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# Homocytotropic Antibody Production in Rats Infected with Moloney Leukemia Virus (38413)

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(Introduced by E. H. Lennette)

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Infection of rats with Moloney leukemia virus (MLV) results in an early and continued viremia with subsequent development of lymphoma. Cells stained for viral antigen by the direct fluorescent antibody technique are usually more abundant in the thymus as compared to other lymphoid organs (1), and the thymus is usually the first organ that shows lymphomatous changes. The initial change appears as thymus atrophy, followed later by thymus enlargement (2).

In the light of current concepts on antibody regulation and of thymus (T) and bone marrow (B) lymphocyte interactions (3), MLV-induced, immunologic defects of antibody synthesis thus far examined appear related to T cell malfunction. IgG synthesis in general and specific antibody responses of the IgG class to certain soluble (T-dependent) protein antigens are severely curtailed (4, 5), while IgM synthesis and specific antibody of the IgM class directed against *Salmonella typhi* (a T-independent antigen) remained within normal limits (6, 7). Indeed, serum IgM levels appear to be higher in unstimulated, infected rats than in unstimulated, noninfected controls.

From studies performed on normal animals, synthesis of homocytotropic antibodies of the IgE class in response to stimulation with hapten-carrier conjugates requires T-cell participation (8–12). If all immunologic responses dependent on T-cell participation are affected by MLV infection, a depression in homocytotropic (reaginic) antibody levels could be anticipated. The present study investigates IgE production in MLV-infected rats using the reagin response to crude *Ascaris suum* antigen as a model (13).

*Materials and Methods. Animals.* Thirty inbred Osborne–Mendel rats were inoculated intraperitoneally (ip) 24–48 hr after birth with 0.05 ml of a $10^{-3}$ dilution of MLV prepared from lymphomatous rat thymuses by the method of Moloney (14). At four weeks of age they were bled and their plasmas were inoculated into newborn BALB/c mice for determining presence or absence of viremia, as evidenced by induction of lymphoma in mice. An equal number of rats were kept as noninfected controls.

*Immunization.* Fifteen infected and 15 normal rats were immunized at 2 months of age with 1 mg crude Ascaris extract (CE) subcutaneously together with $2 \times 10^{10}$ *B. pertussis*-killed organisms[1] intraperitoneally. The remaining infected and noninfected rats were immunized in the same way at the age of 3 months. All animals were bled 14 days after immunization, were sacrificed, autopsied, and their spleens and thymuses were weighed. The sera were stored at −20° until needed.

*Homologous passive cutaneous anaphylaxis (PCA).* The reactions were performed as previously described (13) using female rats from a Sprague–Dawley-derived strain, originally obtained from Berkeley Pacific Laboratories (Berkeley, CA) and maintained in closed colony since 1968. The antigenic challenge was given 48 hr later by intracardial injection of 500 μg CE in 1 ml of 1% Evans blue solution. The PCA titer was established as the highest dilution of serum that gave a positive reaction of at least 5-mm diameter, 10–15 min after challenge. The starting dilution was 1:5.

*Results. Moloney leukemia virus infection of rats.* By 4 weeks of age all of the virus-inocula-

[1] Kindly supplied by Dr. H. B. Devlin, Parke Davis and Co., Detroit, MI.

ted rats had developed viremia as demonstrated by induction of lymphoma in the mice inoculated with their plasma. Prior to reagin testing, one rat in the 2-month-old infected group, five in the 3-month-old infected group, and one in the 3-month-old control group died. None of the 14 rats checked for antibody at 2 months of age had as yet developed gross evidence of lymphoma while five of 10 rats checked at 3 months of age were grossly lymphomatous with thymus weights ranging from 0.61 to 3.70 g/100 g body wt. Three of these rats also had enlarged spleens, with weights of 0.72–2.46 g/100 g body wt (Table I).

*Reaginic antibody titers.* At 2 months of age the geometric mean serum titer of reaginic antibody in infected rats was 1:1.5 and in control rats 1:27. At 3 months of age the respective mean titers for the two groups of rats were 1:5 and 1:44 (Table I). In the 2-month-old group, 11 of 14 infected rats and one of 15 control rats had no detectable reagins at a 1:5 dilution of serum. In the 3-month-old group the number of nonresponders at that dilution was three of 10 infected rats and two of 14 control rats (Fig. 1). No difference in serum titers was seen between infected rats with and without enlarged thymuses or spleens. This would indicate that the suppression in reagin production was related to the viral infection rather than to malignant transformation of the lymphoid cells.

*Discussion.* Neonatal thymectomy of rats has been shown to cause a marked decrease of reaginic (IgE) antibody synthesis to dinitrophenylated Ascaris extracts, although experiments were performed on a very limited number of animals (8). On the other hand, adult thymectomy caused an enhanced and prolonged reagin response following stimulation with the same hapten-carrier conjugate. It has been suggested that two populations of T cells are involved in reagin production, one acting as helper, the other as regulator and that adult thymectomy removes that segment of T cells that may be involved in negative regulation of reagin synthesis (8).

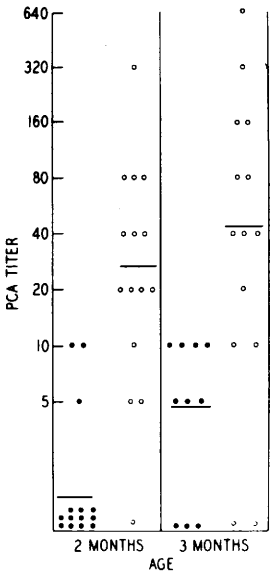


FIG. 1. Passive cutaneous anaphylaxis titers of normal and MLV-infected rats, immunized with crude Ascaris extract at 2 and 3 months of age. ○ Normal rats; ● MLV-infected rats; —— geometric mean.

TABLE I. Reaginic Antibody Titers Determined by Passive Cutaneous Anaphylaxis in Normal Rats and in Those Infected with Moloney Leukemia Virus.

| Rats | Age (months) | Thymus[a] (g) | Spleen[a] (g) | PCA titer |
|---|---|---|---|---|
| Normal | 2 | 0.24[b] | 0.35[b] | 1:27[c] |
| | | 0.20–0.37[d] | 0.30–0.46[d] | < 1:5–1:32[d] |
| Infected | 2 | 0.19 | 0.41 | 1:1.5 |
| | | 0.12–0.31 | 0.26–0.57 | < 1:5–1:10 |
| Normal | 3 | 0.20 | 0.29 | 1:44 |
| | | 0.13–0.24 | 0.24–0.35 | < 1:5–1:640 |
| Infected | 3 | 0.75 | 0.62 | 1:5 |
| | | 0.11–3.70 | 0.23–2.46 | < 1:5–1:10 |

[a] Expressed per 100 g body wt.
[b] Average.
[c] Geometric mean.
[d] Range.

Moreover the possibility of two distinct populations of regulator T cells acting on two different B-derived antibody-forming cell precursors was also envisaged. Our findings thus far on immunosuppression in MLV infection are not sufficiently extensive to make a distinction between different classes of regulatory T cells and/or their influence on different B cell subpopulations. However it seems clear from the present study on reagin production and from our previous work (1, 4–7) that production of antibodies requiring cooperation between T cells and B cells is curtailed in MLV infection.

*Summary*. Rats infected as newborns with MLV had a depressed ability to form reagin antibody to crude *Ascaris suum* antigen. This finding is consistent with the view that immunodepression in MLV infection is related to T cell malfunction.

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