MURINE LEUKAEMIA VIRUS EXPRESSION IN THE AKR FOLLOWING THYMECTOMY

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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 onco-
genomic 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 develop-
ment of the lymphoma which character-
izes the AKR (Furth, Seibold and Rath-
bone, 1933) still remains to be deter-
mined (Miller, 1961). One possible effect of thymectomy may be to suppress the titres of murine leukaemia virus group specific antigens and this has been exam-
ined 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 thymecto-
mized 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 vari-
ous 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 investiga-
ted. 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 anti-
genoms 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 "leukemic 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      | nt    | nt  |
| 2 (9) days          | 4              | 0      | 0      | 0     | 0      | nt    | nt  |
| 3 (+ 100)           | 3              | 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. Title) |
|---|---|---|---|---|---|---|---|--- |
| 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 | 2 | 1 | 2 | 0 | 0 |
| Group B | | | | | | | | | | | |
| Mice killed | 7 | 8 | 1 | 1 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| 2 weeks following operation | 6 | 4 | 0 | 1 | 1 | 0 | 4 | 2 | 1 | 0 | 0 | 0 | 0 |

Note: The table shows the titres of MuLV-gs in various tissues and serum of sham and thymectomized AKR/CRC mice. The titres are expressed as the reciprocal of the I.F.A. titre. The data is divided into groups based on the age and time following thymectomy.
### Table I—continued.

| Group | Age at thymectomy (weeks) | Spleen | Bone marrow | Lymph nodes | Kidney | Serum | Age at thymectomy (weeks) | Spleen | Bone marrow | Lymph nodes | Kidney | Serum |
|-------|---------------------------|--------|-------------|-------------|--------|-------|---------------------------|--------|-------------|-------------|--------|-------|
|       |                           |        |             |             |        |       |                           |        |             |             |        |       |
| C     | 5                         | 2      | 1           | 0           | 0      |       | 4                         | 0      | 0           | 0           | 0      | 0     |
|       | Mice killed               | 4      | 2           | 0           | 2      |       | 4                         | 0      | 0           | 0           | 0      | 0     |
|       | one month                 | 4      | 2           | 0           | 1      | 0     | 4                         | 0      | 0           | 0           | 0      | 0     |
|       | following                 | 4      | 2           | 1           | 1      | 0     | 4                         | 0      | 0           | 0           | 0      | 0     |
|       | operation                 | (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     |
| D     | 5                         | 4      | 0           | 2           | 0      |       | 4                         | 2      | 1           | 2           | 0      | 0     |
|       | Mice killed               | 5      | 4           | 1           | 2      | 0     | 4                         | 1      | 1           | 4           | 0      | 0     |
|       | 2 months                  | 4      | 4           | 0           | 1      | 2     | 4                         | 0      | 2           | 0           | 0      | 0     |
|       | following                 | (5)    | (2)         | (0)         | (2)    | 0     | (0)                       | (0)    |             |             |        |       |
|       | operation                 | (5)    | (2)         | (0)         | (2)    | 0     | (0)                       | (0)    |             |             |        |       |
|       | 6                         | 4      | 1           | 2           | 0      | 0     | 9                         | 4      | 0           | 1           | 0      | 0     |
|       |                            | 6      | 2           | 1           | 0      | 0     | 4                         | 8      | 1           | 0           | 0      | 0     |
| E     | 5                         | 2      | 0           | 0           | 0      | 0     | 4                         | 4      | 0           | 2           | 0      | 0     |
|       | Mice killed               | 5      | 2           | 1           | 1      | 0     | 4                         | 4      | 0           | 1           | 0      | 0     |
|       | 3 months                  | 4      | 2           | 1           | 2      | 0     | 4                         | 0      | 2           | 0           | 0      | 0     |
|       | following                 | 4      | 2           | 0           | 0      | 0     | 4                         | 2      | 1           | 2           | 0      | 0     |
|       | operation                 | 4      | 1           | 0           | 1      | 0     | 4                         | 0      | 1           | 1           | 0      | 0     |
|       |                            | 4      | 2           | 0           | 2      | 1     | 4                         | 0      | 0           | 0           | 0      | 0     |
|       |                            | (5)    | (4)         | (0)         | (1)    | (0)  | (0)                       | (0)    |             |             |        |       |
|       |                            | (4)    | (4)         | (0)         | (0)    | (0)  | (0)                       | (0)    |             |             |        |       |
|       |                            | (4)    | (4)         | (0)         | (0)    | (0)  | (0)                       | (0)    |             |             |        |       |
| 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     1       0       0       | 2*                        -    -    -    1     1       0       0       |
|                                   | 0              -    -    -   0     0       0       0       | 2*                        -    -    -    0     2       0       0       |
|                                   | 4              -    -    -   0     0       0       0       | -                         -    -    -    0     0       0       -       |
|                                   | 2              -    -    -   0     1       2       0       | 2*                        -    -    -    0     2       0       0       |
|                                   | 1              -    -    -   1     0       0       0       | 1*                        -    -    -    2     4       0       0       |
|                                   | (4)            (1)  (0)  (0) (0)                          | 0*                        -    -    -    0     2       0       0       |
| Group B 2 weeks                   |                                                          |                             |
|                                   | 2              0    -    -    0     1       0       0       | 0*                        -    -    -    1     1       0       0       |
|                                   | 1              0    -    -    1     0       0       0       | 1*                        -    -    -    1     1       0       0       |
|                                   | (4)            (1)  (0)  (0) (0)                          | 1*                        -    -    -    0     1       0       0       |
| Group C 1 month                   |                                                          |                             |
|                                   | 2              1*   -    -    0     0       0       0       | 0*                        -    -    -    0     2       0       0       |
|                                   | 1              1*   -    -    0     0       0       0       | 4*                        -    -    -    2     1       0       0       |
|                                   | (2)            (1*) (-)  (0) (0)                          | 2*                        -    -    -    2     1       0       0       |
|                                   | (2)            (1*) (-)  (0) (0)                          | 0*                        -    -    -    0     2       0       0       |
|                                   | (2)            (1*) (-)  (0) (0)                          | 1*                        -    -    -    0     1       0       0       |
|                                   | (2)            (1*) (-)  (0) (0)                          | 2*                        -    -    -    2     1       0       0       |
|                                   | (2)            (1*) (-)  (0) (0)                          | 4*                        -    -    -    4     1       0       0       |
| Group D 2 months                  |                                                          |                             |
|                                   | 4              2*   4*   -    2     2       0       0       | 2*                        -    -    -    1     2       0       0       |
|                                   | 4              2*   -    -    1     1       0       0       | 1*                        -    -    -    0     0       0       0       |
|                                   | 4              -    -    -    1     0       0       0       | 2                         -    -    -    0     0       0       0       |
|                                   | (4)            (-)  (4*) (0)  (0) (0)                    | 2*                        -    -    -    2     0       0       0       |
|                                   | (2)            (2*) (0)  (0)  (0)                         | 2*                        -    -    -    2     0       0       0       |
|                                   | (2)            (2*) (0)  (0)  (0)                         | 1*                        -    -    -    2     0       0       0       |
| Group E 3 months                  |                                                          |                             |
|                                   | 2              2*   1    0    0     0       0       0       | 2                         -    -    -    0     2       0       0       |
|                                   | 2              1    1    0    0     1       0       0       | 1*                        -    -    -    0     1       0       0       |
|                                   | 2              2*   2*   0    2     1       0       0       | 2*                        -    -    -    1     1       0       0       |
|                                   | (2)            (2*) (2*) (0) (0) (0)                     | 2                         -    -    -    1     1       0       0       |
|                                   | (2)            (2*) (2*) (0) (0) (0)                     | 2                         -    -    -    1     1       0       0       |
|                                   | (2)            (2*) (2*) (0) (0) (0)                     | 4                         -    -    -    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 × T6) F1, the resulting leukemic cells were of host origin which suggested that the "thymic influence responsible for leukemic 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).

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