The success in producing a local Schwartzmann reaction in guinea-pigs is attributed to the use of a higher dose (1 mg lipopolysaccharide intradermally followed by 1 mg lipopolysaccharide intravenously) than that used in the only other species, the rabbit, where this reaction has been previously obtained consistently using doses of the order of 10–100 μg. The failure to use higher doses in other species has given rise to the impression that the local Schwartzmann reaction is a peculiarity of the rabbit[3].

The failure to modify this reaction by heterologous antisera other than anti-complement serum, and in a few cases with anti-γ-globulin serum, separates this reaction from other inflammatory haemorrhagic reactions of the skin, such as the Arthus reaction and delayed-type hypersensitivity reactions of the tuberculin type, which have an allergic basis[1,2]. One anomalous finding is the failure to inhibit the haemorrhage of the Schwartzmann reaction with anti-thymocyte serum. The intravenous injection of this serum produces a marked fall in the level of circulating haemolytic complement sufficient to inhibit the development of the haemorrhagic component of the Arthus reaction[1], and the inflammatory components of chemical contact sensitivity and the non-specific reaction to the intradermal injection of turpentine[4]. Similar effects on contact sensitivity and turpentine inflammation by anti-complement serum have been reported previously[5]. This anomaly could be due to the fact that complement contains at least nine components. Anti-complement serum is directed mainly against the third component, whereas the fall in total haemolytic complement as a result of treatment with an antilymphocytic serum could be a result of a fall in other components of complement. Possibly, the Schwartzmann phenomenon needs the third component of complement (among others), while the Arthus reaction, cell-mediated immune reactions, and non-specific inflammation need other components not necessary for the Schwartzmann reaction—components which are "fixed" by the reaction between the antilymphocytic antibody and the lymphocyte cell membrane.

We therefore demonstrate for the first time the consistent production of a "true" Schwartzmann reaction in a species other than the rabbit; furthermore, we elucidate the difference between this reaction and the Arthus reaction in that it can occur despite a drop in the level of total haemolytic complement, produced by an antilymphocytic serum. We suggest, however, that certain complement components are involved, for it is suppressed by an antiserum directed chiefly against the third component of complement.

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Immune Complexes in Mice infected neonatally with Moloney Leukaemogenic and Murine Sarcoma Viruses

Several viruses have been reported to induce immunological tolerance in mice when administered in the immediate postnatal period. Among these are the lymphocytic choriomeningitis (LCM) virus[1,2] and the murine leukaemogenic viruses[3–5]. Moloney leukaemogenic virus (MLV) induces lymphocytic leukaemia in both newborn and adult mice[6]. Inoculation of adults with MLV results, however, in the development of both humoral and cell-mediated immune responses to the virus, whereas in neonatally infected mice these responses are greatly reduced or undetectable[4,7].

Oldstone and Dixon[8,9] have recently reported that mice neonatally infected with LCM virus are not completely tolerant to this agent, but make antibodies detectable only in renal glomeruli where they are presumably deposited in the form of antigen–antibody complexes. Our observations suggest that mice neonatally infected with MLV are also not completely tolerant, but have circulating antigen–antibody complexes which are deposited in renal glomeruli. Similarly, mice infected as newborns with the late lymphocytic component of murine sarcoma virus—Harvey (MSV-H), which is antigenically closely related to, or identical with, MLV—also develop immune complexes detectable in glomeruli.

BALB/c mice were inoculated with 0·1 ml. of MLV intraperitoneally within the first 18 h of life, or were in the third to ninth generation receiving vertically transmitted milk-borne MLV or the lymphocytic leukaemogenic virus separated from MSV-H ("late" MSV)[10]. Uninfected BALB/c mice housed in the same room as infected mice served as controls. Kidneys of 1–5 month old mice were studied by light microscopic and immunofluorescence techniques. Light microscopic sections were fixed in formaldehyde–acetic acid–alcohol, and stained with haematoxylin and eosin or with periodic acid–Schiff reagents. Tissues for immunofluorescence were rapidly frozen in liquid nitrogen and isopentane. Sections (4 microns thick) were washed in phosphate buffered saline (*p*H 7·1) for 1 h to remove any unbound globulin, stained for 30 min with fluorescein isothiocyanate conjugated goat anti-mouse γ-globulin, washed thoroughly and mounted in buffered glycerol. Similar sections were examined for the presence of MLV antigens by an indirect immunofluorescence technique, using hyperimmune rabbit anti-MLV serum and fluorescein isothiocyanate conjugated horse anti-rabbit globulin serum.

Deposition of mouse γ-globulin in glomeruli of MLV or "late" MSV infected animals was demonstrable when they were 70–90 days old. No differences were noted between groups receiving neonatal inoculation of MLV and those receiving vertically transmitted MLV or "late" MSV. Detectable γ-globulin first appeared along glomerular capillary walls in a finely granular pattern. By 100–120 days, deposits had become coarse and lumpy (Fig. 1). All glomeruli appeared to be involved, but other areas of

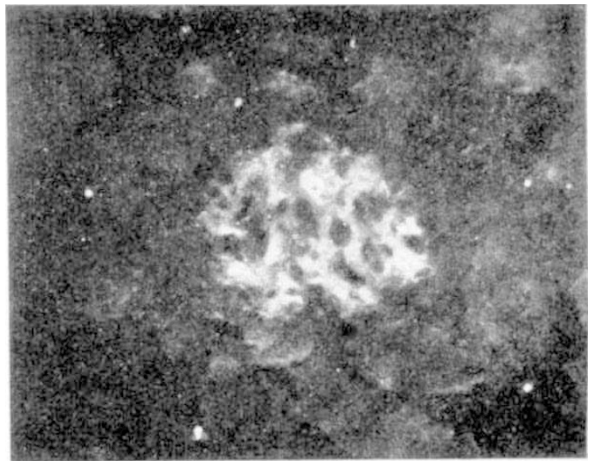

Fig. 1. Fluorescence micrograph of kidney from a 100 day old BALB/c mouse infected with MLV by vertical transmission, showing coarse, lumpy deposition of mouse γ-globulin in glomerular tufts. (Fluorescein isothiocyanate-conjugated goat anti-mouse globulin, × 450.)

the kidney were not affected. Viral antigens were also demonstrable as fine granular deposits throughout the kidney, the heaviest concentrations being in glomeruli. Kidneys of control BALB/c mice of comparable age did not contain γ-globulin or viral antigens demonstrable by immunofluorescence.

Histological lesions appeared in infected mice between 90 and 120 days, first as membranous periodic acid–Schiff positive thickening of glomerular walls. These early changes preceded any signs of leukaemic infiltrations. Later lesions showed proliferation of glomerular and capsular cells, with occasional obliteration of subcapsular spaces. The microscopic findings will be more fully described elsewhere in collaboration with Dr M. Branca. Occasional control BALB/c mice older than 150 days had minimal thickening of glomerular basement membranes, similar to that described previously[11].

γ-Globulin was eluted from kidneys of 3–4 month old mice neonatally infected with MLV and from age-matched uninfected controls. This was done by treatment of repeatedly washed preparations of glomerular basement membranes with 0·2 M citrate buffer (*p*H 3·2) for 2 h at room temperature. The eluate was brought back to neutrality and tested for antibody against MSV–MLV antigens (which are, as yet, indistinguishable). Equal amounts of eluates and undiluted sarcoma-inducing preparations of MSV were mixed and incubated at 37° C for 1 h. The mixtures were then inoculated intraperitoneally in 0·1 ml. amounts into newborn random-bred Parkes albino mice. Twenty-one days after inoculation, the mice were killed and their spleens weighed. The spleen weight assay has been found in this laboratory to be an effective and reproducible method of titrating potency of MSV preparations[12]. Spleen weights of mice receiving mixtures of MSV and eluates from neonatally infected animals were significantly lower than those receiving MSV and uninfected eluates ($P < 0.05$) (Table 1).

Table 1. EFFECTS OF CITRATE ELUATES FROM SALINE WASHED KIDNEY HOMOGENATES ON MSV-H INDUCED SPLENOMEGALY OF NEWBORN MICE

Group	No.	Mean spleen weights ± *S.E.* (g)
Neonatally infected MLV	19	0·100* ± 0·015
Control BALB/c	16	0·149 ± 0·008

Equal amounts of eluates and undiluted sarcoma inducing preparations of MSV-H were mixed and incubated at 37° C for 1 h. Newborn Parkes mice were inoculated intraperitoneally with 0·1 ml. of the mixtures and were killed 21 days later.

* Mean significantly less than control mean ($P < 0.05$; Dunnett's procedure).

To examine the possibility that infectious virus–antibody complexes were present in the circulation of MSV or MLV infected mice, aliquots of serum from infected mice were mixed with equal amounts of either unconjugated rabbit anti-mouse globulin, normal rabbit serum (NRS), or saline for 1 h at 37° C and inoculated intraperitoneally (0·1 ml.) into newborn mice. All twenty mice inoculated with MSV–NRS or MSV–saline mixtures developed characteristic MSV tumours and fatal erythroblastic splenomegaly between 14–21 days after infection, whereas only one of twelve animals inoculated with MSV–anti-mouse globulin mixtures developed sarcomas and erythroblastosis, and none developed later lymphocytic leukaemia although observed for a period of 6 months. Similarly, nine of ten animals inoculated with mixtures of MLV plasma and normal rabbit serum developed characteristic lymphocytic leukaemias, whereas only one of thirteen animals receiving mixtures of MLV plasma and anti-mouse globulin became leukaemic during a 6 month observation period.

In an attempt to determine which immunoglobulin classes were responsible for the complexing of circulating virus, aliquots of serum from infected mice were mixed with equal amounts of specific rabbit antisera against mouse immunoglobulins IgG, IgM and IgA, as well as with saline, for 1 h at 37° C and inoculated intraperitoneally (0·1 ml.) into newborn mice. Twenty-one days later the mice were killed and their spleens weighed. Mice receiving anti-IgM serum had significantly lower spleen weights than any of the other groups (Table 2) ($P < 0.05$), whereas no significant differences were noted between groups receiving saline, anti-IgA serum or anti-IgG serum. Immunodiffusion studies of glomerular basement membrane eluate from mice neonatally infected with MLV also indicated that antibody was predominantly of the IgM class.

Table 2. EFFECTS OF ANTI-IMMUNOGLOBULIN SERA ON SPLENOMEGALY INDUCED BY MSV FROM SERUM OF NEONATALLY INFECTED MICE

Group	No.	Mean spleen weights ± *S.E.* (g)
Anti-IgM	15	0·107* ± 0·010
Anti-IgA	8	0·176 ± 0·015
Anti-IgG	14	0·159 ± 0·015
Saline control	23	0·159 ± 0·011

Equal amounts of infectious serum and rabbit anti-mouse immunoglobulin serum were mixed and incubated at 37° C for 1 h. Newborn Parkes mice were inoculated intraperitoneally with 0·1 ml. of the mixtures and were killed 21 days later.

* Mean significantly less than control mean ($P < 0.05$; Dunnett's procedure).

These studies suggest that mice neonatally infected with MLV and MSV do not become completely tolerant immunologically, but have circulating virus–antibody complexes. In this respect they resemble persistent infections with lactate dehydrogenase virus (LDV) and LCM virus[8,9,13]. As with persistent LCM virus infections[8,14], these complexes are gradually deposited in the renal glomeruli, resulting in an immunopathological glomerulonephritis. The complexes appear to be of infectious virus and IgM anti-viral antibody, strongly suggesting that the antibody is not of maternal origin, but rather produced by the neonatally infected mouse. Whether animals having persistent viral infections have split tolerance, involving only failure to develop cell-mediated immune responses, remains to be determined.

Our findings do not necessarily apply to all murine leukaemias, particularly to those such as the "spontaneous" leukaemias of AKR mice in which the virus may be transmitted before birth, rather than by milk as with MLV and late MSV. Similar glomerular lesions have, however, also been described in AKR mice with spontaneous lymphatic leukaemias[15] as well as in murine infections with Rauscher, Friend and Passage A leukaemogenic viruses[15,16], suggesting possible common factors in pathogenesis such as immune complex deposition.

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