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THE EFFECT OF IMMUNOSUPPRESSION ON VIRAL ENCEPHALITIS, WITH SPECIAL REFERENCE TO CYCLOPHOSPHAMIDE

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SUMMARY.—Cyclophosphamide altered not only the pathological picture of virus encephalitis, but also enhanced the invasiveness of viruses and produced fatal forms of disease to which monkeys were otherwise resistant. Normal patas monkeys infected either i.c. or intranasally (i.n.) with louping ill developed clinical encephalitis from which they recovered, but when cyclophosphamide was administered the disease proved fatal. Normal rhesus monkeys inoculated i.n. with virulent western equine encephalitis virus developed neither clinical disease nor CNS lesions, but after treatment with cyclophosphamide they developed fatal encephalitis. The viruses of louping ill, Venezuelan equine encephalitis and Western equine encephalitis when given without an immunosuppressant usually gave rise to acute inflammatory CNS pathology, but when the monkeys were given cyclophosphamide, the inflammatory reaction was replaced by a degenerative process causing both neuronal necrosis and spongy degeneration.

THE purpose of this communication is twofold: firstly to present evidence which elucidates the role of the cellular inflammatory reaction in virus encephalitis; and secondly to emphasize some possible dangers in the use of immunosuppressive and cytotoxic drugs.

The cellular reaction, expressed as perivascular cuffings and focal or diffuse infiltrations, which is recognized as the characteristic pathology of virus encephalitis, is not due to direct action by the virus, but to immunological reactions with the virus. Thus immunological responses play a major part both in the pathogenesis of and in recovery from virus encephalitis (Webb and Smith, 1966; Webb, Wight, Platt and Smith, 1968; Webb, 1969). Although the effect of inflammation on the final outcome of the disease process is not yet fully understood and probably varies from case to case, the impression has been gained that it may, in sum, be more harmful than beneficial. However, the fact that severe inflammatory reactions can be seen in inapparent infections of the CNS suggests that in such circumstances these reactions have been beneficial, and may have prevented more serious consequences (Zlotnik, 1968; Zlotnik, Keppie and Grant, 1970).

The availability of immunosuppressants has provided new opportunities for elucidating these problems of virus encephalitis (Murphy and Glasgow, 1967; Cole and Nathanson, 1968; Hirsch and Murphy, 1968; Robinson, Curlton and Heath, 1969; Medawar, 1969; Nathanson and Cole, 1970) and it is now obvious that, although immunosuppressants greatly reduce inflammatory reactions, the resulting disease is more severe and deaths occur where, without immunosuppression, only inapparent infections would have resulted. Thus a balanced view must be reached

about the relative importance of the harmful and beneficial effects of immunological reactions in the CNS.

MATERIALS AND METHODS

Rhesus (*Macaca mulatta*) and patas (*Erythrocebus patas*) monkeys were infected with the following arboviruses: (1) Louping ill virus, 6th mouse brain passage of the Moredun strain; dose, 10^7 mouse LD_{50}; (2) Venezuelan equine encephalitis (VEE), (a) Colombian strain (VEE-9), isolated from man, passaged once through rhesus monkeys then twice in suckling mouse brain; dose, 10^9 pfu/ml.; (b) Trinidad strain, originally grown in chick embryo, 1st suckling mouse brain passage; dose, 10^8 pfu/ml.; (3) Western equine encephalitis (WEE), (a) virulent WEE + 4, isolated in suckling mice and passaged in hamsters; 6th hamster brain passage; dose, 10^7 pfu/ml.; (b) Attenuated strain (Johnson, 1963), B.628, Clone 15, 3rd egg yolk passage; dose, 10^8 pfu/ml. Cyclophosphamide (Endoxana, Ward, Blenkinsop and Co. Ltd.) was given s.c. (75 mg./kg. body weight) on the first day after virus inoculation, followed by 50 mg./kg. on 3rd and 6th days.

Virus was inoculated either i.c. into the thalamus in a volume of 1·0 ml., or the same volume was given intranasally (i.n.), or virus was administered by the respiratory route in the Henderson apparatus (Henderson, Peacock and Belton, 1956). Sick animals were either allowed to die, or killed when moribund and brains and cords removed immediately after death for virus assay and histological examination.

RESULTS

Louping ill

When inoculated either i.c. or i.n. into patas monkeys, louping ill virus caused clinical signs and inflammatory lesions of encephalitis, but the animals normally recovered after 3–6 weeks (Zlotnik, unpublished). In a recent experiment, 6 patas monkeys were given virus i.c. and 6 were inoculated i.n. Three monkeys in each group were left untreated while the other 6 received cyclophosphamide. The untreated monkeys developed signs of encephalitis 6 days after i.c. and 10 days after i.n. infection, but clinical improvement began 7 days after the onset of disease and clinical recovery was complete 2 weeks later. Histological examination of the brains and cords of these monkeys, killed 4–6 weeks after infection, revealed widespread inflammatory lesions and a moderate degeneration of neurons in various centres, but the Purkinje cells of the cerebellum were most severely affected, irrespective whether virus was given i.c. or i.n.

The cyclophosphamide-treated patas monkeys developed signs of encephalitis somewhat later, 7 days after i.c. and 12 days after i.n. infection. However the disease in these monkeys was more persistent; the animals failed to show any signs of improvement and died 10–24 days after i.c. and 24–26 days after i.n. infection. The histological changes in the CNS of these monkeys were striking, in that apart from a moderate meningeal exudate and a few perivascular cuffings mostly in the vicinity of the needle track in the thalamus of i.c. inoculated monkeys, all the lesions were of a degenerative necrotizing character. Most severely affected was the cerebellum where almost all the Purkinje cells were either absent or exhibited very advanced degeneration and necrosis. In addition to widespread spongy degeneration in the Purkinje layer with empty baskets, there was also oedema of the white matter, proliferation of Bergmann cells, severe astrocytic fibrosis in the molecular layer and marked depletion of granule cells (Fig. 1). In the brain of i.n. infected monkeys there were also foci of malacia in the white matter of the cerebellum with microcavitation and Gitter cell formation. In other parts of the brain the most conspicuous lesions, consisting of chromatolysis and necrosis of neurons,

were seen in the red nucleus, substantia nigra and the motor tegmentopeduncular nuclei of the midbrain (Fig. 2). In the spinal cord, apart from severe degeneration and disappearance of ventral horn neurons, there were varying degrees of microglial infiltration.

Venezuelan equine encephalitis

Cyclophosphamide did not alter the susceptibility or the course of the disease in rhesus monkeys infected by any of the 3 routes, but in each case the pattern of the histological lesions changed from a predominantly inflammatory character with perivascular cuffings, to a degenerative one with neuronal necrosis and a great tendency to spongy degeneration in subcortical centres, especially the thalamus, midbrain and pons.

In a series of experiments with the 2 strains of VEE, groups of 2 monkeys were treated as follows: (a) Colombian strain given i.n. or by the respiratory route with or without cyclophosphamide treatment; (b) Trinidad strain given by the i.c., i.n. or respiratory routes with and without cyclophosphamide. Clinical signs appeared on the 5th day and they died on 6th or 7th day. The highly virulent Colombian strain proved fatal to rhesus monkeys when inoculated by either the i.n. or the respiratory route, but the less virulent Trinidad strain was fatal to monkeys only after i.c. inoculation; all other routes proved ineffective and produced neither clinical signs nor lesions.

Western equine encephalitis

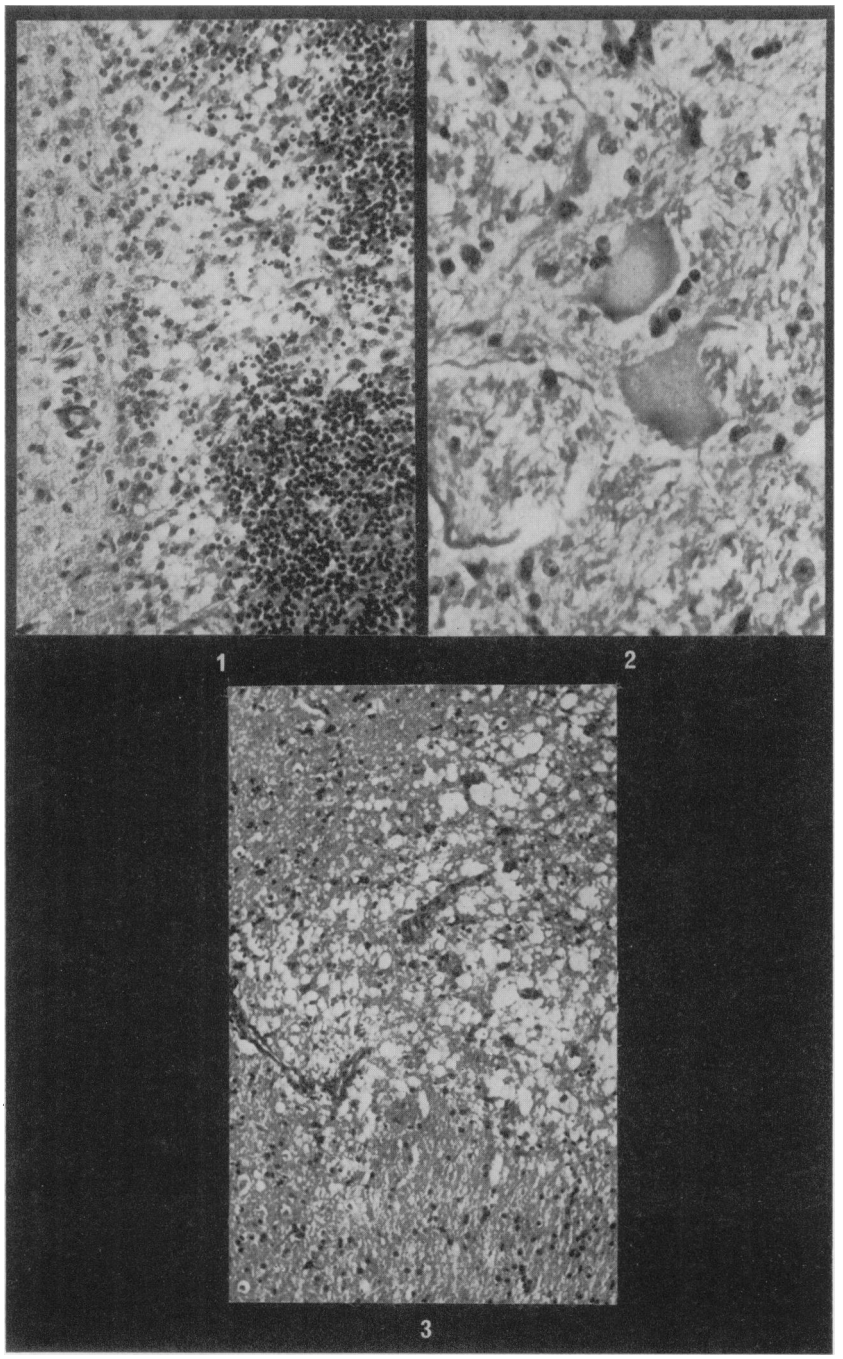
The virulent (WEE + 4) strain was found pathogenic for rhesus monkeys only by the i.c. route; other routes of infection (intravenous, intranasal, subcutaneous or respiratory) caused neither clinical signs nor CNS lesions. The immunity acquired after respiratory infection protected only 1 in 2 monkeys when they were subsequently challenged by the i.c. route, the monkeys dying with signs of encephalitis. Similar results were obtained when the attenuated strain of WEE (Johnson, 1963) was given by the respiratory route and 2 weeks later the monkeys were challenged i.c. with virulent WEE + 4. Histological examination of the CNS of monkeys that died 5 days after i.c. infection with WEE + 4 alone, revealed very severe inflammatory lesions with cellular infiltrations and marked perivascular cuffings throughout the leptomeninges, brain and cord. However in monkeys immunized by the respiratory route with either the virulent or the non-virulent strains and dying 5 days after i.c. challenge (WEE + 4), the CNS changes were mostly degenerative, inflammatory lesions being limited to the vicinity of the needle track. The striking lesion here was very severe spongy degeneration causing disruption of subcortical centres throughout the neuraxis from the paraterminal body, along the thalamus, mesencephalon, pons, medulla to the spinal cord.

EXPLANATION OF PLATE

FIG. 1.—Cerebellum of a patas monkey showing widespread destruction of Purkinje cells and depletion of granule cells—louping ill with cyclophosphamide. H. and E. × 168.

FIG. 2.—Neuronal necrosis in the red nucleus of a patas monkey—louping ill (i.n.) with cyclophosphamide. H. and E. × 375.

FIG. 3—Severe spongy degeneration in the thalamus of a rhesus monkey. WEE + 4 (i.n.) with cyclophosphamide. H. and E. × 105.



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Cyclophosphamide given after i.c. or respiratory infections with either the virulent or the non-virulent strain did not alter the susceptibility of the animals. Thus cyclophosphamide-treated monkeys infected i.c. with WEE + 4 died at the same time as similarly infected normal animals. However, they had severe degenerative changes in the CNS similar to those observed in monkeys following challenge infections. Those infected by the respiratory route with the virulent or non-virulent virus remained unaffected and when destroyed showed no lesions in the CNS.

In a further experiment, 2 rhesus monkeys were exposed by the respiratory route to virulent and 2 monkeys to non-virulent virus and then treated with cyclophosphamide. These animals were challenged with WEE + 4 virus i.c. 2 weeks after infection. All 4 died 5 days after challenge and their brains showed very severe degenerative lesions with little inflammatory change.

The most dramatic results were obtained when 3 rhesus monkeys were given virulent WEE + 4 i.n. and were then treated with cyclophosphamide. Monkeys that received only virus remained unaffected for 4 weeks and when destroyed had no lesions whatsoever in the CNS. However all the cyclophosphamide-treated monkeys developed clinical signs between the 7th and 9th day after infection and died 2 days after the onset of disease. The CNS changes resembled those of the fatal cases among immunized monkeys in that they had hardly any cellular infiltrations, but showed very severe and widespread spongy degeneration, neuronal necrosis, and oedema in most subcortical centres (Fig. 3).

It is important to note that in all the cyclophosphamide-treated animals dying from the above 3 virus infections there was a marked depletion of lymphocytes in the white pulp of the spleen. In many cases the process caused complete disappearance of lymphocytes from the Malpighian bodies. These splenic changes were not, however, present in monkeys immunized with WEE and dying from a necrotizing encephalitis after subsequent i.c. challenge.

DISCUSSION

This work shows that treatment with cyclophosphamide after infection with some viruses may cause far reaching effects not only in increasing the susceptibility of animals, but also in changing the character of the disease so that degenerative and necrotic lesions predominate and cause more deaths and disabling sequelae. Moreover, it is clear that the inflammatory reaction is not by itself an expression of direct action by virus, and that fatal disease may result without any sign of inflammation. It appears therefore that treatment with cyclophosphamide reduces or eliminates the inflammatory reaction in virus encephalitis, presumably because its cytotoxic action prevents the proliferation of specifically sensitized lymphocytes and thus the development of both humoral and cell-mediated immunity (Fairly and Simister, 1965; Aisenberg and Davis, 1968; Cole and Nathanson, 1968; Robinson *et al.*, 1969). Thus the relationship between CNS inflammation in virus infections and immunity becomes very obvious and it is very plausible that inflammation in virus encephalitis is mediated by cell immunity and the perivascular cuffings consist of immunologically competent cells. Both lymphocytic cuffings and perivascular astrocytic gliosis can be interpreted as an attempt to prevent the penetration of virus from the blood into the brain.

The results in patas monkeys infected with louping ill virus by either the i.c. or

the i.n. route were similar to those of Nathanson and Cole (1970), where severity of Japanese encephalitis was aggravated and caused deaths in spider monkeys, whereas in the absence of the immunosuppression the same infection caused only mild disease at the most. The results of intranasal infection with WEE + 4 with and without cyclophosphamide show, however, an even greater effect, as in the absence of the drug, no signs of disease or CNS lesions have been observed.

Peripheral immunization with WEE does not always protect animals against i.c. challenge and the absence of an inflammatory response in animals which die of a purely degenerative process after challenge suggests that the immunological processes in the CNS have not been primed by the earlier peripheral infection. Treatment with cyclophosphamide aggravated this phenomenon in that all the immunized animals died from challenge rather than a half of those animals which did not receive immunosuppressive treatment.

The effects of cyclophosphamide are in many respects very similar to those of anti-lymphocytic serum and in both cases the action is probably mediated through a depression of cell mediated immunity (Hirsch and Murphy, 1968; Medawar, 1969). Thus at least a large part of the inflammatory reaction in the CNS is an expression of the cell-mediated immunity and as a whole should be considered beneficial and leading to the recovery of the animal from the disease. However during the acute stage of the disease, the inflammatory reactions release pharmacologically active substances and cause tissue changes, particularly oedema, which may lead to at least temporary dysfunction of neurons. This temporary dysfunction may be sufficient to cause death at the height of the disease and the rapid functional recovery which follows survival of the acute stage is attributable to diminution of the inflammatory lesions and recovery of temporarily disabled neurons.

In some fatal progressive encephalopathies such as scrapie (a transmissible disease) no immunological responses have ever been demonstrated and the CNS lesions are non-inflammatory with spongy degeneration and neuronal necrosis very similar to those observed in cyclophosphamide-treated monkeys infected with WEE or VEE (Zlotnik, 1962; Zlotnik and Rennie, 1965).

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