# Antibody Response and Leukemia Development in Mice Inoculated Neonatally with the Moloney Virus[1]

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SUMMARY

Mice infected neonatally with homogenates from lymphomas induced by the Moloney virus showed a deficient or delayed antibody response, measured by the indirect fluorescent antibody test against Moloney lymphoma target cells in comparison with mice infected as adults. In neonatally infected mice there was a correlation between the development of antibodies and the length of the preleukemic latency period.

Adult mice inoculated with homogenates or cells from lymphomas induced by the Moloney virus form antibodies capable of neutralizing the virus, killing Moloney lymphoma cells *in vitro* in the presence of complement and specifically attaching to the membrane of living Moloney cells, as demonstrated by the indirect fluorescent antibody reaction (4, 5). The treated mice are also resistant to Moloney lymphoma isografts (4, 15). Analogous reactions have been demonstrated with other virus induced mouse leukemias (for a review see Ref. 10).

While these studies have conclusively shown that the fate of a Moloney lymphoma isograft can be influenced by immunologic means, it is not known what role immunologic reactions may play during leukemogenesis itself. Their possible importance is suggested by the fact that newborn animals are considerably more susceptible to leukemogenesis than adults, the latter being quite resistant in some strains (2, 9). This is even more pronounced with some other oncogenic viruses and particularly striking in the mouse-polyoma system. It cannot be ruled out, however, that the age dependence of susceptibility to viral oncogenesis may be due (or partly due) to other differences than those concerning immunologic responsiveness.

Infection of *adult* mice with Moloney virus is regularly followed by persistent antibody production after a latency period which is inversely related to the virus dose. Subsequently, the antibody titer rises to a high level that was found to be maintained during the whole observation period (approximately 1 year), and probably through the entire lifetime of the animal. This antibody response can be adapted to serve as a sensitive virus assay system (5).

The present paper describes studies concerning the antibody response of mice inoculated with Moloney virus *neonatally*. There were considerable differences compared to adults and a certain relationship could be demonstrated between antibody response and the length of the latency period prior to leukemia development.

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## METHODS

Antibodies reacting specifically with Moloney lymphoma cells were demonstrated by the indirect fluorescent antibody method. Moloney lymphoma cells of the YLD type were used as targets, derived from the C57leaden strain. Living cell suspensions were prepared from a mechanically dissociated solid tumor. Samples containing $2 \times 10^6$ cells were incubated with 0.05 ml undiluted test serum or control serum for 20 min at 37°C. After washing they were stained with fluorescein-conjugated rabbit antimouse serum at 37°C for 20 min. The proportion of cells showing dye binding at the cell membrane was established by differentially counting 100–150 cells in each sample, using the criteria described previously (4). A "fluorescence index" (F.I.) was calculated for each antiserum sample, by subtracting the percentage of unstained cells in the test sample from the corresponding control value and dividing the difference with the control figure.

According to our previous experience, when the F.I. of undiluted sera reaches 0.8–0.9, further differences in the strength can be measured by titrating the sera. Titer variations between 1:8 and 1:64 were obtained with YLD target cells. This was not done in this work and high values therefore merely represent a threshold above which no further comparisons were considered necessary for the purposes of the present paper.

## RESULTS

In experiments designed to induce leukemias in various genotypes, virus was inoculated into newborn mice. Two different preparations were used, obtained by standard procedure, and preserved at −79°C. Each mouse received 0.1 ml, diluted 1:5, 1:10, or 1:100. Exploratory tests showed that such mice were char-

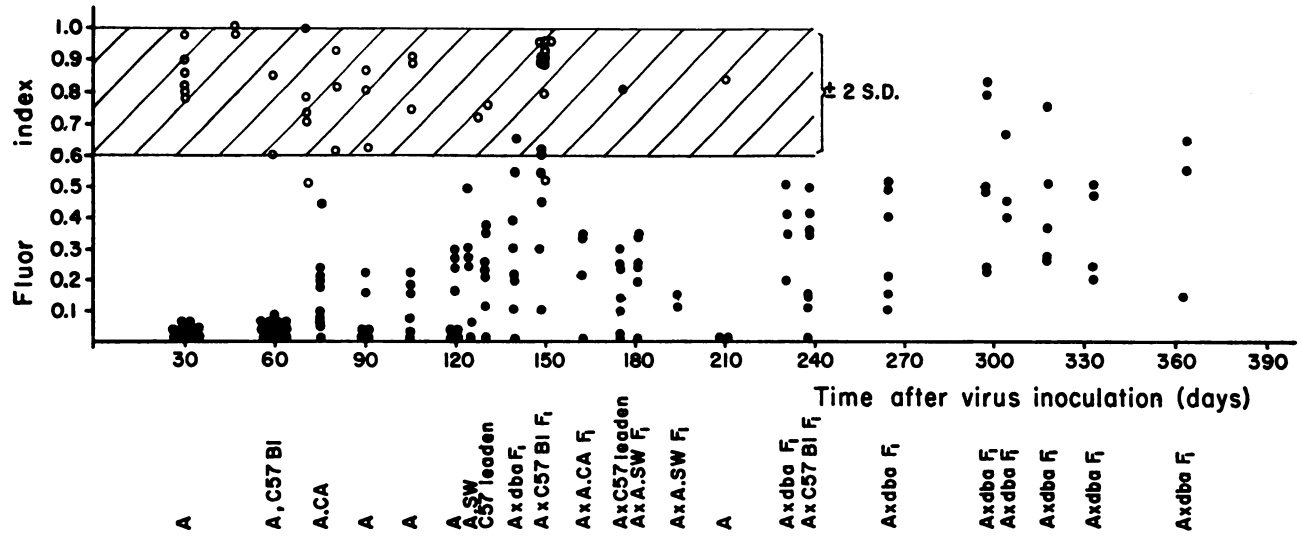


CHART 1.—Fluorescent index obtained with sera of individual mice, sampled at different times after Moloney virus infection. *Open circles*, strain A mice infected as adults (6–10 weeks old); *closed circles*, mice infected neonatally, derived from the strains indicated.

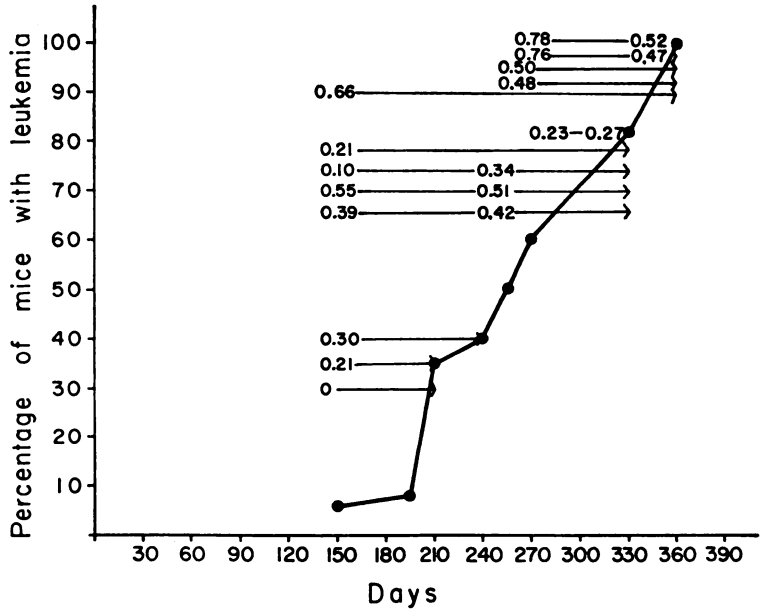


CHART 2.—Cumulative incidence of leukemia in 22 A × DBA $F_1$ mice, infected neonatally with 0.1 ml of a $1:10^{-3}$ diluted virus preparation. The fluorescence index of some mice is entered at the time for serum sampling. The latency period of the corresponding leukemia is indicated by the *arrow*.

acterized by a high individual variation with regard to the development of antibody response, in contrast to mice inoculated as adults. Some remained entirely negative and those that reacted did so with considerable delay.

A comparison of the response of adult A mice with neonatally infected animals of various genotypes is given in Chart 1. All animals have been tested individually. The prompt reaction of adult mice is in sharp contrast to the delay and wide scatter of the response in neonatally infected mice where high values were seen only occasionally. The antibody level attained 4–5 months after infection in a given animal was maintained at comparable level as shown by repeat tests, exemplified in Chart 2. This is reminiscent of the findings of Rubin *et al.* (13) with the avian lymphomatosis virus, finding no substantial changes in antibody titer over an interval of 5 months. The data suggest a difference in the response of mice of different strains. A.SW and C57leaden reacted better than A and C3H, as shown in Table 1.

Susceptibility to leukemogenesis in C3H and C57leaden was also compared in one experiment. Five newborn C3H litters and 4 C57leaden litters were injected with virus at the same time. After a 9-month observation period, all (16/16) C3H mice succumbed with leukemia (mean latency period 139 days; range 112–181). Only 43% (6/14) C57leaden developed leukemia (mean latency period 144 days; range 116–181).

In all groups of animals inoculated neonatally, mice

responding with high antibody levels tended to survive longer than poor antibody producers. Chart 2 shows the cumulative leukemia incidence in 22 A × DBA $F_1$ mice. The antibody level of randomly tested animals is indicated by the F.I. The horizontal position of the values indicates the time for serum sampling while their vertical position corresponds to the appearance of leukemia in the same animal in relation to the cumulative incidence curve.

Chart 3 shows the F.I. of all tested sera, in relation to the latency period prior to leukemia development. There was a significant positive correlation between the F.I. values and the length of the latency period prior to leukemia development. ($n = 48$, $r = 0.76$, $z \pm \sigma_z = 0.89 \pm 0.15$, $P \leqq 0.01$). A more detailed statistical analysis will be presented in a future paper.

Since the sera tested were sampled at different times, the question arose whether the correlation shown in Chart 3 could be an artifact, related to the interval between sampling and the manifestation of leukemia and due to the fact that the latter leads to a paralysis of antibody formation. Chart 4 shows the same data plotted as a function of time between sampling and the appearance of leukemia. Since the values are randomly distributed, such an artifact seems to be excluded.

TABLE 1

| Genotype | No. of mice[a] | Fluorescence index | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0–0.1 | 0.1–0.2 | 0.2–0.3 | 0.3–0.4 | 0.4–0.5 | ≧0.5 |
| A.SW | 7 | 2 | | 3 | | 1 | 1 |
| A | 27 | 14 | 5 | 5 | 3 | | |
| C3H | 16 | 10 | 3 | 3 | | | |
| C57leaden | 27 | 3 | 6 | 7 | 6 | 3 | 2 |

[a] Number of animals yielding the indicated range of fluorescence index when undiluted sera are tested against YLD target cells. The mice were injected neonatally with 0.1 ml of 1:5 diluted homogenate of virus induced leukemia YHA, and the serum collected 4 months later.

## DISCUSSION

A possible role played by immunologic tolerance in viral oncogenesis was already suggested by findings in a number of other tumor virus systems. Rubin *et al.* (13, 14) found that infection transmitted through the egg was associated with persistent viremia, lack of antiviral antibody and a sixfold increase of visceral lymphomatosis, all in comparison with birds infected after hatching. Axelrad's results (1) suggest that the vertical transmission of the Gross virus in AKR mice leads to tolerance to the specific antigen of Gross lymphoma cells, in contrast to the sensitized state in mice of other strains, exposed to the virus as infants or adults, but at a later stage of their development. In these studies the immunologic status of the mice was judged by the number of spleen colonies formed after intravenous inoculation of isologous Gross lymphoma cells. Mice inoculated with polyoma virus neonatally develop high titers of antiviral antibodies and

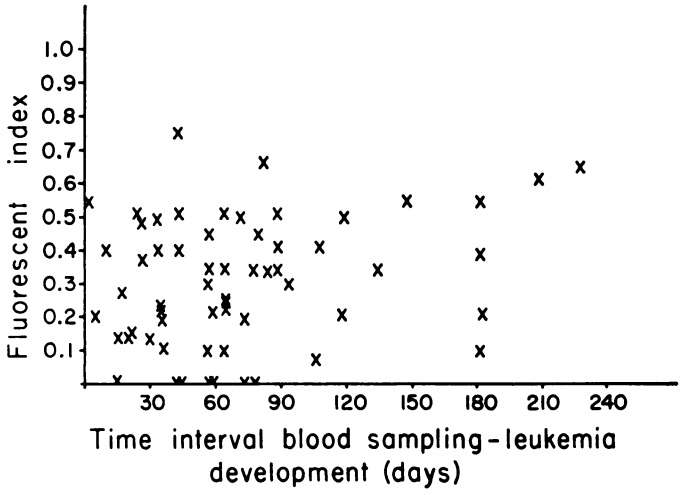


CHART 4.—Fluorescence index obtained with sera of individual mice infected neonatally as related to the time interval between blood sampling and the appearance of leukemia.

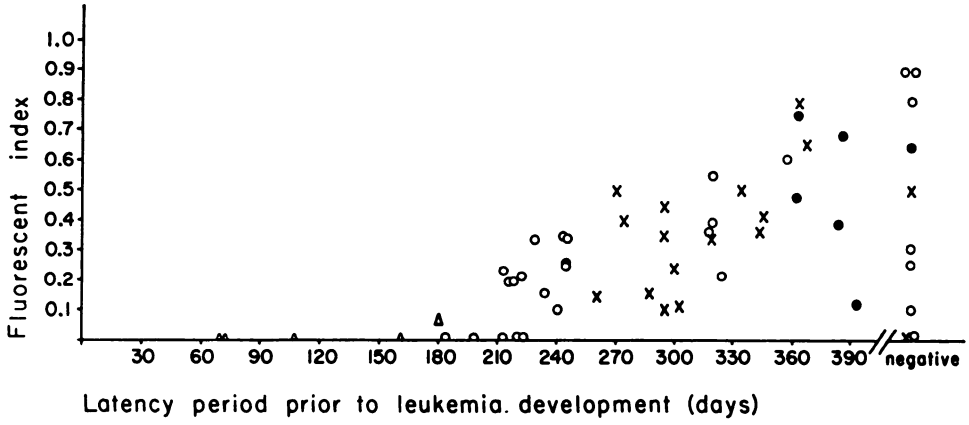


CHART 3.—Fluorescence index obtained with sera of individual mice infected neonatally. The data plotted against the latency period of leukemia development in the same mice. Interval between virus infection and serum sampling: △ less than 100 days; ○, 100–200 days; ×, 200–300 days; ●, 300–400 days. The right-hand column of symbols designated as "negative" stands for animals that have been killed or died without showing any signs of leukemia after an observation period of at least 350 days.

are resistant against polyoma tumor isografts. In comparison with infection at adult age, their immunologic response is delayed, however. According to Habel (3) adults develop resistance against isografts 3 days after virus inoculation while mice infected as newborn require 24 days. Thymectomy of the newborn considerably increases the incidence of polyoma tumors, even in the relatively resistant C57BL strain (8); this is also in line with the possibility that immunologic tolerance plays a role in polyoma oncogenesis.

The findings presented in this paper suggest that immunologic host defense is playing a role in counteracting the growth of antigenic tumor cells in the primary host, even if this role is only a relative one. In poorly responding animals the unopposed proliferation of antigenic leukemia cells seems to lead to early leukemia development, while mice with a demonstrable immune response seem to carry on a retarding and finally unsuccessful struggle against the neoplastic cells. This ultimate breakdown of defense raises some intriguing questions. It is possible that some antigenic cells "sneak through," i.e., escape immune destruction by chance. Alternatively, antibody coating may even enhance their growth. Still another possibility is that progressive involvement of the lymphoid system in the neoplastic process by an accumulation of virus transformed cells leads to a diminution of the immune response. A selection of less antigenic neoplastic cells, suggested by findings on other antigenic tumor systems may also play a role (12). The importance of the thymus for the maturation of immunologic functions (7) only partially clarified, makes detailed hypothesis difficult at present since the thymus represents a primary target organ for the action of this leukemia agent. It may be recalled that the antibody response to T-2 phage was impaired in newborn C3H mice infected with Gross passage A virus, similarly to thymectomized mice (11).

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