Allergic Neuritis Induced in Rats Without the Use of Mycobacteria.* (28524)

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Experimental allergic neuritis has been produced in rabbits, guinea pigs, mice(1), rats(2) and monkeys(3) by injection of peripheral nerve tissue with Freund's complete adjuvant (killed mycobacteria in mineral oil ± emulsifier). However, mycobacteria are not essential components of the adjuvant used with central nervous system tissue for production of the analogous disease, experimental allergic encephalomyelitis (EAE), in rats(4). Similarly, the following experiments demonstrate that mycobacteria are not essential for production of neuritis, inasmuch as a single injection of nerve tissue with "incomplete" adjuvant (oil and emulsifier without mycobacteria) suffices to produce severe disease in rats.

Methods. Rat or guinea pig sciatic nerves and brachial plexuses and human lumbosacral plexuses were dissected as free as possible of fat and connective tissue, stored in frozen state, and cut twice on a freezing microtome. Diverse CNS tissues, also stored frozen, did not require pretreatment. Nerve or CNS homogenates were prepared with water at 40% w/v concentration. Incomplete adjuvant consisted of 8.5 parts of mineral oil (Bayol F) and 1.5 parts of emulsifying agent (Arlacel A). Complete adjuvant contained, in addition, killed dried tubercle bacilli, 4 mg/ml. Water-in-oil emulsions were prepared by cycling equal volumes of tissue homogenate and adjuvant between 2 syringes connected by a double-hubbed needle. Female rats of various strains, 9-11 weeks old, maintained on Purina Laboratory Chow and tap water *ad libitum,* were challenged by a single injection of 0.05 ml emulsion in the right hind foot pad. Signs of neuritis and EAE appeared after 7-14 days. Loss of weight, weakness, paralysis and urinary incontinence occurred in both conditions. Complete paraplegia and incontinence were more common in EAE than in neuritis. Limpness or dragging of the tail were frequent in both, but were not scored because of poor correlation with histologic lesions. Mortality was insignificant and spontaneous recovery was the rule. Rats were exsanguinated under chloroform anesthesia 3 weeks after challenge. Entire spinal cord with attached roots, hindbrain, both sciatic nerves, both brachial plexuses, spinal ganglia, inoculation site (foot pad) and right popliteal lymph node were studied histologically in hematoxylin-eosin, Luxol fast blue, or cresyl violet stains. Neuritis and EAE were graded histologically from 1 to 4 according to number and severity of lesions. Slides were randomized and scored without knowledge of the experimental conditions.

Results. EAE and neuritis in various strains. The choice of rat strain for neuritis experiments was based on experiences with EAE. Previous work had indicated that the Hemlock Hollow (HH) Wistar strain was more susceptible to EAE than 5 other strains (5). The recent appearance on the commercial market of 2 inbred rat strains prompted an evaluation of their susceptibility to EAE. Following challenge with an emulsion of guinea pig cord and incomplete adjuvant, one of these, the Charles River CD F (Fischer 344) strain, proved more susceptible to EAE than 4 other strains carried by the same producer (Charles River Breeding Laboratories) or than the HH Wistar strain (Table I). The incidence of neuritis following challenge with an emulsion of guinea pig nerve and incomplete adjuvant was comparable in 2 different experiments; all of 16 CD F rats had weakness or paralysis of both hind limbs, corroborated by severe histologic nerve lesions. Only 3 out of 10 HH Wistar rats had clinical signs of neuritis (Table II). Addition of mycobacteria to the adjuvant increased the severity of neuritis in either strain only slightly (Table II).

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TABLE I. EAE Produced with CNS Antigens and Incomplete Adjuvant.

Rat strain*	CNS antigen	No. of rats	Clinical EAE (incidence)	Histologic EAE (severity) †
HH	Guinea pig cord	19	11%	1.3
W	" " "	10	0	.5
SD	" " "	10	0	1.7
CD	" " "	10	20	2.3
CD W/Fu	" " "	20	0	1.9
CD F	" " "	41	61	3.4
"	Human white matter	31	0	.4
"	Human cord	10	0	1.3
"	Dog cord	10	0	.8
"	Rat cord	10	0	.5
"	Chicken cord	6	0	.1

* Hemlock Hollow descendants of Wistar (HH); Charles River descendants of Wistar (W), Sprague-Dawley (SD), cesarian-derived Sprague-Dawley (CD), cesarian-derived Furth's W/Fu (CD W/Fu), and cesarian-derived Dunning Fischer 344 (CD F).
† Group avg, graded individually from 0 to 4 according to severity.

TABLE II. Allergic Neuritis Produced with Nerve Antigens and Incomplete or Complete Adjuvant.

Rat strain	Adjuvant	Source of nerve antigen	No. of rats	Clinical neuritis (incidence)	Histologic neuritis (severity)*
CD F	Incomplete	Guinea pig	16	100%	3.2
HH	"	" "	10	30	1.4
CD F	Complete	" "	10	100%	3.6
HH	"	" "	10	30	3.0
CD F	Incomplete	Rat	6	0	0
"	"	Human	16	25	.3
HH	Complete	Rat	9	33	2.2
"	"	Human	10	30	1.6

* Group avg, graded individually from 0 to 4 according to severity.

Antigens. Differences in encephalitogenic potency of various CNS tissues have been recorded(4,5,6). CD F rats were challenged with emulsions of diverse CNS or peripheral nerve tissues and incomplete adjuvant. Guinea pig spinal cord gave a higher incidence of EAE than human, dog, rat or chicken CNS tissues (Table I). Guinea pig nerve was superior to human or rat nerve in neuritogenic potency (Table II). The incidence of neuritis with human or rat nerve was higher when these antigens were combined with complete adjuvant (Table II).

Histopathology. After inoculation with peripheral nerve antigen and adjuvant, there were inflammatory and demyelinating lesions in nerves, roots and ganglia. There were no qualitative differences that depended on rat strain, type of adjuvant, or source of nerve antigen. Nerves exhibited no abnormalities of mast cell number, size, or granulation. Neuritis was more severe in sciatic nerves than in brachial plexuses. Right and left sciatics were occasionally dissimilar, but there was no consistent lateralization of neuritis despite the presence of the inoculation site in the right foot. Root and nerve lesions were not necessarily proportional. The CNS did not escape completely. Most CNS lesions were minor mononuclear cell infiltrates in meninges adjacent to spinal nerve roots. However, many rats had a few perivascular infiltrates in the CNS parenchyma, and some had severe and even destructive lesions. Most CNS lesions were in the spinal cord, but some were in the cerebellum, far removed from nerve root attachments; hence they could not be interpreted as mere "overflow" of root inflammation. These CNS lesions were indistinguishable from lesions of EAE.

"Cross reactions" occurred also in the opposite direction. That is, rats with EAE fol-

lowing inoculation of CNS antigen with complete or incomplete adjuvant, frequently had lesions in nerves and roots in addition to CNS. Cross reaction in this direction was more frequent and severe than that described above. In general, cross reactions were more frequent when the primary disease was severe. Despite cross reactions, neuritis and EAE in the rat were distinctly separate entities on the basis of predominant involvement of the tissue corresponding to the inoculated antigen.

Lymph nodes draining inoculation sites of either CNS or nerve antigen with incomplete adjuvant had many small empty vacuoles representing oil droplets from the adjuvant. Focal suppuration, as seen following injection of complete adjuvant(5) was absent. There was only slight epithelioid cell response around oil droplets except in the CD F strain, in which the granulomatous response was strong. This outstanding reaction of CD F rats was observed following injection of either CNS or nerve antigen in incomplete adjuvant. The correlation of lymph node lesions and propensity to auto-allergic disease has been noted before(2,5).

Discussion. The production of EAE previously (Paterson and Bell(4)) and of allergic neuritis in the present work, with antigen and incomplete adjuvant, shows that mycobacteria are not essential for development of autoimmune disease in the rat, provided one employs a highly reactive strain. Application to auto-allergies of other tissues may be of interest. It is curious that guinea pig CNS and nerve were especially potent antigens for EAE and neuritis under the particular conditions of our experiments (combined with incomplete adjuvant and injected in CD F rats). This cannot be ascribed entirely to ancillary adjuvant activity of foreign proteins (or Forssman antigen) contained in the heterologous guinea pig tissue, because other heterologous tissues (Forssman positive dog and chicken, Forssman negative human) were much less active in CD F rats.

Cross reactions occurred in both directions in EAE and neuritis produced by CNS and nerve antigens in the rat. This places the rat in the same category as the guinea pig, and opposed to rabbit and mouse in which cross reactions occur in one direction only (no CNS lesions occur after sensitization with nerve)(1). However, such distinction is subject to qualification because cross reactions appear to depend on the severity of the primary disease, and neuritis in the mouse, for example, was mild(1).

Summary. Experimental allergic neuritis was produced in rats by a single injection of peripheral nerve antigen with "incomplete" adjuvant. Thus, mycobacteria were not essential components of the adjuvant. The incidence of neuritis depended on the strain of rats and the source of antigen. There was some overlap ("cross reactions" in both directions) in the involvement of rat peripheral and central nervous systems following challenge with peripheral and central antigens.

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