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The Reticuloendothelial System in Scrapie Pathogenesis

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SUMMARY

A single injection of 1 mg dextran sulphate 500 per mouse considerably prolonged incubation times and produced survivors, even when given 3 days after intravenous or intraperitoneal scrapie infection. Since this compound could be detected in mononuclear phagocytes of spleen and lymph nodes for up to 7 months, it is suggested that it impairs a particular step in the infectious process in these cells of the lymphoreticular system. Blockage of the reticuloendothelial system by trypan blue and silica did not alter the pathogenesis.

The scrapie agent causes a slow progressive, always lethal disease of the central nervous system (CNS) in sheep and goats. Following propagation of the scrapie agent in mice (Chandler, 1961), quantitative data have led to a reasonable understanding of the pathogenesis of scrapie. It is a widely accepted view that after extraneural infection an early, probably obligatory replication phase occurs in one or more as yet unidentified cell types of the lymphoreticular system (LRS). This concept is based on the following observations: (i) an early rise in titre in the LRS long before the appearance of the agent in the CNS (Eklund et al., 1967; Kimberlin & Walker, 1979), (ii) prolonged incubation periods in spleenless (Fraser & Dickinson, 1970, 1978) or immunosuppressed mice (Outram et al., 1974), and (iii) enhancement of susceptibility by immunostimulants (Kimberlin & Cunningham, 1978; Dickinson et al., 1978).

The agent possibly replicates in either lymphocytes or the phagocytes of the reticuloendothelial system (RES). Separation of spleen cells of scrapie-infected mice by centrifugation on a discontinuous albumin gradient resulted in a cell population with high specific infectivity containing lymphoblasts, myeloblasts and macrophages (Lavelle et al., 1972). In several virus–host systems the RES plays an important role not only in providing resistance against viral infections (Mims, 1964) but also in supporting viral replication (Allison, 1974; Mims, 1978). Therefore, a recent report which suggested inactivation of the scrapie agent by mouse peritoneal macrophages in vitro (Carp & Callahan, 1982) is of interest.

To elucidate further the role of the RES in scrapie pathogenesis we report here the effect of three RES-blocking substances, dextran sulphate (DS) 500, silica and trypan blue, on the pathogenesis of the 139A strain (Kimberlin & Walker, 1978) of scrapie agent in STU inbred mice (Schäfer, 1979).

Silica is selectively cytotoxic for macrophages (Allison et al., 1966). Like DS 500 and trypan blue it causes a short-term impairment of RES function (Levy & Wheelock, 1975; Kripke et al., 1977; H. Hahn, personal communication). Thus, these substances lead to an increase in morbidity and mortality rates due to virus infections taking place during the time of impaired RES function (Zisman et al., 1971; Mogensen & Andersen, 1977; McGeorge & Morahan, 1978; Frank et al., 1978).

To demonstrate a similar effect in scrapie pathogenesis we first confirmed that the dosages used by others affected RES function in STU mice. This was done by examination in vitro of phagocytic activity of peritoneal macrophages collected after treatment in vivo. Briefly, groups

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of five mice were injected intraperitoneally (i.p.) with DS 500 [Serva, 1 mg in 0.5 ml phosphate-buffered saline (PBS)], silica [Sigma, 40 mg in 0.5 ml PBS + 5% foetal calf serum (FCS)], or trypan blue (Merck, 1 mg in 0.5 ml PBS). Mice treated with PBS or PBS + 5% FCS served as controls. Twenty-four or 2 h (groups treated with silica or PBS + 5% FCS) later, peritoneal cells were harvested from the peritoneal cavity, cultivated for 2 h on coverslips, and the adherent cells were incubated with zymosan (Sigma, 0.5 mg per coverslip) for 1 h. After Giemsa staining, cells that had interiorized one or more zymosan particles were counted. Treatment with the three compounds was effective in STU mice and led to a 30 to 60% reduction of cells exhibiting phagocytic activity.

We now focused on the question whether blockage of the RES enhances susceptibility to scrapie infection. This was done by comparing incubation periods of groups of scrapie-infected mice (Hunter et al., 1963; Dickinson et al., 1969; Millson et al., 1979). Groups of six to ten female mice were infected either i.p. with 0.1 ml of brain homogenate containing 25 to 250 i.p.-LD$_{50}$ or intravenously (i.v.) with 0.2 ml containing 150 to 1500 i.v.-LD$_{50}$ of scrapie agent. The animals were treated as above with DS 500, silica or trypan blue. In addition, i.v. and i.p. infected animals also received silica i.v. (15 mg silica in 0.2 ml PBS + 5% FCS). Different groups were treated with these compounds 24, 6 or 2 h before scrapie infection, to cover sufficiently the time period of impaired RES function. Administration 3 days after infection served as a control [scrapie infectivity is known to disappear from the blood within a few hours after infection (Millson et al., 1979; Hotchin et al., 1983) and entry of the agent into the permissive cells within 3 days can therefore be expected]. In addition, for each treated group a control group was established which received only the appropriate diluent without the blocking compound. Furthermore, uninfected animals were treated with the blocking substances to ensure that the compounds themselves did not cause neurological disorders. All mice were monitored for clinical signs of scrapie twice weekly. In a few instances the clinical status of scrapie was confirmed by histological examination of brain sections.

Throughout the experiment all treated, uninfected animals remained healthy. The i.p. and i.v. infected control groups that did not receive any of the compounds gave mean incubation periods of 152 to 165 and 155 to 171 days, respectively, without survivors.

In Fig. 1 the effects of the three blocking substances on the incubation periods are summarized. To allow a direct comparison of the changes in incubation times, the values of the control groups are defined as zero and the difference in incubation period of every treated group is plotted.

None of the three compounds produced the shortening of incubation period which might have been expected with conventional infectious processes, where blockage of the RES would compromise degradation of infectivity. No matter whether infection was by the i.p. (Fig. 1 a) or the i.v. (Fig. 1 b) route, the i.p. treatment with trypan blue or the i.v. and i.p. treatments with silica did not alter the incubation periods significantly ($P > 1\%$). This holds true for any time of treatment prior to infection as well as for the control treatment 72 h after infection. Survivors were not observed.

Treatment with a single injection of 1 mg DS 500 at 24, 6 or 2 h before infection and even 72 h after infection increased the incubation periods significantly ($P < 1\%$) in the i.p. as well as in the i.v. infected animals. The average increase in incubation period was 50 days. In addition, some of the animals survived (healthy at 300 days post-infection; see Fig. 1 a, b). This indicates a loss in titre of 90 to 99%.

None of the three RES-blocking substances enhanced susceptibility to scrapie infection; this is in accordance with a recent report showing that clearance of scrapie agent from the blood is unchanged after RES blockage with silica and carbon black (Hotchin et al., 1983). This might be explained by the fact that scrapie infectivity exhibits highly hydrophobic properties (Prusiner et al., 1981) and after infection will bind rather non-specifically to cells. Short-term blockage of phagocytic activity would not influence this process and probably would not change the proportion of cells infected in the LRS. This would lead finally to identical incubation periods and this is what we indeed observed with silica or trypan blue treatment.

The unexpected prolongation of incubation periods after treatment with DS 500 seems to
Fig. 1. Influence of DS 500, trypan blue and silica on incubation periods, when administered 24, 6 or 2 h before or 72 h after intraperitoneal (a) or intravenous (b) scrapie infection. For details see text. The ordinate shows the differences of the mean incubation periods, i.e. mean value of treated group - mean value of control group. The 95% confidence limits are given. * The mortality rates of the groups treated with DS 500 not showing 100% mortality are given.

indicate impairment of early events in scrapie pathogenesis. Since spleen and lymph nodes are major early replication sites in mouse scrapie, we compared the histology of these tissues after treatment with the three compounds. Clear histological differences were seen only after treatment with DS 500 (Fig. 2 and 3). Firstly, metachromatic material was observed in reticulendothelial cells of spleen and lymph nodes for at least 7 months (Fig. 2a, b). Such material has been shown to consist of DS 500 (Walton, 1954; Hahn & Bierther, 1974). Secondly, a reduction in small lymphocytes was found in the white pulp of spleen and lymph nodes, which was most striking about 6 h after injection (Fig. 3a). Proliferation of lymphoblastoid cells in the follicles was observed over the following 2 days, and normal appearances were restored after 3 to 4 days. Similar observations were made in rat spleens after treatment with a dextran sulphate of 5000 mol. wt. (Sasaki & Suchi, 1967). These authors demonstrated that the transient disappearance of
Fig. 2. Histological sections of mouse lymph node (a) and spleen (b) stained with toluidine blue 4 days and 2 months, respectively, after i.p. injection of 1 mg DS 500. The arrows indicate phagocytic cells with diffusely distributed dark granules within their cytoplasm. In the stained sections, the DS 500 shows a distinctive violet colour within these granules. Bar markers represent 25 μm.

Fig. 3. Loss of small lymphocytes from white pulp of mouse spleen 6 h after i.p. injection of 1 mg DS 500. Note the cortex (arrows) around the germinal centre of the follicle of a treated mouse depleted of cells (a) compared with the follicle of a control mouse (b). Staining: Giemsa. Bar markers represent 62.5 μm.
lymphocytes from white pulp of spleen was caused by mobilization of the cells into the bloodstream. This mobilization of lymphocytes from the lymphoid organs could perhaps cause a prolongation of incubation periods, but the occurrence of survivors is hard to explain by this effect.

DS 500 has been reported to stimulate antibody production (Diamantstein et al., 1971). It is a B-cell mitogen (Dörries et al., 1974) and an adjuvant for cell-mediated immune responses (McCarthy et al., 1977). However, other immunostimulatory substances used to alter scrapie pathogenesis have been shown to shorten rather than lengthen incubation periods (Dickinson et al., 1978; Kimberlin & Cunnington, 1978).

On the other hand, DS 500 has been reported to form non-infectious complexes with encephalomyocarditis virus (Liebhaber & Takemoto, 1963) and to inhibit the replication in vitro of herpes simplex virus (Takemoto & Fabisch, 1964). A similar effect might be responsible for its action on scrapie infection in the mouse. We have shown that DS 500 is detectable in vivo in the cells of the RES for at least 7 months and that treatment with this compound reduces susceptibility to scrapie infection, even when the compound and the agent are injected at different sites and even when DS 500 is injected 3 days after infection. We therefore put forward the hypothesis, that DS 500 interacts inside the cells of the RES in spleen and lymph nodes either with the scrapie agent itself or with its replication.

This is the first report showing a single administration of a compound to be effective in protection against the scrapie agent. The substance is active with i.p. and i.v. infection and thus might be active with the horizontal route of natural scrapie infection in sheep. Even when administered 3 days after infection, DS 500 exhibits its effect. Preliminary experiments indicate that a single injection 1 week before infection also results in an increased incubation period and that multiple applications of DS 500 are even more effective.

It seems, therefore, that DS 500 may be of future interest in two areas of research: protection and control of diseases like scrapie which cannot otherwise be treated and (ii) the elucidation of early peripheral events in scrapie pathogenesis preceding the entrance of the agent into the CNS.

While preparing this manuscript we learned that Dr R. H. Kimberlin, studying the effect of polyanions on scrapie disease (Kimberlin & Walker, 1983), as well as Dr A. G. Dickinson working on immunomodulators in scrapie, independently of each other and of us discovered the same beneficial effect of a single injection of DS 500 on the course of the disease (unpublished results).

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REFERENCES


SHORT COMMUNICATION


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