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The Apparent Lack of Association of Antibody Activity to Murine Leukemia Virus and Lymphoma Development in (AKR × CBA) F_1 Mice

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Abstract—*We have in the past proposed the hypothesis that virus-associated lymphoma development in AKR mice might be in some way related to the occurrence of antibody activity to murine leukemia virus (MuLV). Here we have attempted to test this hypothesis using the ^{125}I-protein A radioimmunoassay recently developed in this laboratory. Previous findings in naturally derived (AKR × CBA/H-T6Crc) F_1 confirmed the presence of MuLV, moreover, at levels comparable to or indeed occasionally in excess of, the AKR. Despite this, tumours were uncommon during the first year of life and no antibody activity could be detected at this time. It was later noted that lymphomas did occur in the F_1 but in the second year. To test our hypothesis that there may be a possible association between tumour resistance and antibody activity, these hybrids have been investigated further during this later period.*

In addition, we have examined other groups of mice in which we have attempted to prematurely induce antibody activity experimentally.

In both these situations there was no apparent relationship between tumour development and the presence of anti-MuLV activity.

INTRODUCTION

OUR INTEREST in antibody activity to MuLV was stimulated by the remarkable lack of renal antigen–antibody complexes observed in a group of very old embryo-aggregation-derived tetraparental AKR↔CBA chimaeras [1, 2]. Although there were various possible explanations, absence of detectable antibody activity has been held to be responsible [3–5]. The fact that tumours were delayed, or in certain cases absent, was particularly remarkable since these chimaeras were shown to be predominantly AKR in composition [6–8]. Apparent lack of antibody and delayed tumour development in these chimaeras led us to suggest that the two phenomena might be related [9]. There was further evidence to support this possibility in (AKR × CBA) F_1 mice in which tumours were uncommon in the first year of life, a period during which no antibody activity could be detected [10].

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It is now known that tumours do develop in the (AKR × CBA) F_1, but during their second year. Since we have recently described an improved method for detecting antibodies to MuLV we have further investigated the antibody status of these mice, paying particular attention to the second year of life when tumours are expected. In addition, attempts have been made to experimentally induce anti-MuLV activity early in the (AKR × CBA) F_1 to see if this results in earlier tumour development.

MATERIALS AND METHODS

Mice

Conventionally housed reciprocal crosses between AKR/Crc and CBA/H-T6Crc (AKR and CBA in text) were used.

1. *Untreated (AKR × CBA) F_1*

Seventy-four untreated reciprocal (AKR × CBA) F_1 aged 4–112 weeks were bled and

their sera tested for antibody activity to MuLV using the ^{125}I-protein A radioimmunoassay [11].

Detection of anti-MuLV activity has been modified from the earlier method described by Ihle [12]. The original competition radioimmunoassay against radiolabelled intact Gross virus has now been replaced by a quicker assay using radiolabelled protein A, a substance derived from *Staphylococcus aureus* which is known to bind specifically to the Fc region of many immunoglobulins. This method has been described elsewhere [11] but is basically that of Colombatti and Hilgers [13] who used it to detect certain type B and type C RNA tumour viruses. The wells of a polystyrene microtitre plate were first coated with Gross virus and then, after washing, treated with the heat inactivated serum to be tested. After the specific antibody–antigen reaction has taken place any residual serum was washed off leaving behind antibody–antigen complexes which were then reacted with ^{125}I-labelled protein A. After further washing the ^{125}I-labelled protein A was eluted and radioactivity measured. The amount of protein A calculated from this was, in turn, directly related to amount of antibody. Adsorption of antisera with Gross virus confirmed the specificity of the anti-MuLV screening procedure [11].

2. *Treated* (*AKR* × *CBA*) F_1

The aim here was to transfer anti-MuLV activity by means of 'primed' spleen cells from donor CBA immunized with a single dose of gradient-purified AKR virus injected one year before. (Using the ^{125}I-protein A radioimmunoassay, immunity can be detected after this interval [11].)

Spleen cell preparations were made using a Potter Mill from immunized and age-matched untreated controls. Recipients were injected at 6 weeks of age and in certain cases irradiated (400 rad) immediately prior to adoptive immunization in an attempt to increase the survival of the grafted cells (Table 1).

Samples of tail blood from one or two animals from each group were taken for cytogenic analysis 7 and 8 weeks after adoptive immunization. Examination for the presence of the CBA (T6T6) chromosome marker, i.e. persistence of injected cells, was performed according to the method of Burtenshaw [14]. Serum samples from each group were also taken 3 weeks after treatment to test for antibody response. Individual mice were examined clinically throughout life for signs suggestive of a tumour. When these became apparent the mice were sacrificed, serum obtained for antibody assay, and tumours were confirmed by histology.

Table 1. *Groups of treated* (*AKR* × *CBA*) F_1

Group (No. of mice)	Irradiation of recipient (F_1) (400 rad)	Unprimed CBA spleen cells (3×10^7)	Primed CBA spleen cells (2.6×10^7)
A (19)	−	−	+
B (15)	+	−	−
C (20)	+	−	+
D (16)	−	−	−
E (16)	+	+	−

RESULTS

1. *Untreated* (*AKR* × *CBA*) F_1

The incidence of lymphomas in untreated F_1 (mean age at death 84.4 weeks) and AKR (mean age at death 48.6 weeks) is shown in Fig. 1. This figure incorporates data which has been published previously [10] and is only presented here for completion and comparison.

Levels of anti-MuLV activity (as pg of ^{125}I-protein A bound by a 1/40 dilution of serum) in 74 AKR × CBA reciprocal crosses (Fig. 2) ranged from 0 to 144 pg (mean 12.3 pg) over an age range of 4–112 weeks. The majority of values were low over the entire age span but there were a few sporadic

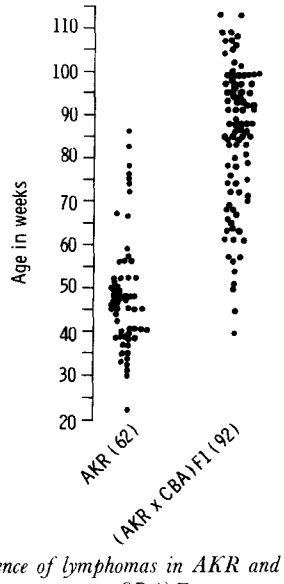

Fig. 1. *Incidence of lymphomas in AKR and reciprocal* (*AKR* × *CBA*)F_1.

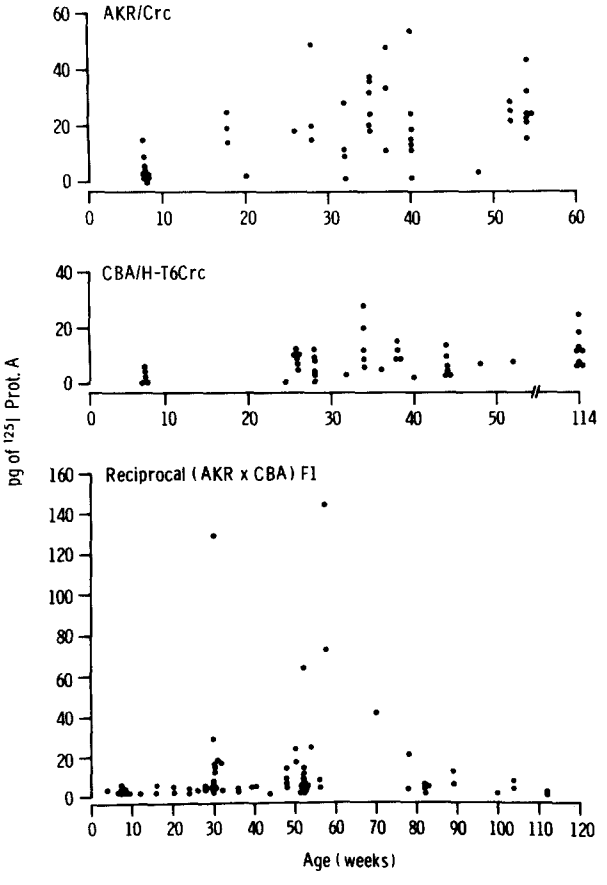

Fig. 2. Anti-MuLV activity (^{125}I Prot. A assay) in AKR, CBA and reciprocal $(AKR \times CBA)F_1$.

high values. When compared to the levels of anti-MuLV activity in the parental strains (also shown in Fig. 2) it can be seen that, with a few notable exceptions, the levels of antibody activity in the F_1 were closer to those found in the CBA than the AKR, and that this pattern appears to continue throughout life.

2. *Treated* $(AKR \times CBA)$ F_1

Table 2 shows mean antibody levels and the percentage chimaerism from test bleeds taken 3–8 weeks after treatment. Sera were pooled from two sets of animals from each treatment group.

It can be seen that antibody levels of recipients of primed cells 3 weeks after injection were similar whether or not they had been irradiated. The inoculation of unprimed cells into irradiated animals apparently elicited a slight response in one of the two sets of animals examined.

Cultures of blood taken from recipients 7–8 weeks after treatment confirmed the continuing presence of T6T6 cells in groups A and E, although, unfortunately, no suitable cells were found in the cultures from group C.

The incidence of lymphoma and the range of anti-MuLV activity found in the various groups of experimental mice at autopsy is summarized in Table 3. It is apparent from the results that the administration of primed or unprimed spleen cells with or without irradiation did not significantly alter the incidence of lymphoma within the various groups. The range of antibody levels in post mortem serum samples was very varied and did not appear to be related to time of death with lymphoma. The lowest mean antibody levels were seen in mice injected with primed spleen cells with [group C (4.8)] or without [group A (8.5)] irradiation whilst those receiving irradiation alone (group B) gave the highest levels (26.5 pg).

The antibody level at autopsy differed from those seen 3 weeks after treatment when the groups that had been injected with primed cells gave the highest response (71 and 74 pg) whether or not they were irradiated, and irradiated animals that did not receive cells gave the lowest response (13 pg). The intermediate response given by the mice receiving the normal CBA spleen cells (30 pg) was possibly attributable to the fact that MuLV expression is absent in the CBA (unpublished data) and that their injected cells were therefore capable of reacting to virus expressed in the F_1. The mean values for the untreated controls in group D were similar at the test bleed at 9 weeks of age (19 pg) and at sacrifice with lymphoma (14.8 pg). These were also comparable with the mean values found in the first group of 74 untreated mice throughout the 2-yr period during which they were tested (12.3 and 16.1 pg).

DISCUSSION

In any discussion of the present, or for that matter, our earlier results, it must be stressed that we have continued to work with one particular subline of CBA, namely CBA/H-T6Crc. The CBA/H-T6Crc appears to lack expression of ecotropic or xenotropic MuLV which may be the factor that renders them capable of developing a good immune response to MuLV when injected in adult life [10, 11]. Indeed, we have previously shown that adult CBA injected with MuLV develop high levels of antibody (binding in excess of 200 pg ^{125}I-protein A). Moreover, we feel justified in considering them a 'negative'

Table 2. Antibody levels and percentage chimaerism 3–8 weeks after treatment

Group	Treatment	Anti-MuLV activity	(mean)	Percentage chimaerism†
A	Primed T6 spleen cells only	45 97	(71)	16 (3/19)
B	Irradiation only	17 8	(13)	(0/23)
C	Primed T6 spleen cells and irradiation	85 63	(74)	no suitable cells
D	No treatment	13 24	(19)	(0/9)
E	Unprimed T6 spleen cells and irradiation	41 19	(30)	9.5 (2/21) 20 (3/15) 44 (4/13) 27 (3/11)

*pg bound ^{125}I-protein A, 3 weeks after treatment.
†No. of T6T6 cells/No. of F_1 cells, 7–8 weeks after treatment.

Table 3. Incidence of lymphoma and anti-MuLV levels in treated (AKR × CBA) F_1 and controls

Group	Treatment	Confirmed lymphoma	Age at sacrifice (wks)	Anti-MuLV levels* (mean)	No. tested	Range	(mean) (±S.E.)
A	26×10^6 primed CBA spleen cells	17/19 (89%)	51–100	(73.3)	12	1–54	(8.5±4.3)
B	Irradiation (400 r)	15/15 (100%)	48–104	(74.1)	12	2–119	(26.5±9.8)
C	26×10^6 primed CBA spleen cells + irradiation (400 r)	19/20 (95%)	55–103	(73.9)	17	0–22	(4.8±1.3)
D	No treatment	15/16 (94%)	62–102	(84.4)	14	0–37	(14.3±3.1)
E	30×10^6 normal CBA spleen cells + irradiation (400 r)	14/16 (88%)	64–103	(86.5)	13	1–71	(20.5±6.5)

*pg bound ^{125}I-protein A.

strain in terms of spontaneous anti-MuLV levels (generally binding less than 20 pg ^{125}I-protein A).

The 'spontaneous' development of antibody activity to MuLV in most strains of mice poses interesting questions. In spite of early acquisition upon the germ line, tolerance to endogenous C-type murine leukaemia virus is a short-lived phenomenon not yet understood. One possible explanation is the delay of antigenic expression of virus [15] and we have suggested that it is this which renders the murine host 'tolerant' to the virus [16]. We have been particularly interested in the AKR strain which expresses high levels of MuLV, develops lymphoma and produced spontaneous anti-MuLV antibodies (42% of 48 AKR tested bound more than 20 pg ^{125}I-protein A) [11]. Although the development of anti-MuLV reactivity is a common phenomenon [17], we have described two notable exceptions [AKR↔CBA T6T6 chimaeras and (AKR × CBA) F_1] and originally suggested that their lack of antibody activity might be in some way related to tumour resistance [3–5, 9, 10]. Had our hypothesis been correct we would have expected to find anti-MuLV activity in the second year of life in the (AKR × CBA) F_1, the time when tumours develop: generally this was not the case. Levels of bound ^{125}I-labelled protein A were similar in the first (tumour 'resistant') and second (tumour 'susceptible') years of life. However, it must remembered that the ^{125}I-protein A radioimmunoassay used here detects only free antibody, mainly of the IgG immunoglobulin

class and it is conceivable that virus–antibody complexes or antibody of other immunoglobulin classes may have been present.

The hypothesis that anti-MuLV activity and tumour development are related also appears to have been disproven by the adoptive immunisation experiments described here. Although it appears from the 3-week post-treatment test sera that the recipients were responding with antibody production, there was no difference in tumour incidence between the groups, neither was there significant antibody production within any of the groups tested at autopsy. Thus in both the natural and experimental situation described here, it appears that we have failed to demonstrate any association between anti-MuLV activity and tumour development. However, it could be argued that lymphomas in the F_1 at around 80 weeks of age, due to blocking factors which involve natural anti-viral antibodies, leads to accelerated lymphoma development. Equally, it can be argued that adoptively transferred spleen cell induced antibody may be of a different kind to the 'natural' antibodies and these have no blocking effect. Apart from these possibilities the results have failed to demonstrate any association between anti-MuLV activity and tumour development.

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