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Cell-Mediated Immunity in Experimental Allergic Encephalomyelitis: Cross Reactivity Between Myelin Basic Protein and Mycobacteria Antigens[1] (38723)

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Myelin basic protein can initiate an immunologically mediated disease of the central nervous system (experimental allergic encephalomyelitis, EAE) if injected with adjuvant containing mycobacteria. Neither basic protein nor mycobacteria injected individually, however, produces the severe neurological sequelae of classical EAE. The requirement of mycobacteria in inducing EAE is not well understood, although it is likely that the mycobacteria may augment the cell-mediated immune (CMI) response to the encephalitogenic basic protein antigen (1). Since mycobacteria (2) or basic protein (3) exposure prior to an encephalitogenic challenge confers protection against EAE, a more direct relationship between basic protein and mycobacteria antigens is strongly suggested. Apparently there are not antigenic similarities between these agents since mycobacteria pretreatment does not induce antibody against basic protein (4). However, this does not exclude cross-reacting determinants that may only stimulate cell-mediated immunity. Cross reactivity at the cellular level would be of particular interest since it is believed that EAE is mediated by CMI against myelin determinants. This report documents the dermal cross reactivity between mycobacteria and both guinea pig and human basic protein.

Materials and Methods. Experimental protocol. In these experiments randomly selected male or female Hartley and Rockefeller strain guinea pigs (Life Systems, Box 25093, Portland, OR) were used. Animals received no injection or were injected in the front footpads (0.1 ml/footpad) with one of the following: (a) Freund's incomplete adjuvant (FIA, DIFCO); (b) FIA with 2.5 mg *Mycobacterium tuberculosis* H37RA (DIFCO); (c) FIA with 20 mg guinea pig spinal cord (GPSC); (d) FIA with 100 μg guinea pig basic protein[2] (GPBP); (e) FIA with 100 μg human basic protein (HBP); or (f) FIA with 2.5 mg bovine serum albumin (BSA). All animals were skin tested intradermally 5–8 wk after injection with combinations of the following: (a) finely powdered *Mycobacterium tuberculosis* H37RA (100 μg in 0.1 ml saline); (b) tuberculin, purified protein derivative (PPD, Parke-Davis) (10 μg); GPBP (20 and 100 μg); HBP (100 μg), BSA (20 μg). Skin test sites were observed by two separate investigators for erythema and induration at 6, 24, and 48 hr. Data presented here represent the diameter of induration of the test site 24 hr after skin testing. Positive induration at 24 hr always persisted through 48 hr, although erythema was usually diminished by the later reading.

Blood from some animals was cultured according to the method of Han and Pauly (6). Briefly, heparinized blood was diluted 1/33 in RPMI 1640 (GIBCO) with 25 m*M* Hepes buffer and antibiotics and was dispensed in 3-ml aliquots. The cultures were incubated in duplicate with GPBP (20 μg), HBP (20 μg), PPD (10 μg), or without addition for 6 days, the last day with 1 μCi ^{3}H-thymidine (6.7 Ci/mmole, New England Nuclear). Cultures were harvested on glass fiber filters, rinsed with 3% acid, and counted by standard liquid scintillation techniques.

Results. Response of animals receiving FIA with neural tissue antigens. Rockefeller

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[2] Highly purified GPBP and HBP was kindly donated by Dr. Marian Kies, NIH, Bethesda, MD. Some of the GPBP used in these experiments was prepared by the authors according to the method of Oshiro and Eylar (5).

strain guinea pigs, injected with guinea pig spinal cord emulsified in FIA exhibited a significant dermal response (7 and 9 mm induration) when tested with 100 μg GPBP. A lower dose of (20 μg) GPBP failed to elicit substantial dermal reactivity in either Hartley or Rockefeller guinea pigs immunized with the same inoculum. These Hartley and Rockefeller animals, however, showed significant dermal reactivity to tuberculin ($\bar{x}$ = 12 and 21.3 mm induration, respectively) as well as to mycobacteria ($\bar{x}$ = 9.0 and 16.7 mm induration, respectively) even though they had no previous exposure to mycobacteria (Table I). The Rockefeller guinea pigs injected with FIA emulsified with GPSC also expressed cross reactivity to PPD as measured by lymphocyte transformation. Tuberculin stimulated 24,000 ± 2600 cpm of ^{3}H-thymidine incorporation in the cultures from these animals. This response was significantly greater ($P < 0.001$) than the response of similar cultures containing no antigen (183 ± 9 cpm) or the response to PPD of cultures from untreated or FIA-treated animals (750 ± 250 and 495 ± 304 cpm, respectively). Basic protein did not significantly stimulate lymphocyte cultures from animals receiving FIA with GPSC.

Hartley guinea pigs that were injected with FIA emulsified with purified GPBP showed a dermal response to GPBP ($\bar{x}$ = 8 mm induration), HBP ($\bar{x}$ = 8.0 mm induration) and a cross reactivity to PPD ($\bar{x}$ = 11 mm induration). Hartley animals injected with FIA with purified HBP also showed a substantial response to GPBP ($\bar{x}$ = 10.5 mm induration), HBP ($\bar{x}$ = 13 mm induration) and to PPD ($\bar{x}$ = 14.7 mm induration) (Table I).

Response of animals receiving FIA with Mycobacterium tuberculosis. In reciprocal experiments, Hartley guinea pigs injected with FIA with *Mycobacterium tuberculosis* showed strong dermal reactivity to both PPD ($\bar{x}$ = 24.5 mm induration) and mycobacteria ($\bar{x}$ = 22 mm induration) as expected, but responded weakly to 20 μg GPBP. Rockefeller guinea pigs injected with FIA with mycobacteria reacted strongly to PPD ($\bar{x}$ = 23.3 mm induration) and to mycobacteria ($\bar{x}$ = 21 mm induration) but also showed a significant cross reactivity to 100 μg GPBP ($\bar{x}$ = 8.5 mm induration) and to 100 μg HBP ($\bar{x}$ = 7.8 mm induration) (Table I). Lymphocyte cultures from all the animals injected with FIA with mycobacteria showed a significant transformation response to PPD as expected but failed to respond significantly to either GPBP or HBP tested over a wide concentration range.

Response of control animals. Six control animals receiving no injection or FIA emulsified with saline had skin tests of less than 5 mm induration to all the test antigens. Additionally, six animals receiving FIA with BSA responded substantially to BSA skin tests (14 mm induration, Table I), but did not respond to intradermal challenge by GPBP or PPD.

Discussion. These data clearly show dermal cross recognition of basic protein and mycobacteria antigens by two strains of guinea pigs. These data also provide evidence for antigenic similarities in GPBP and HBP. That is, animals injected with either FIA with GPBP or FIA with HBP respond to both GPBP and HBP as well as to PPD. Additionally, animals injected with FIA with mycobacterium respond to both GPBP and HBP.

Significant dermal response to BP required a high concentration of BP even in animals immunized with neural tissue antigens. Initial testing with 20 μg GPBP failed to demonstrate significant skin test reactivity; subsequently we found that 100 μg of both GPBP and HBP elicited substantial skin tests in the experimental groups without causing toxic reactions in the control groups. The conditions which were employed above appear to be important in demonstrating skin reactivity to BP in animals injected with FIA with mycobacterium (i.e., sensitization with 2.5 mg mycobacterium followed 5–8 wk later with skin tests using 100 μg BP). Previous reports (7–9) indicated that animals injected with Freund's complete adjuvant (with or without other antigens) did not show dermal reactivity to BP. However, the experimental conditions that were used in these reports included lower immunizing doses of mycobacterium, lower skin test concentrations of BP, and a shorter immunization period.

TABLE I. DERMAL REACTIVITY OF GUINEA PIGS TO BASIC PROTEIN AND MYCOBACTERIA PREPARATIONS[a]

Sensitization-animal group[b]	Basic protein			Mycobacteria preparations	
	Guinea pig (20 μg)	Guinea pig (100 μg)	Human (100 μg)	Tuberculin (10 μg)	Mycobacteria (100 μg)
FIA + GPSC	—	ND	ND	10	9
Hartley strain	—	ND	ND	8	—
	—	ND	ND	14 (B)	12
	—	ND	ND	16 (B)	11
Rockefeller strain	—	7	ND	22 (BN)	16 (B)
	5	ND	ND	24 (BN)	17 (B)
	—	9	ND	18 (B)	17 (B)
FIA + GPBP	ND	7	8	10	ND
Hartley strain	ND	5	8	12 (B)	ND
	ND	10 (B)	8 (B)	12	ND
	ND	10 (BN)	8 (BN)	10	ND
FIA + HBP	ND	6	12	17 (BN)	ND
Hartley strain	ND	9	10	15	ND
	ND	17 (B)	18 (BN)	12	ND
	ND	10	12	15	ND
FIA + mycobacteria	—	ND	ND	28 (N)	25 (BN)
	—	ND	ND	22 (N)	25 (BN)
Hartley strain	—	ND	ND	25 (N)	22 (BN)
	—	ND	ND	23 (N)	16 (B)
Rockefeller strain	5	11	ND	24 (N)	22 (BN)
	ND	10	ND	28 (N)	20 (BN)
	—	7	8	31 (BN)	ND
	—	12	10	17 (B)	ND
	—	6	7	28 (BN)	ND
	—	5	6	12	ND
Untreated	—	—	—	—	—
Hartley strain	—	—	—	—	—
FIA + saline	—	—	—	—	5
Hartley strain	—	—	—	5	5
	—	—	—	—	—
	—	—	—	—	—
FIA + BSA[c]	ND	—	ND	—	ND
	ND	—	ND	—	ND
	ND	—	ND	—	ND
	ND	—	ND	—	ND
	ND	—	ND	—	ND
	ND	—	ND	—	ND

[a] Number represents diameter (mm) of induration at 24 hr skin tests; — = <4 mm diameter of induration; ND = Not done; B = Blanched center of skin test; N = Central necrosis at skin test site.

[b] FIA = Freund's Incomplete Adjuvant; GPSC = Guinea pig spinal cord; GPBP = Guinea pig basic protein; HBP = Human basic protein.

[c] This group of animals showed a significant response to 25 μg BSA ($\bar{x}$ = 14 mm induration).

Lymphocyte cultures from FIA–GPSC-immunized animals showed a significant transformation response to PPD, whereas cultures from control animals were negative. The lymphocyte response to PPD confirms the dermal reactivity of FIA–GPSC-immunized animals to tuberculin. However, lymphocyte responsiveness to basic protein was not demonstrated in these animals or in animals immunized with mycobacterium. This suggests that lymphocyte transformation to BP is a poor correlate of dermal reactivity to the same preparation.

The cross reactivity described in this report may help explain the role of mycobacteria in the induction of EAE or protection

against it. The addition of mycobacteria to the encephalitogenic mixture may simply represent an increase in disease-inducing antigenic load. That is, mycobacteria may stimulate a larger population of potentially destructive lymphocytes which could react against myelin basic protein. This alternative does not seem completely sufficient since in the guinea pig, mycobacteria alone does not cause neurological sequelae. It is possible, however, that the cross-reacting antigen is not the rigidly defined nonapeptide sequence (residues 114–122) that is the major encephalitogenic determinant in the guinea pig. The cross-reacting structure might reside on a nonencephalitogenic portion of the basic protein. This possibility is consistent with other data (10) which suggest that in the guinea pig, disease-inducing and disease-protecting sites on the basic protein molecule are separate. Thus, injection of mycobacteria alone would not induce EAE but could afford some protection against subsequent encephalitogenic injections by inducing a population of sensitized lymphocytes, reactive to a nonencephalitogenic moiety of basic protein, which could essentially eliminate a subsequent injection of basic protein.

Cross reactivity of BP and mycobacteria in other species was previously suggested by our observation that Freund's complete adjuvant (FCA)-pretreated rats were resistant to subsequent encephalitogenic injections (unpublished observation). Additionally, the injection of mycobacteria (FCA) in rats can induce adjuvant arthritis which is occasionally accompanied by neurological sequelae similar to that seen in mild EAE (11). Thus, in the rat the cross-reactive site may be found within the encephalitogenic sequence (which includes residues 45–86) (12).

Cross reactivity of HBP and mycobacterium may be important in the induction of or protection against human demyelinating diseases such as multiple sclerosis. That is, if the cross-reacting structure is found on the encephalitogenic portion of HBP, mycobacterial exposure could initiate an autoimmune response against HBP that could lead to neurological sequelae. If the structure is found on a nonencephalitogenic portion of HBP, mycobacterial exposure could protect the individual against autoimmune damage that might be initiated by another agent.

Summary. Guinea pigs injected with Freund's incomplete adjuvant emulsified with guinea pig spinal cord, purified guinea pig myelin basic protein, or human myelin basic protein showed dermal reactivity to both of the basic proteins as well as to mycobacteria antigens. Animals receiving only mycobacteria antigens expressed dermal reactivity to the sensitizing antigen in addition to basic protein. This cross reactivity may help explain the role of mycobacteria in inducing and protecting against EAE, and may have important implications concerning human demyelinating diseases.

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