Genetic Control of Susceptibility to Experimental Allergic Encephalomyelitis in Rats

Abstract. *Rats of the inbred strains Lewis and DA are highly susceptible to the induction of experimental allergic encephalomyelitis (EAE) while Brown Norway rats are resistant to this disease. Evidence has been obtained which suggests that a single dominant gene is associated with susceptibility to EAE. The locus controlling EAE susceptibility is closely linked to the* Ag-B *histocompatibility locus but is not identical to it.*

Experimental allergic encephalomyelitis (EAE) can be readily produced in several species of rats by the injection of spinal cord tissue emulsified in complete Freund's adjuvant (CFA). The immunological basis of this disease was established by Paterson's demonstration that EAE could be passively transferred in rats by sensitized lymphoid cells (*1*). In order for passive transfer to be successful, donor and recipient must be compatible at the major histocompatibility locus (*2*).

Although EAE can easily be produced in Lewis and DA rats by injection of guinea pig spinal cord in CFA, rats of Brown Norway (BN) strain are not susceptible to this disease (*3*). In examining the genetic basis of this difference we have observed that a single gene determining resistance to induction of EAE is linked to the *Ag-B* histocompatibility locus.

All rats used in this experiment were obtained from the animal colonies of the Department of Human Genetics, University of Pennsylvania, and the Wistar Institute. The animals in the (Lewis × BN)F_1 × BN generation were typed for *Ag-B* alleles by the mixed leukocyte culture (MLC) test (*4*), since no recombinants between the serologically defined *Ag-B* locus and the locus determining MLC reactivity have yet been reported (*5*).

Guinea pig spinal cords (Pel-Freeze Biologicals) were stored at −60°C until use. Rats were injected intradermally in the hind footpads with 110 mg of guinea pig spinal cord in an equal volume (0.35 ml) of CFA (Difco). The rats were killed 16 days later, and the spinal cords with roots and cauda equina of all animals and the cerebrum and cerebellum of a few animals were removed, fixed in 4 percent paraformaldehyde in 0.1*M* phosphate buffer, and embedded in paraffin. The embedded roots were cut in a longitudinal plane. The spinal cords were processed as follows. Serial sections of alternating 2-mm transverse sections, and 5-mm longitudinal sections were obtained from the entire cord. Each block was sectioned semiserially, and an average of eight to ten sections, stained with hematoxylin and eosin, were studied from each block. Four to six coroneal sections were processed from cerebrum and cerebellum. The affected areas in order of severity were (i) segments of spinal roots adjacent to the cord and the cauda equina, (ii) the lumbar and thoracic cords, and least of all (iii) the cerebrum and cerebellum.

Since under the conditions of our experiment clinical signs of EAE were not always recognized with ease, grading of lesions of EAE was done on histologic sections alone. Grading was done by means of a modification of a previously described system (*6*) by one observer (N.K.G.) who did not know the identity of the slides being examined (Table 1).

Of more than 200 Lewis rats examined, not one failed to develop EAE after the above-mentioned treatment. Ten BN rats were examined histologically and none developed EAE. All eight (Lewis × BN)F_1 hybrids developed EAE and, therefore, susceptibility is inherited as a dominant trait. Among 26 (Lewis × BN)F_1 × BN backcross rats, 12 developed EAE and 14 did not, a result consistent with the 1 : 1 ratio expected on the basis of a single gene trait. These backcross rats were classified according to *Ag-B* type and severity of EAE (Table 1). All 11 of the $Ag\text{-}B^3/Ag\text{-}B^3$ homozygotes were resistant to the disease, while 12 of the 15 $Ag\text{-}B^1/Ag\text{-}B^3$ heterozygotes were susceptible, with varying degrees of histologic damage to the spinal cord. The correlation between EAE susceptibility and *Ag-B* type is highly significant ($\chi^2 = 13.28$; $P < .005$) and suggests that the genetic locus controlling susceptibility to EAE is either identical to the *Ag-B* locus or is closely linked to it. The fact that three $Ag\text{-}B^1/Ag\text{-}B^3$ heterozygotes did not develop the disease suggests either that there is recombination between *Ag-B* and the locus controlling EAE susceptibility or that there is nongenetic variation in this system.

Table 1. Comparison of EAE susceptibility with *Ag-B* type among (Lewis × BN)F_1 × BN backcross rats. The spinal cord ratings are as follows: 0, no infiltration of mononuclear cells; 1, one infiltrate in spinal root; 2, many infiltrates in spinal roots, and occasional infiltrates in spinal cord; 3, many root and cord infiltrates; and 4, many confluent root and cord infiltrates.

$Ag\text{-}B^1/Ag\text{-}B^3$		$Ag\text{-}B^3/Ag\text{-}B^3$	
Animal number	Spinal cord histology	Animal number	Spinal cord histology
532	0	529	0
535	3	538	0
536	3	540	0
617	4	541	0
664	4	542	0
530	0	618	0
531	2	736	0
728	0	665	0
729	3	564	0
735	3	561	0
737	1	669	0
738	2		
667	3		
663	2		
672	2		

In order to discriminate between these two possibilities, which are not mutually exclusive, we examined rats of strains DA and BN.B4. Strain BN.B4 is a congenic strain which has the genetic background of strain BN, but is homozygous for the $Ag\text{-}B^4$ allele of strain DA (*7*). Strain DA has been found to be susceptible to EAE (*3*), and in our hands eight of ten rats of this strain developed the disease. When 14 BN.B4 rats were tested, 13 were completely resistant to the induction of EAE, and 1 was scored "questionable."

We therefore conclude that susceptibility to EAE in rats is determined by a single dominant gene that is closely linked but not identical to *Ag-B*. It appears that in the derivation of BN.B4, a crossover occurred between these two loci. If we assume that the three *Ag-B* heterozygotes in Table 1 which did not get EAE are recombinants and that these are the only recombinants out of the 26 rats tested, then the recombination frequency between the *Ag-B* locus and the locus determining susceptibility to EAE is 11.5 ± 7.9 percent. This must be considered a very crude estimate because we do not yet know how much nongenetic variability is involved in this system. However, since the BN.B4 rats were resistant to the disease, we are confident that the locus controlling EAE susceptibility is not *Ag-B*.

There are basically three mechanisms by which this gene might operate. (i) The allele possessed by BN might code for a protein which is not encephalitogenic, so that an immune response to guinea pig spinal cord does not react with self-antigens in a destructive manner. (ii) BN might possess an encepha-

litogenic protein but be unresponsive to it. Many examples of such immune response (Ir) genes are now known, and many of these are linked to major histocompatibility loci such as *Ag-B* (*8*). (iii) An entirely nonimmunological mechanism could be involved.

At present there is not enough information to establish which of these mechanisms is correct, but the work of Kornblum (*3*) strongly supports possibility (ii). Sensitized lymph node cells from Lewis but not BN rats were able to react against guinea pig spinal cord in the irradiated hamster test. If possibility (ii) is correct, it is interesting to note how the EAE gene differs from *Ir-1* of mice. *Ir-1* maps inside the *H-2* region (*9*), whereas the EAE gene is separable from *Ag-B* by recombination. Second, the *Ir-1* locus of mice is known to cause stimulation in the MLC test (*10*), but this is not the case with the EAE gene. Cells of DA and BN.B4 do not stimulate each other in the MLC test, but those of BN.B4 and BN cause mutual stimulation in mixed culture (*11*). Since BN.B4 and BN have the same susceptibility to EAE, the locus associated with this disease is distinct from that causing MLC stimulation.

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Clarification of the Chemical Status of the Pink Bollworm Sex Pheromone

Abstract. *Propylure, 10-*n*-propyl-*trans*-5,9-tridecadienyl acetate, and deet,* N,N,-*diethyl-*m*-toluamide, were previously reported as the sex pheromone and a sex pheromone activator, respectively, of the pink bollworm. Neither chemical in three extracts of female moth abdomen tips could be detected by gas-liquid chromatographic analysis. These compounds, alone or in combination, exhibited little or no biological activity in the laboratory or in the field. Hexalure,* cis*-7-hexadecenyl acetate, a synthetic attractant for pink bollworm males, could not be detected in female moth abdomen tip extracts. The pink bollworm sex pheromone was identified as a mixture of* cis,cis *and* cis,trans *isomers of 7,11-hexadecadienyl acetate.*

Considerable confusion exists concerning the status of female sex pheromones (*1*) or sex attractants (*1*), of the pink bollworm moth, *Pectinophora gossypiella* (Saunders) (Gelechiidae). In 1966, Jones *et al.* (*2*) isolated and identified a compound, 10-*n*-propyl-*trans*-5,9-tridecadienyl acetate (propylure), from an extract made from whole bodies of virgin female moths. They reported that propylure caused a sexual response in caged male moths, thus qualifying it as a sex pheromone. However, their subsequent studies indicated that propylure was not attractive to male moths in the field (*3*). In 1968, Jones and Jacobson (*3*) stated that for attractancy in the field, propylure requires the simultaneous presence of a naturally occurring activator. The activator, which they found in methylene chloride extracts of female pink bollworm moths, was *N,N*-diethyl-*m*-toluamide. This compound is commercially available as the insect repellent "deet" and had been found earlier to be moderately attractive to male pink bollworm moths (*4*).

The claim that propylure was a sex pheromone for the pink bollworm was challenged by Eiter *et al.* (*5*) in 1967. They synthesized the compound independently and reported that it did not excite male moths in the laboratory. Jacobson (*6*) then presented data showing that as little as 15 percent of the *cis* isomer of propylure could completely mask the biological activity of the natural *trans* isomer. This masking, he explained, was the reason for the lack of activity of Eiter's preparation, which was a 1 : 1 mixture of *cis* and *trans* isomers.

At about the same time, through empirical screening of synthetic compounds, an unrelated chemical was found to be an attractant for pink bollworm males in the field (*7*). This compound, *cis*-7-hexadecenyl acetate, was called hexalure.

Because of this confused situation, we undertook a reinvestigation of propylure (*8*), deet (*9*), and hexalure (*10*), with regard to their possible presence in the female moths and their biological activities in the laboratory and the field.

The natural sex pheromone was characterized by determining its gas-liquid chromatographic (GLC) retention time, with the use of a hydrogen flame ionization detector in parallel with a cage of male moths. An ether extract of the abdomen tips of 200 adult virgin female moths, 2 to 4 days old (*11*), was filtered to remove particulate matter and a portion was used for quantitative bioassays. The ether was removed from the remainder by evaporation at room temperature with a stream of nitrogen. The residue was taken up in a known volume of carbon disulfide and analyzed by GLC (*12*). A "splitter" diverted nine parts of the effluent to the cage of male moths used as a biological detector while one part

Table 1. Gas-liquid chromatographic characteristics for hexadecyl acetate, hexalure, propylure, deet, and extracted pink bollworm sex pheromone.

Liquid phase	Relative retention time (hydrogen flame)				
	Hexadecyl acetate	Hexalure	Propylure	Deet	Extracted pheromone
Apiezon L	1.00	0.83	0.51	0.15	0.80
SF-96	1.00	.86	.62	.15	.78
NPGA	1.00	1.01	.71	.44	1.03
Carbowax 20M	1.00	1.05	.83	.87	1.24
QF-1	1.00	.91	.63	.60	.86