Lack of Effect of Immunosuppression on Scrapie Infection in Mice

(Accepted 24 July 1971)

Although scrapie has been studied for many years, its pathogenesis is still poorly understood. It has not been possible to demonstrate any immune response to the scrapie agent, and it is not known if immune mechanisms play any role in defence against scrapie infection (Gibbs, Gajdusek & Morris, 1965; Clarke & Haig, 1966). It has been suggested that the disease caused by the scrapie agent may be analogous to experimental allergic encephalomyelitis (EAE), and that an immune response may actually cause the observed pathological lesions (Paterson et al. 1966). Because of this uncertainty about the role of the immune system in scrapie, experiments were undertaken to determine the effect of long-term immunosuppression with cyclophosphamide (Cytoxan) on the outcome of scrapie infection in the mouse.

Cytoxan was obtained from Mead Johnson and a solution in phosphate buffered saline at a concentration of 20 mg./ml. was prepared just before use. A titrated pool of the scrapie agent in mouse brains was obtained from Dr C. J. Gibbs, Jun. at the National Institutes of Health; this strain was originally obtained from a sheep and was subsequently passaged in goats in England. It was further passaged eight times through Swiss mice before these experiments.

Two experiments were made, each in four groups of thirty 4-week-old GP Swiss female mice. In Experiment A, mice in groups 1, 2 and 3 were inoculated intraperitoneally (i.p.) with 10^3 LD50 of scrapie agent in 0.2 ml. of Eagle's medium. The agent was previously titrated intracerebrally in 3-week-old Swiss mice. Twenty-four hr later mice in groups 1 and 2 received Cytoxan as i.p. injections of 90 mg./kg. body weight. Mice in group 1 were injected with Cytoxan once a week until they died or were killed; mice in group 2 received a similar weekly dose of Cytoxan for only 3 months. Mice in control group 3 received only 10^3 LD50 of scrapie agent, while mice in control group 4 received only 90 mg./kg. of Cytoxan weekly until the experiment was terminated. Experiment B was identical to Experiment A except that groups 1, 2 and 3 received 10^3 LD50 of scrapie agent intracerebrally (i.c.) rather than i.p. Each experimental group again consisted of 30 mice.

Immunosuppressed and control mice developed clinical signs after about 6½ months with no difference in incubation period or development between the groups (Fig. 1a; Experiment A). There was also no difference between the times of death for each group. Cytoxan therapy also failed to influence the incubation period of the disease when the scrapie agent was inoculated i.c. (Fig. 1b; Experiment B). There was again no difference between the times of death for the groups. In both experiments, four mice from each group were examined histologically and the presence of typical brain lesions verified by using Cajal gold stain.

Cytoxan has been shown to be a potent suppressor of cellular and humoral immunity (Gabrielsen & Good, 1967) and to potentiate virus, bacterial and protozoal infections in mice (Cole & Nathanson, 1968; Tripathy & MacKaness, 1969; Luckins, 1969). It has been effective in protecting against diseases considered to have an immunological basis, such as renal disease in NZB mice, acute and chronic lymphocytic choriomeningitis virus (LCM)
disease in mice, and EAE in rats (Russell & Hicks, 1968; Sharon, 1970; Nathanson & Cole, 1970; Paterson et al., 1966). The schedule of Cytoxan doses which prevents renal disease in NZB mice and in mice chronically infected with LCM virus was similar to that used in the present experiments (Russell & Hicks, 1968; Sharon, 1970). In our laboratory this identical schedule of Cytoxan administered to GP Swiss female mice very markedly suppressed the

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\caption{(a) Effect of Cytoxan treatment on development of scrapie infection following i.p. injection of scrapie agent. Group 1 (\textbullet{} - - - \textbullet{}) received $10^3$ LD$_{50}$ of scrapie agent and Cytoxan weekly until death. Group 2 (\texttriangle{} - - - \texttriangle{}) received $10^5$ LD$_{50}$ of scrapie agent and Cytoxan weekly for 3 months: Group 3 (\textcircled{} - - - \textcircled{}) received only $10^5$ LD$_{50}$ of scrapie agent. Group 4 (\texttriangle{- - - \texttriangle}) received only Cytoxan weekly until the end of the experiment. (b) Effect of Cytoxan treatment on development of scrapie following i.e. injection of scrapie agent. Group 1 (\textbullet{} - - - \textbullet{}) received $10^3$ LD$_{50}$ of scrapie agent and Cytoxan weekly until death. Group 2 (\texttriangle{} - - - \texttriangle{}) received $10^5$ LD$_{50}$ of scrapie agent, and Cytoxan weekly for 3 months. Group 3 (\textcircled{} - - - \textcircled{}) received only $10^5$ LD$_{50}$ of scrapie agent. Group 4 (\texttriangle{} - - - \texttriangle{}) received only Cytoxan weekly until the end of the experiment.}
\end{figure}

formation of neutralizing antibody to lactic dehydrogenase virus (H. duBuy and M. Worthington, unpublished observation). The results thus suggest that in scrapie infection in the mouse, the immune system does not play a major role in development of, or protection against, the observed pathological lesions. It is of interest that it has been observed that long-term treatment with rabbit anti-mouse thymocyte sera does not influence the course of scrapie infection in mice (Hirsch, 1970).

We thank Drs S. Baron and C. J. Gibbs for their helpful advice in planning these experiments and in preparing the manuscript.
Short communications

Supported in part by Special Fellowship 5F03AI42860-02 MCBY from the National Institute of Allergy and Infectious Diseases.

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REFERENCES


(Received 18 March 1971)

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