LACK OF NEUROLOGICAL ABNORMALITIES IN LEWIS RATS WITH EXPERIMENTAL CHRONIC SERUM SICKNESS

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SUMMARY

Serum sickness in man may occur after treatment with foreign proteins such as tetanus or diphtheria antisera, and in some patients leads to neurological complications such as neuropathy or encephalomyelitis. Many of the effects of serum sickness are associated with the deposition of antigen-antibody complexes in the tissues. Chronic serum sickness in the rabbit has previously been shown to cause perivascular inflammation and demyelination in the nervous system. We induced chronic serum sickness in the Lewis rat by daily intraperitoneal injections of bovine serum albumin (BSA) in male rats that had previously received footpad inoculations of BSA. Two animals died of anaphylaxis and 15 were observed for periods of 39 to 142 days. Three animals injected with 3 mg or 4 mg/day of BSA, and 6 animals injected with up to 16 mg/day of BSA had no clinical abnormalities when sacrificed. Six animals were injected with 36 to 40 mg BSA/day and, at the time of sacrifice, were lethargic and had ruffled fur, but no neurological signs. In these animals, the production of chronic serum sickness was confirmed by the presence of immune complex deposits in the kidneys. In the nervous system, there was no evidence of inflammatory cell infiltration either in the parenchyma or the vessel walls. Immunofluorescence studies identified deposits of immunoglobulin in the choroid plexus of chronic serum sickness rats but not in controls. Staining with antibodies to immunoglobulin, complement and BSA showed marked staining of blood vessels of the nerve roots of the animals with chronic serum sickness. There was also some minor immunofluorescent staining with these markers in the blood vessels of the nerve roots of control animals, but this was always less than in chronic serum sickness animals.

Serum sickness due to the injection of foreign proteins is an immune-complex-mediated disease[1] and affects the nervous system in a small percentage of patients[2,3,4]. Serum sickness is no longer a common disease, but there is evidence implicating immune complexes in other diseases affecting the nervous system, including systemic lupus erythematosus[5] and post-infectious and post-vaccination syndromes[3,6,7], particularly the neuro-pathy associated with hepatitis-B infection[8]. Furthermore, immune complexes have been implicated in the pathogenesis of inflammatory demyelinating diseases including multiple sclerosis[9,10,11], the Guillain-Barré syndrome[9] and chronic inflammatory demyelinating polyradiculoneuropathy[12].

We were therefore interested to know whether circulating immune complexes can deposit in the nervous system and cause neuropathological changes. Experimental chronic serum sickness is a well established model of immune complex disease and has been used to study glomerulonephritis[13]. Previous studies in rats have shown that, in experimental serum sickness, immune complexes are deposited in the choroid plexus[14]. In rabbits, chronic serum sickness has been shown to cause vasculitis in the coronary arteries[1] and vasculitis and perivascular demyelination and axonal damage in the central and peripheral nervous systems[15,16]. To determine the effects of circulating immune complexes on the nervous system of the Lewis rat, we produced chronic serum sickness and studied the clinical, pathological and immunopathological consequences.

MATERIALS AND METHODS

ANIMALS

Male Lewis rats (JC strain) aged 3 to 4 months were obtained from the animal breeding facility of the University of Queensland.

INDUCTION OF CHRONIC SERUM SICKNESS

Chronic serum sickness was induced using the technique of Arisz et al.[13] which requires pre-inoculation with BSA followed by the gradual introduction of daily BSA injections. We used intraperitoneal inoculation which results in a higher incidence of immune complex deposition in extrarenal sites than does intravenous injection[17].

CLINICAL ASSESSMENT

Animals were weighed daily. They were assessed for general well-being including activity level, fur texture and cleanliness, and for neurological impairment using the scale devised by Pender[18].

HISTOLOGICAL STUDIES

At appropriate times animals were perfused with glutaraldehyde/formaldehyde[18] or with 4% formaldehyde in 0.1M phosphate buffer. Tissues were processed for routine histological examination in HistoResin (LKB Bromma) and stained with haematoxylin and eosin or cresyl fast violet[19]. Frozen sections were stained with haematoxylin and eosin or prepared for immunofluorescence. For immunofluorescent staining, 5 μm frozen sections were prepared, air dried, fixed in acetone, and then incubated with fluorescein-conjugated rabbit antibodies to rat immunoglobulin, complement and bovine serum albumin or fluorescein-conjugated F(ab')$_2$ fragments of goat antibodies to rat immunoglobulin (all antibodies from Cappel). The sections were examined with a Zeiss Axiophot microscope.

RESULTS

CLINICAL OBSERVATIONS

A total of 17 animals were injected with BSA as described above. Eight animals of the same age were untreated and were observed as controls. In the group injected with BSA, 2 animals died of anaphylaxis and 15 were observed for periods of 39 to 142 days. Three animals were given low doses (3 mg or 4 mg/day) of BSA. These animals had no clinical abnormalities when sacrificed at 46 to 67 days. Six animals were given increasing daily doses of albumin until day 39, when the daily dose was 16 mg/day. These animals had no abnormalities when sacrificed at 39 days. Six animals were injected with 36 to 40 mg/day of BSA and, when sacrificed at days 79 to 142, were lethargic and had ruffled fur, but had no neurological signs. One chronic serum sickness animal given 3 mg/day of BSA and one control animal were perfused with glutaraldehyde/ formaldehyde. Two chronic serum sickness animals given 36 to 40 mg/day of BSA and 2 control animals were perfused with formaldehyde for preparation of frozen sections.

HISTOLOGICAL FINDINGS

Kidney and heart

Kidneys and hearts from a control and a chronic serum sickness animal were

examined histologically. The kidneys of the chronic serum sickness animal showed mild cellular infiltration when compared to the control. The hearts of chronic serum sickness and control animals were histologically normal.

Nervous System

No abnormalities were found in the spinal cord, dorsal roots or sciatic nerves of the control or the chronic serum sickness animal.

IMMUNOFLUORESCENT STUDIES

Kidney

The kidneys of one chronic serum sickness and one control animal were studied. There was deposition of immunoglobulin, complement, and BSA in the glomeruli of the kidneys of the chronic serum sickness animal but not of the control.

Nervous System

In the choroid plexus of 2 chronic serum sickness animals but not of 2 controls there was deposition of immunoglobulin. There was deposition of immunoglobulin, complement and BSA in the blood vessel walls in the nerve roots of 2 chronic serum sickness animals. There was also some occasional immunofluorescent staining in the blood vessel walls in the nerve roots of control animals but this was always less than in chronic serum sickness animals. Haematoxylin and eosin staining of the sections was normal.

DISCUSSION

Lewis rats have previously been found susceptible to the development of chronic serum sickness[17]. In the present study, Lewis rats injected with high doses of BSA for 79 to 142 days became lethargic and had ruffled fur and evidence of immunoglobulin and complement deposition in the kidneys, confirming the production of chronic serum sickness.

However, in the present study no rats with chronic serum sickness developed clinical signs of neurological disturbance. One chronic serum sickness rat was examined histologically and had no abnormalities in the spinal cord or nerve roots. Two chronic serum sickness rats had evidence of immunoglobulin deposition in the choroid plexus and of immunoglobulin, complement and BSA deposition in the vessels of the nerve roots. Although immunoglobulin,

complement and BSA deposition was also present to some extent in the nerve roots of controls, it was always more prominent in chronic serum sickness animals. In previous studies Peress *et al.*[14] found immune complex deposits in the choroid plexus but not in the brain capillaries of Wistar rats with passive serum sickness. Peress and Tompkins[20] found no pathology or immune complex deposits in the brains of Wistar rats given weekly tail vein injections of albumin. However, these studies used low doses of antigen, and did not examine the nerve roots, and so would not have noted any immunoglobulin or complement deposition in the nerve roots as detected in the present study. It might be expected that immune complexes would deposit in the nerve roots because the blood-nerve barrier is relatively permeable in these regions[21,22].

Despite the evidence of immune complex deposition in the nerve roots we found no clinical or pathological evidence of damage to the nervous system such as observed in some cases of serum sickness in humans[1,2] or rabbits[15,16]. This may be because the dose of antigen was not comparable. Another possible explanation for this difference is that there are species differences in susceptibility to damage to the nervous system by immune complexes, and that the rat is relatively resistant to immune complex damage of the nervous system. Several possible mechanisms for such resistance can be suggested. Firstly, *in situ* formation of immune complexes may be pathogenic whereas deposition of pre-formed complexes, as presumably occured in the present study, may not be. *In situ* immune complex formation in the choroid plexus of rats has been demonstrated by Huang *et al.*[23] but there is no information about *in situ* complex formation in other parts of the nervous system. Secondly, it is possible that the immune complexes of only certain relevant antigens will deposit in the nervous system and cause damage. There is evidence that immune complexes deposit in the peripheral nerves in the neuropathy associated with hepatitis B[8] and that complexes of galactocerebroside and anti-galactocerebroside can cause neuropathy[24]. Thirdly, it may be that immune complexes *per se* are insufficient to cause damage but have a role in enhancing other immunological mechanisms, perhaps by damaging the blood-brain barrier thus facilitating access to the nervous system of lymphocytes and/or antibodies specific for neural antigens. The work of Colover[25], which demonstrated that experimental allergic encephalomyelitis was more severe if animals were pre-immunized with ovalbumin, may be explained by these postulated mechanisms.

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