that has been suggested earlier (Barnes and Tuffrey, 1974).

REFERENCES

- BARNES, R. D. & TUFFREY, M. (1974) Lymphoma Susceptibility of the AKR Mouse Strain Acquired Before the Stage of Implantation. *Br. J. Cancer*, **26**, 400.
- BARNES, R. D., TUFFREY, M. & FORD, C. R. (1973) Suppression of Lymphoma Development in Tetraparental AKR Mouse Chimaeras Derived from Ovum Fusion. *Nature, New Biol.*, **244**, 282.
- FURTH, J., SEIBOLD, H. R. & RATHBONE, R. R. (1933) Experimental Studies on Lymphomatosis of Mice. *Am. J. Cancer*, **19**, 521.
- GROSS, L. (1951) "Spontaneous" Leukaemia Developing in C3H Mice following Inoculation in Infancy, with AK-leukemic Extracts or AK-embryos. *Proc. Soc. exp. Biol., N.Y.*, **76**, 27.
- GROSS, L. (1957) Studies on the Nature and Biological Properties of a Transmissible Agent causing Leukemia following Inoculation into Newborn Mice. *Ann. N.Y. Acad. Sci.*, **68**, 501.
- HILGERS, J., DECLEVE, A., GATESLOOT, J. & KAPLAN, H. S. (1974) Murine Leukaemia Virus Group-specific Antigen Expression in AKR Mice. *Cancer Res.*, **34**, 2553.
- LAW, L. W. & MILLER, J. H. (1950) The Influence of Thymectomy on the Incidence of Carcinogen-induced Leukemia in Strain DBA Mice. *J. natn. Cancer Inst.*, **11**, 425.
- MCENDY, D. P., BOON, M. C. & FURTH, J. (1944) On the Role of Thymus, Spleen and Gonads in the Development of Leukemia in a High-Leukemia Stock of Mice. *Cancer Res.*, **4**, 377.
- MILLER, J. F. A. P. (1960) Recovery of Leukaemogenic Agent from Non-leukaemic Tissues of Thymectomised Mice. *Nature, Lond.*, **187**, 703.
- MILLER, J. F. A. P. (1961) Analysis of the Thymus Influence in Leukaemogenesis. *Nature, Lond.*, **191**, 248.
- NAKAKUKI, K., SHISA, H. & NISHIZUKA, Y. (1967) Prevention of AKR Leukemia by Thymectomy at Varying Ages. *Acta Haemat.*, **38**, 317.
- OLDSTONE, M., TISHON, A., TONIETTI, G. & DIXON, F. (1972) Immune Complex Disease Associated with Spontaneous Murine Leukaemia Incidence and Pathogenesis of Glomerulonephritis. *Clin. Immunol. Immunopath.*, **1**, 6.
- ROWE, W. P. & PINCUS, T. (1972) Quantitative Studies of Naturally Occurring Murine Leukaemia Virus Infection of AKR Mice. *J. exp. Med.*, **135**, 429.
- TUFFREY, M., KINGMAN, J. & BARNES, R. D. (1973) The Failure to Transfer the NZB Disease following Implantation of NZB Thymus containing Chambers. *Eur. J. Immunol.*, **3**, 519.
- WOODS, A., WIVEL, N. A., MASSICOT, J. G. & CHIRIGOS, M. A. (1970) Characterisation of a Rapidly Growing AKR Lymphoblastic Cell Line Maintaining Gross Antigens and Viral Replication. *Cancer Res.*, **30**, 2147.