References and Notes

1. J. Roth, *Metab. Clin. Exp.* **22**, 1059 (1973).
2. J. S. Flier, C. R. Kahn, J. Roth, R. S. Bar, *Science* **190**, 63 (1975).
3. C. R. Kahn, J. S. Flier, R. S. Bar, J. A. Archer, P. Gorden, M. M. Martin, J. Roth, *N. Engl. J. Med.* **294**, 739 (1976).
4. J. S. Flier, C. R. Kahn, D. B. Jarrett, J. Roth, *J. Clin. Invest.* **58**, 1442 (1976).
5. L. C. Harrison, J. S. Flier, J. Roth, F. A. Karlsson, C. R. Kahn, *J. Clin. Endocrinol. Metab.*, **48**, 59 (1979).
6. Since insulin can block the binding of ^{125}I-labeled antibody to the receptor, the observation that antibody can precipitate solubilized insulin receptors labeled with [^{125}I]insulin may appear contradictory. We characterized this phenomenon in detail elsewhere (*5*) and demonstrated that solubilized receptors labeled with tracer amounts of [^{125}I]insulin are precipitated by excess receptor antibody. The explanation is probably that receptors in solution are multimeric and under conditions of tracer [^{125}I]insulin binding additional sites for insulin and receptor antibody are available. These sites are blocked by saturating concentrations of insulin.
7. D. B. Jarrett, J. Roth, C. R. Kahn, J. S. Flier, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4115 (1976).
8. C. R. Kahn, K. Baird, J. S. Flier, D. B. Jarrett, *J. Clin. Invest.* **60**, 1094 (1977).
9. Y. LeMarchand-Brustel, P. Gorden, J. S. Flier, C. R. Kahn, P. Freychet, *Diabetologia* **14**, 311 (1978).
10. L. C. Harrison, T. Billington, I. J. East, R. J. Nichols, S. Clark, *Endocrinology* **102**, 1485 (1978).
11. G. Saviolakis and L. C. Harrison (unpublished observation) showed that gel filtration to separate labeled receptor is unnecessary and that the presence of free insulin has no effect on the immunoassay under the conditions described.
12. J. R. Gavin III, J. Roth, D. M. Neville, Jr., P. De Meyts, D. N. Buell, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84 (1974).
13. Our estimate of assay sensitivity may depend on the validity of using a single-site cooperative model instead of a multiple-site model for insulin binding. If the multiple-site model were correct and the antibody bound only to high-affinity sites, our estimate of sensitivity obtained by extrapolation to the horizontal axis of the Scatchard plot (*11*) would be spuriously low. Thus, the estimated sensitivity presented here may be the minimum value.
14. J. R. Gavin III, P. Gorden, J. Roth, J. A. Archer, D. N. Buell, *J. Biol. Chem.* **248**, 2202 (1973).
15. J. Roth, *Methods Enzymol.* **37**, 223 (1975).
16. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).

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Transfer of Experimental Allergic Encephalomyelitis with Guinea Pig Peritoneal Exudate Cells

Abstract. *Incubation with specific antigen, myelin basic protein, greatly enhances the ability of guinea pig peritoneal exudate cells to transfer experimental allergic encephalomyelitis. Reproducibly successful transfers are obtained with 10^7 cells. With this relatively small number of cells, in vitro studies to determine the immunologic mechanisms involved in the disease process are now possible.*

Experimental allergic encephalomyelitis (EAE) is a cell-mediated autoimmune disease induced in susceptible animals by sensitization with central nervous system (CNS) myelin basic protein (BP) (*1*). Successful adoptive transfer of full-blown EAE in guinea pigs was originally reported by Stone (*2*), who found that transfer depended not only on viable histocompatible lymph node cells (LNC) but also required sensitization of donors with a large amount (2.5 mg) of mycobacteria in conjunction with whole spinal cord antigen. Falk *et al.* (*3*) confirmed these results and reported that EAE could also be transferred with cells from donors sensitized with large amounts of BP and mycobacteria (0.5 mg each). We later showed that a more reproducible transfer could be achieved (paralysis and CNS lesions in all recipients) by sensitization of donors with 0.5 mg of BP plus the larger amount of mycobacteria originally suggested by Stone (2.5 mg) (*4*). The number of LNC required for successful transfers was of the order of 10^9 regardless of whether whole tissue or BP was the sensitizing CNS antigen. On the basis of cell numbers, freshly harvested peritoneal exudate cells (PEC) are probably no better than LNC for disease transfer, since 5×10^8 PEC induced only mild disease in recipients (*5*).

Because of the large number of cells required, cellular transfer has not been extensively used for examining the specific immunologic mechanisms of EAE. We report here that in vitro culture of BP-sensitized PEC with specific antigen (BP) enhances the ability of PEC to transfer disease, reducing the number of cells required to induce both clinical and histologic signs of EAE in the recipients.

Male guinea pigs (strain 13) weighing 400 to 700 g (Division of Research Services, NIH) were used in all experiments. Donors were sensitized with either 100 μg of BP, 100 μg of BP plus 100 μg of ovalbumin (Sigma), or saline in 0.1 ml of complete Freund's adjuvant (CFA), containing 100 μg of H-37R$_v$, in a single intracutaneous site on the chest. Ten days after sensitization, the animals were injected in the peritoneum with 25 to 30 ml of sterile paraffin oil. Three days later the animals were anesthetized, and their peritoneal cavities were washed with 200 to 300 ml of Hanks balanced salt solution (HBSS).

After two washings in HBSS, the cells were suspended in RPMI 1640 medium containing 5 percent fetal calf serum, glutamine (300 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml). These cells (2×10^7 to 4×10^7) were then plated in 100-mm plastic tissue culture dishes for 2 hours at 37°C in a humidified air mixture containing 5 percent CO_2. After incubation the nonadherent cells were removed, and the plates were washed once with medium. The pooled nonadherent cells were centrifuged and resuspended in medium. Viability was determined by trypan blue exclusion, and the number of phagocytic cells was determined by latex bead ingestion. All cell recoveries are based on viable cell counts. The cells were incubated for 72

Table 1. Transfer of EAE with cultured PEC.

Donor sensitization*	Culture	Antigen in culture		Cells (10^7)	No. sick / No. recipients	D.I.† (mean ± S.E.)
		Concentration (μg/ml)	Type			
100 μg of BP + CFA	−			4.0	0/2	0
	−			2.0	0/1	0
	+	10	BP	4.0	6/6	8.4 ± 0.6
	+			3.5	2/2	9.0 ± 0.0
	+			2.0	5/5	9.1 ± 0.6
	+			1.0	10/10	8.0 ± 0.4
	+			0.5	3/6	8.0, 9.0, 7.5‡
	+			0.25	2/4	8.0, 9.0‡
	+			0.1	2/3	6.0, 9.0‡
100 μg BP + 100 μg Ova + CFA	+	None		3.0	0/1	0
	+			2.0	0/1	0
	+	10	BP	2.0	1/1	7.5
	+			1.0	1/1	9.0
	+			0.6	1/1	7.5
	+	100	Ova	4.0	0/1	0
	+			2.0	0/1	0
CFA	+	10	BP	4.0	0/2	0

*Myelin basic protein was isolated from guinea pig CNS tissue (*10*) and purified by ion exchange chromatography (*11*). †D.I. = Disease index—severity of disease based on a combination of clinical and histologic signs of disease (*6*). ‡Disease indices of sick animals.

hours in flat-bottom plastic trays (Costar, Tissue Culture Clusters[24], Cambridge, Mass.) at a concentration of 1×10^6 cell/ml. The concentration of antigen was 10 μg/ml for BP, and 100 μg/ml for ovalbumin.

Portions of each cell preparation (0.2 ml) were also incubated in quadruplicate in 96-well flat-bottom microtiter tissue culture plates (1×10^6 cell/ml). After 2 days, 1 μCi of [^{3}H]thymidine (6.0 Ci/mmole, Schwarz-Mann) was added to each well, and 18 to 20 hours later the cells were harvested with a semi-automatic cell harvester (MASH II, Microbiological Associates). The radioactivity on the glass fiber discs was counted in a Packard Tri-Carb liquid scintillation counter. The stimulation index (S.I.) is equal to the ratio of the mean number of counts per minute of cultures containing antigen to that of cultures without antigen.

After a 3-day incubation period, with or without antigen, the cells intended for transfer were harvested and counted, and the number of phagocytic cells was determined. Cultured cells were then injected intraperitoneally into recipient strain 13 guinea pigs, which were weighed daily and checked for clinical signs of EAE. Positive animals developed clinical signs within 4 to 6 days. They were anesthetized when acutely ill. Negative animals were anesthetized 15 days after the injection of cells. The CNS tissues of all recipients were fixed for histologic examination (*6*).

The average phagocytic cell content of PEC was 63 percent. Plating for 2 hours reduced the content of phagocytes to 46 percent, with a recovery of 66 percent of the viable nonphagocytic cells. After 72 hours in culture, the average recovery of viable nonphagocytic cells was 44 percent of those put into culture. The final cell suspension injected into the recipient contained 69 percent viable nonphagocytic cells.

The cell transfer experiments are summarized in Table 1. Sensitized cells transferred after plating but without culture failed to transfer EAE even when 4×10^7 cells were used. In contrast, 1×10^7 of these cells incubated with BP for 72 hours transferred acute EAE to recipient animals. This result was completely reproducible, both clinically and histologically. Transfer of full-blown disease was also accomplished in two of three recipients with as few as 1×10^6 cells.

When cells from BP-sensitized animals were incubated without BP, and when cells from CFA-injected animals were incubated with BP, the disease was not transferred. The result in the latter case ruled out the possibility of active sensitization of recipients by BP nonspecifically bound to cells. The specificity of the effect of BP in culture on transfer of EAE was further confirmed by double sensitization of donors with BP and ovalbumin in CFA. Cells from these animals were subsequently cultured with BP or ovalbumin. Only the cells incubated with BP were capable of transferring disease.

The S.I. of cells from BP-sensitized animals, incubated with BP was variable, ranging from 1.7 to 9.6 with a S.I. for 15 experiments of 5.3 ± 0.6 (mean ± S.E.M.). Cells from doubly sensitized donors responded to both ovalbumin (S.I. = 13.9) and to BP (S.I. = 7.1).

From our S.I. data it is obvious that some BP-specific cells are proliferating in culture. This does not prove that the proliferating cells are the ones which transfer disease. In other immune systems it has been possible to separate cells that proliferate in vitro from the cells responsible for specific immune functions (*7*). It appears that the immunologic function of cells that proliferate in response to antigen is still poorly understood.

Recently, Panitch and McFarlin (*8*) described a system for adoptive transfer in the rat in which relatively small numbers of spleen cells could transfer EAE after culture with concanavalin A (Con A). Spleen cells not cultured with Con A were inactive. They postulated that the cells stimulated by the mitogen were "memory" T cells because they were still responsive to Con A after the donors had recovered from EAE. We have found that in vitro incubation with BP as well as in vitro incubation with Con A confers on sensitized rat spleen cells the ability to transfer EAE (*9*).

The method of transferring EAE with viable cells reported here provides an effective tool for examining immunologic and pathogenetic mechanisms of EAE in guinea pigs. Such studies were not feasible with the larger cell numbers previously required. Further studies are necessary to explain the effect of BP in culture and to identify the T cell subset (or subsets) responsible for the transfer of EAE.

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References and Notes

1. R. H. Laatsch, M. W. Kies, S. Gordon, E. C. Alvord, Jr., *J. Exp. Med.* **115**, 777 (1962).
2. S. H. Stone, *Science* **134**, 619 (1961).
3. G. A. Falk, M. W. Kies, E. C. Alvord, Jr., *J. Immunol.* **101**, 638 (1968).
4. B. F. Driscoll, M. W. Kies, E. C. Alvord, Jr., *ibid.* **114**, 291 (1975).
5. ——, unpublished data.
6. E. C. Alvord, Jr., and M. W. Kies, *J. Neuropathol. Exp. Neurol.* **18**, 447 (1959).
7. J. W. Moorhead, *J. Immunol.* **120**, 137 (1978); R. L. Evans, J. M. Breard, H. Lazarus, S. F. Schlossman, L. Chess, *J. Exp. Med.* **145**, 221 (1977).
8. H. S. Panitch and D. E. McFarlin, *J. Immunol.* **119**, 1134 (1977).
9. J. R. Richert, B. F. Driscoll, M. W. Kies, E. C. Alvord, Jr., *ibid.*, in press.
10. G. E. Deibler, R. E. Martenson, M. W. Kies, *Prep. Biochem.* **2** (No. 2), 139 (1972).
11. G. E. Deibler and R. E. Martenson, *J. Biol. Chem.* **248**, 2392 (1973).
12. Supported in part by grants from the National Multiple Sclerosis Society (RG 1037A17, RG 805C18, and RG 828C6).

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Rotaviral Immunity in Gnotobiotic Calves: Heterologous Resistance to Human Virus Induced by Bovine Virus

Abstract. *The possibility of immunizing human infants against rotaviruses, which cause severe dehydrating diarrheal disease, may depend on the use of a related rotavirus, derived from another animal species, as a source of antigen. To test the feasibility of this approach, calves were infected in utero with a bovine rotavirus and challenged with bovine or human type 2 rotavirus shortly after birth. Infection in utero with bovine rotavirus induced resistance to diarrheal disease caused by the human virus as well as the homologous bovine virus. These data suggest that the bovine virus is sufficiently related antigenically to the human type 2 virus to warrant further evaluation of the former as a source of vaccine.*

Rotaviruses are a major cause of serious, acute gastroenteritis in human infants up to 2 years of age (*1, 2*). Any attempt to immunize children should therefore be made early in infancy. Resistance to rotaviral disease appears to be mediated in man and in animals by local immunity at the epithelial surface of the small intestine (*3–5*). Because of the importance of local immunity, a live attenuated vaccine given orally is likely to be more effective than an inactivated

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