Acute and Chronic Autoimmune Encephalomyelitis: Age, Strain, and Sex Dependency The Importance of the Source of Antigen[1] (34210)

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Experimental autoimmune encephalomyelitis (AE), which has been proven to be an autoimmune disease by fulfillment of the appropriate Witebsky's postulates (1) and by the adoptive transfer of the disease (2–4), may take various forms which depend upon the strain and age of animals employed (5–7). Although all newborn animals had been considered completely resistant (8, 9), recently AE has been induced in neonatal guinea pigs (6) and in neonatal monkeys (7). Furthermore, the disease has shown strain-dependent differences within neonates of a given species. For example Strain 2/N guinea piglets did not develop overt disease; Strain 13/N neonates regularly developed a chronic type of disease and random-bred Hartley newborns showed resistance or chronic or acute AE which varied from litter to litter (6).

In previous studies on adult guinea pigs, Strain 2/N animals were resistant to AE in contrast to Strain 13/N guinea pigs (5). However, an occasional adult Strain 2/N animal proved susceptible. Hence, in view of the strain-dependent results in newborns, it was thought desirable to determine the interrelated influences of age, strain, sex, and the source of antigen in experiments inducing AE in guinea pigs. The results established a pattern ranging from optimal conditions for acute AE (using homologous spinal cord antigen in adult Strain 13/N) to striking resistance to AE (using rabbit spinal cord antigen in newborn Strain 2/N guinea pigs), between which there were a variety of conditions favoring production of chronic forms of AE. This chronic form, as described previously (6), is characterized by a long latent period, protracted downhill course with or without remissions, paralysis and wasting of rear extremities, and lesions concentrated in the lumbosacral cord. In the cord there is, in addition to the perivascular distribution of mononuclear cells, extensive and sometimes massive infiltration by lymphocytes, monocytes, and some plasma cells, with resultant marked destruction of white and grey matter (10).

Materials and Methods. Young and mature inbred histocompatible Strain 2/N and Strain 13/N guinea pigs[2] were used. Young guinea pigs were 220–350 g, mature Strain 2/N were 600 g or larger and mature Strain 13/N were 750 g or larger. (Strain 13/N guinea pigs attain larger weights than do Strain 2/N guinea pigs.) Guinea pig (Strain 2) and rabbit whole spinal cord preparations were emulsified in complete Freund's adjuvant containing killed and dried *M. tuberculosis*. Materials used per animal: 0.5 ml of water-in-oil emulsion containing 0.25 ml of 50% suspension of spinal cord, 0.25 ml of Arlacel-Bayol (1.5:8.5) and 2.5 mg of killed *M. tuberculosis*, injected in a single dose intracutaneously in four sites in the nuchal area and one site in each hind foot pad. Control animals were injected with lung tissue in complete Freund's adjuvant or with the adjuvant emulsion alone.

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[2] Strains 2/N and 13/N refer to inbred histocompatible Strain 2 and 13 guinea pigs bred at NIH. Such designations become necessary as many centers are now breeding these animals.

TABLE I. Age, Strain, and Sex-Dependent Differences in Autoimmune Encephalomyelitis after Injection of Spinal Cord Antigens.

Sex	No.	Antigen	Acute	Chronic	Sex	No.	Antigen	Acute	Chronic
		Mature strain 13/N					Young strain 13/N		
M	10	Guinea pig	10	—	M	10	Guinea pig	0	10
F	10	Guinea pig	10	—	F	9[a]	Guinea pig	8	9
M	10	Rabbit	10	—	M	10[a]	Rabbit	1	2
F	10	Rabbit	10	—	F	8[a]	Rabbit	8	2
		Young strain 2/N					Mature strain 2/N		
M	10	Guinea pig	0	0	M	10[a]	Guinea pig	6	2
F	10	Guinea pig	0	4	F	9	Guinea pig	9	—
M	10	Rabbit	0	0	M	10	Rabbit	0	0
F	10	Rabbit	0	0	F	10	Rabbit	0	0

[a] Some animals recovered from acute disease and developed chronic disease at a later stage.

Guinea pigs were observed at regular intervals and weighed. Loss of weight is usually the first sign of acute encephalomyelitis. This is followed by wetness of mouth, impacted feces, paresis, and paralysis. Brain, spinal cord, and other organs were fixed in neutral formalin, embedded in paraffin, sectioned, and preparations were stained with hematoxylin and eosin.

Results. Table I shows the results when mature, susceptible guinea pigs (Strain 13/N) were sensitized with homologous or heterologous spinal cord: all animals developed severe or lethal acute AE. Young animals of the same Strain 13/N, between 200 and 300 g, presented a different picture. When young animals were used, differences appeared in susceptibility based on sex of animals and type of antigen. Immunized with homologous cord, males showed no acute clinical disease, and only females showed mild but significant acute symptoms. The acute disease in these young females was mild and transient, but about 2 months later both males and females which had received guinea pig cord developed slow loss of weight, incontinence, paralysis, and then wasting of the hind legs. Rabbit spinal cord was less encephalitogenic; females developed acute AE, but later on, only a few of the guinea pigs which had received rabbit cord eventually developed chronic disease. When young animals of resistant Strain 2/N were sensitized there was a striking absence of clinical acute disease, but some females receiving guinea pig spinal cord eventually showed typical chronic disease. Moreover, the Strain 2/N was not *absolutely* resistant to the acute disease, nor were mature animals in general *absolutely* resistant to the chronic form. When guinea pig spinal cord was used as sensitizing antigen older female Strain 2/N animals regularly developed severe or lethal acute AE. Most large males showed acute disease. The two males which survived the acute phase developed chronic disease. When rabbit cord was used as sensitizing antigen, no disease was produced in Strain 2/N's.

The details of the clinical and pathological aspects of chronic AE were outlined in (6). A striking result in the present study was that in Strain 2/N guinea pigs which remained clinically healthy, histologic lesions characteristic of severe AE were found in the CNS. These histologic lesions occurred in nearly all young Strain 2/N's, and especially in those receiving rabbit spinal cord. The lesions were of acute aspect at a time after immunization which corresponded with the appearance of acute clinical disease in Strain 13/N animals (2–3 weeks), and at subsequently later stages appeared subacute and chronic.

Discussion. Two facts help to pinpoint the observed effects of strain and age differences: (A) young or newborn Strain 13/N guinea pigs possess the appropriate *target* organ for severe or lethal acute disease, as shown by adoptive transfer of the disease by lymph

node cells from mature donors (6); (B) sensitized young male Strain 2/N guinea pigs, although overtly healthy, show the histological pattern of *severe* acute or chronic AE during the *corresponding stages* when susceptible animals are clinically ill. The distinction between the clinical and histological forms of AE is emphasized by the use of relatively resistant animals, and may or may not be demonstrable in the relatively susceptible Strain 13/N. The possibility of encountering *mild histologic* AE without clinical symptoms has been pointed out by Lumsden (11) who did not accept as "positive" any guinea pig which did not show paralysis without remission. However, the finding of relatively advanced severe histological involvement consonant with severe paralytic or lethal disease, but with no accompanying symptomology, was unexpected. It is worthy of note in this regard that severe histological involvement of the central nervous system was observed in methotrexate-treated mice surviving after infection with otherwise lethal doses of lymphocytic choriomeningitis virus. Mice given this immunosuppressive drug remained clinically healthy despite the presence of extensive acute or chronic lesions comparable to those seen in untreated animals (12).

This observation stresses the importance of making critical distinction between the histological and the clinical aspects of AE. It also engenders interesting speculations as to the mechanisms operative between the inflammatory process within the central nervous system and actual functional damage. The failure of Strain 2/N to show clinical disease despite the anatomic lesions in the CNS indicates a "deficiency" at a very late stage in the pathogenesis of autoimmune encephalomyelitis. If, as has been proposed, the migration inhibition factor (MIF) is responsible for immobilizing mononuclear cells in perivascular sites (13, 14), the paralyzing material suggested here would be a different factor, released or taking effect at an even later stage. A "lymphotoxin" similar to that described by Granger and Kolb, directly damaging neuronal cells would be the type of factor to consider (15).

The present studies provide evidence that several of the effects of varying age, sex, and antigens could be measured only by using relatively resistant animals (all Hartley guinea pigs more than a few weeks old are extremely susceptible to AE). Further, they show that suboptimal conditions of sensitization of guinea pigs may lead to chronic types of AE. It is also evident that experimental conditions which elicit acute AE in certain mature animals induce a chronic form in immature individuals. Although guinea pig, rabbit, or even bovine central nervous system antigens are interchangeably effective in susceptible mature Hartley guinea pigs (16), it is clear from the present work that guinea pig antigen is more efficacious than rabbit antigen. Years ago, even in random-bred guinea pigs, Lumsden found human and goat brain less effective than homologous brain (11). Finally, species differences observed in the amino acid sequences of antigenically active polypeptide or protein isolated from the myelin serve to negate the image of absolute lack of species specificity (17). It should be pointed out that in the present work the "homologous" antigen was homologous for both strains, but in addition was isologous for 2/N.

The relative efficacy of Strain 2 and Strain 13 spinal cord antigens for inducing AE in adult Strain 2 and Strain 13 guinea pigs has been studied previously (5), with no differences observed. Possible differences of these antigens in young animals are the subject of current investigations.

A statement as to the identity of the chronic form of AE may be warranted. In response to some opinions that this was not in fact a distinct and different chronic form of AE, but was instead a quantitatively different mild form of acute AE, it can be reiterated that a disease which is characterized clinically by paraplegia, extreme debilitation, and death cannot be considered mild. The histopathologic finding of massive necrosis in the lumbosacral region of the spinal cord was of a most impressive severity. The appearance of clinical signs at a time later than the entire course of the acute disease obviously removes this from the acute category. The clinical course is one of slow deterioration,

with or without remissions, from onset of signs to paralysis, with marked debilitation and culmination in death.

Knowledge of the existence of *at least two* forms of experimental autoimmune encephalomyelitis may help to reconcile some of the theories regarding its mechanism. The heterogenity of the immune response at both humoral and cellular levels, including the existence of enhancing or protective antibodies specific for myelin antigens, could account for several distinct pathogenetic mechanisms which might otherwise be confused with variations of a single process. Since it has already been accepted that the *acute* form is a fair counterpart to postrabies vaccine encephalomyelitis, if not to postvaccinal encephalitis, the significance of the chronic form vis-à-vis other human demyelinating diseases should be worth investigation.

With regard to the relevance of these findings to certain demyelinating diseases of man, it should be pointed out that postvaccinal encephalitis is very rare in children under 2 years of age (18) and postrabies vaccine encephalomyelitis is not seen in children under 11 years old (19). This is aside from the constitutional or familial predispositions observed in these diseases (18, 19). The familial or geographic, sex- and age-dependency of multiple sclerosis is well known and has been discussed widely (20, 21).

Summary. A variety of conditions favoring the production of *chronic* forms of AE existed between the optimal conditions for *acute* AE (guinea pig antigen in adult Strain 13/N) and for strong resistance to AE (rabbit antigen in newborn Strain 2/N). The resistance of Strain 2/N guinea pigs to autoimmune encephalomyelitis is not absolute. Older Strain 2/N female guinea pigs regularly develop severe or lethal AE when sensitized with guinea pig spinal cord in Freund's complete adjuvant. Guinea pig spinal cord is a more potent encephalitogen than rabbit spinal cord in Strains 2/N and 13/N guinea pigs. Sensitized Strain 2/N guinea pigs of an age group showing no overt clinical signs of AE nevertheless showed striking histological lesions of AE in the central nervous system.

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