Allergic Encephalomyelitis: Inhibition of Cellular Passive Transfer by Exogenous and Endogenous Steroids[1]

Experimental allergic encephalomyelitis (EAE), a form of delayed hypersensitivity induced by active immunization with neural tissue and Freund's adjuvant, has been inhibited by non-specific stress[2] and by exogenous adrenal corticosteroids[3]. Stress and/or steroids can inhibit active immunization of various types including others related to delayed hypersensitivity: tuberculin reaction[4,5], contact sensitivity to chemical allergens[6,7], homograft reaction[8,9]. In the present work, passive transfer techniques ('adoptive immunization') have been used to study fully immunized lymphoid cells. The results indicate that stress or steroids can reduce or prevent the effects of fully immunized, encephalitogenic lymphoid cells.

The donors of lymphoid cells were 8–10 weeks old female Lewis rats, 150–200 g, immunized with 0.05 ml of a water-in-oil emulsion of 40% guinea-pig spinal cord homogenate in an equal volume of Freund's complete adjuvant. The emulsion was injected intradermally into a right hind foot pad, and 0.1 ml pertussis vaccine concentrate (20 billion organisms) was injected into the dorsum of the same foot for additional adjuvant effect. Seven days later, when the donors began to develop signs of EAE, the right popliteal, lumbar, sacral, renal, inguinal and axillary lymph nodes were removed, dissociated into a cell suspension and injected i.v. into the recipients[10,11]. The recipients were immunologically naive, isogenic, male Lewis rats. Three days before cell transfer, the recipients' adrenals were removed in order to eliminate endogenous corticosteroids, and a closed thermal brain injury was inflicted in order to reduce the threshold for EAE[10,11]. Recipients were maintained on saline as sole fluid thereafter. One hour after the cell transfer, dexamethasone-21-phosphate disodium salt was injected i.p. in a volume of 1 ml/100 g[12]. The recipients were killed 24 h later.

Histologic scoring of lesions in randomized slides revealed prevention of EAE by 500 or 50 γ/100 g dexamethasone but not by 5 γ/100 g or less (Table). The larger doses also caused decrease of thymus and spleen weights and marked pyknosis and karyorrhexis of thymocyte nuclei.

Attempts were made to suppress EAE by endogenous adrenal steroids, secreted in response to the stress of forcible restraint (recipient rats immobilized prone by taping all extremities to a board). The stress was instituted 1–3 h before transfer of Lewis lymph node cells and was maintained until sacrifice 24 h after transfer. Because this procedure prevented access to food and water, control groups were starved for the same length of time. All recipients had thermal brain injuries inflicted 3 days earlier.

Histologic scoring of randomized slides revealed definite suppression of EAE by restraint stress in 2 experiments (results combined in the Table). Thymuses of stressed rats had marked nuclear pyknosis due to hypersecretion of corticosteroids. Starved controls had as much EAE as controls with access to food and water, presumably because the period of starvation was brief.

The recipients in the stress experiments were Fischer 344 rats. This strain accepts Lewis lymphoid cells for the short 24 h duration of the transfer[13]. It was used in order to test the effect on passive transfer of an ACTH-secreting pituitary tumor, MtT/F4[14], that can be transplanted in Fischer 344 rats[15] (a similar tumor in mice interfered with an immunologic reaction[16]). The tumor was implanted i.p. or s.c. 4–5 weeks before passive transfer. The tumor-bearing recipient rats had extremely enlarged adrenals (0.5 g/pair) and markedly atrophic thymuses due to excess secretion of ACTH and corticosteroids. There was complete prevention of EAE (Table). In addition, however, the zone of cerebral coagulation necrosis induced by the prior thermal injury did not undergo the expected degree of repair. The organization of a brain injury is known to be delayed or impaired by excess corticosteroids[17,18]. Therefore, the absence of EAE might have been caused by inadequate capacity of the thermal injury to localize EAE[11] as well as by steroid

Inhibition of passive transfer of allergic encephalomyelitis (EAE) by steroids or stress

Treatment of recipients[b]	No. of rats with EAE scores[a] 0 or ±	1+	2+	Thymus average weight
None		3	5	0.36 g
Dexamethasone 0.5 γ/100 g			3	0.40
5 γ/100 g	1	1	6	0.31
50 γ/100 g	4			0.22
500 γ/100 g	4			0.26
None		2	2	
Starved		3	6	
Restraint	3	10		
MtT/F4 tumor	13			

[a] EAE lesions at periphery of zone of thermal coagulation necrosis in brain. [b] Recipients adrenalectomized in dexamethasone experiments only.

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[2] S. LEVINE, R. STREBEL, E. J. WENK and P. J. HARMAN, Proc. Soc. exp. Biol. Med. *109*, 294 (1962).
[3] E. A. KABAT, A. WOLF and A. E. BEZER, J. Immun. *68*, 265 (1952).
[4] S. HARRIS and T. N. HARRIS, Proc. Soc. exp. Biol. Med. *74*, 186 (1950).
[5] V. J. DERBES, J. H. DENT, N. K. WEAVER and D. D. VAUGHAN, Proc. Soc. exp. Biol. Med. *75*, 423 (1950).
[6] A. NILZEN, J. Invest. Derm. *18*, 7 (1952).
[7] D. H. PITKIN, Proc. Soc. exp. Biol. Med. *120*, 350 (1965).
[8] P. B. MEDAWAR and E. M. SPARROW, J. Endocr. *14*, 240 (1956).
[9] R. WISTAR and W. H. HILDEMANN, Science *131*, 159 (1960).
[10] S. LEVINE and E. J. WENK, J. Immun. *99*, 1277 (1967).
[11] S. LEVINE and E. M. HOENIG, J. Immun. *100*, 1310 (1968).
[12] We are indebted to W. B. GALL, Merck Sharp & Dohme, Rahway, N.J., for a gift of dexamethasone.
[13] S. LEVINE, E. J. WENK and E. M. HOENIG, Transplantation *5*, 534 (1967).
[14] J. FURTH and K. H. CLIFTON, in *The Pituitary Gland* (Ed. G. W. HARRIS and B. T. DONOVAN; University of California Press, Berkeley 1966), p. 460.
[15] We are indebted to Dr. J. FURTH, Delafield Hospital, New York, for supplying this tumor.
[16] S. MALKIEL and B. J. HARGIS, Int. Archs Allergy appl. Immun. *26*, 319 (1965).
[17] E. J. FIELD, J. Neuropath. exp. Neurol. *16*, 48 (1957).
[18] N. HOURI, Yonago Acta Med. *11*, 77 (1967).

effects on the transferred cells. This problem was not encountered in the dexamethasone or stress procedures because these treatments were initiated *after* the thermal injury was established.

This demonstration that exogenous or endogenous steroids inhibited passive transfer of EAE is complemented by the previous report of enhancement of passive transfer by adrenalectomy[19]. The responsiveness of the fully immunized rat lymphoid cell to the steroid level in its environment is in accord with the inhibition of transfer of tuberculin hypersensitivity by cortisone in guinea-pigs[20]. However, the same steroid in the same species did not inhibit transfer of contact sensitivity to 2,4-dinitrochlorobenzene[21]. It remains for future research to clarify this discrepancy and to determine whether steroids have a direct effect on the specifically immunized lymphoid cells or interfere with a non-specific host component in the passive transfer recipient.

Zusammenfassung. Injektionen des Antientzündungssteroids Dexamethasone oder eine Hypersekretion des endogenen Kortikosteroids als eine Reaktion zum unspezifischen Stress verhindern die zelluläre passive Übertragung allergischer Enzephalomyelitis. Deshalb kann angenommen werden, dass Steroide die enzephalitogene Wirkung von vollkommen immunisierten Lymphoidzellen verhindern.

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[19] S. Levine and E. J. Wenk, Proc. Soc. exp. Biol. Med. *121*, 301 (1966).
[20] M. M. Cummings and P. C. Hudgins, J. Immun. *69*, 331 (1952).
[21] P. M. Seebohm, M. M. Tremaine and W. S. Jeter, J. Immun. *73*, 44 (1954).

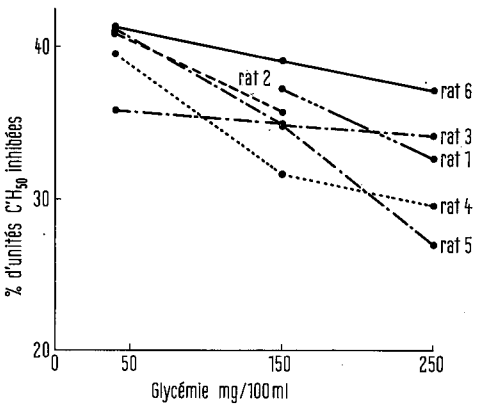
Etude de l'activité anticomplémentaire du sérum décomplémenté de rats hypo-, normo- et hyperglycémiques

Les séra frais ou décomplémentés de plusieurs espèces animales[1–3], inclusivement le rat[4], démontrent une activité anticomplémentaire. Dans le présent travail nous avons recherché, chez le rat une relation entre l'activité anticomplémentaire du sérum décomplémenté et l'état glycémique.

Méthodes. Un système hémolytique fut utilisé consistant de tampon veronal-saline au pH 7,5[5]; de complément lyophilisé de cobaye (Certified Blood Donor Service Inc.) reconstitué et dilué 1:400 et d'érythrocytes frais de mouton à 2,8% (Institut de Microbiologie, Université de Montréal) sensibilisés pendant 15 min à la température ambiante avec un volume égal de la dilution optimale d'hémolysine glycérinée de lapin[6] (Baltimore Biological Laboratory).

La titration du complément total fut faite par la méthode d'hémolyse à 50%[6]. Le pourcentage d'hémolyse fut déterminé au photocolorimètre (Klett-Summerson, 500–570 nm), après incubation de 30 min à 37 °C d'un volume total de 2 ml, consistant de 0,4 ml d'érythrocytes sensibilisés et de 1,6 ml de différentes dilutions de complément dans le tampon. Les dilutions du complément furent obtenues en ajoutant des volumes de complément dilué 1:400 et du tampon en quantité inversement proportionnelle dans le volume total de 1,6 ml. Le titre du complément fut exprimé en unités $C'H_{50}$/ml du sérum de cobaye non dilué.

L'activité anticomplémentaire des séra de rats fut exprimée par le pourcentage d'inhibition de l'hémolyse à 50%. Le sérum fut préalablement décomplémenté en l'incubant à 56 °C pendant 20 min et dilué 1:8 avec le tampon. Un volume constant de 0,4 ml de ce sérum fut substitué pour une partie du tampon dans 1,6 ml des différentes dilutions du complément. A ceci fut ajouté 0,4 ml d'érythrocytes sensibilisés. Le tout fut incubé et le degré d'hémolyse fut estimé comme auparavant. La différence entre les titres du complément de cobaye en absence et en présence du sérum de rat représentait l'activité anticomplémentaire de ce sérum. Cette différence fut exprimée en pourcentage du titre du complément en absence du sérum de rat auquel on allouait la valeur de 100%.



Activité anticomplémentaire du sérum décomplémenté, en fonction de la glycémie chez 6 rats individuels. L'activité anticomplémentaire des séra décomplémentés diminue régulièrement en allant de l'état hypo à l'état hyperglycémique chez les rats étudiés.

[1] L. H. Frommhagen et H. Fudenberg, J. Immun. *89*, 336 (1962).
[2] V. Bech, Acta path. microbiol. scand. *49*, 107 (1960).
[3] Ch. L. Christian, J. Immun. *84*, 112 (1960).
[4] Ch. E. Rice et C. N. Crowson, J. Immun. *65*, 201 (1950).
[5] E. A. Kabat et M. M. Mayeu, *Experimental Immunochemistry* 2nd edn (Charles C. Thomas, Springfield, Ill. 1964).
[6] *Standardized Diagnostic Complement Fixation Method and Adaptation to Micro Test* (Public Health Monograph No. 74; U.S. Department of Health, Education and Welfare; Public Health Service).