

Lymphocyte–Antigen Interaction in Electrophoretic Mobility Test for Cellular Sensitization

A SENSITIVE electrophoretic method for measuring lymphocyte sensitivity has been developed and applied to the study of human disease^{2,3} (including cancer⁴) and to the mode of action of antilymphocytic serum⁵. In principle, the method depends on the observation that a sensitized lymphocyte interacts with its specific antigen (but not with others) to liberate into the ambient medium some material which has the property of causing electrophoretic slowing of normal macrophages. The latter are used merely as an indicator system of lymphocyte–antigen interaction.

Table 1 Effect of Protein Synthesis Inhibitors on Lymphocyte–Antigen Interaction as measured by Macrophage Slowing Test

Antigen	Inhibitor	% Electrophoretic mobility reduction		
		30 min before	simultaneously	30 min after
PPD	None	23	—	—
	Puromycin	–3	0	20
	Cycloheximide	–2	–1	20
	Actinomycin D	–1	NT	19

To study the nature of the lymphocyte–antigen interaction various protein inhibitors were added to the reaction vessel. Results are shown as percentage reduction of migration speed. Thus if t_c is the migration time of macrophages over given distance (at fixed voltage and current) in the absence of antigen, and t_e is the migration time in the presence of antigen, then the percentage reduction is $\frac{t_e - t_c}{t_c} \times 100$. For these experiments,

guinea-pigs were immunized with purified protein derivative (PPD) of *Mycobacterium tuberculosis* (in Freund's complete adjuvant) and PPD itself used as the test antigen. All the protein inhibitors tested (Table 1) eliminated production of the macrophage slowing factor, a process which would thus seem to involve protein synthetic activity by the lymphocyte. While the slowing factor has many similarities to the macrophage migration inhibition factor (MIF) which is produced by lymphocytes in similar conditions and is known to be a protein⁶, there are some ways in which the present material may differ from MIF. Although, in general, the macrophage migration inhibition test runs parallel with the electrophoretic slowing test⁷, there are quantitative differences which may not be entirely due to the physical conditions under which the macrophages move. In the migration test they are moving out from the mouth of a capillary tube onto a glass surface⁸, whilst in the electrophoretic test^{1–3} they move under the influence of an electric field freely in a balanced salt medium. Moreover, whilst a mixed lymphocyte reaction does not occur between human blood lymphocytes and normal guinea-pig macrophage exudate (which contains 10–20% of lymphocytes), it does occur in the electrophoretic test. This may reflect the sensitivity of the latter method. The mixed lymphocyte reaction is also blocked by protein synthesis inhibitors⁹. Our results thus bring the lymphocyte–antigen interaction in the macrophage electrophoresis slowing test into line with what is known of both the macrophage capillary tube migration test⁶ and the mixed lymphocyte reaction which leads to lymphocyte transformation⁹.

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¹ Field, E. J., and Caspary, E. A., *J. Clin. Pathol.* (in the press).

- ² Field, E. J., Caspary, E. A., Hall, R., and Clark, F., *Lancet*, i, 1144 (1970).
³ Caspary, E. A., and Field, E. J., *Europ. Neurol.* (in the press).
⁴ Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).
⁵ Caspary, E. A., Hughes, D., and Field, E. J., *Clin. Exp. Immunol.*, 7, 395 (1970).
⁶ Bloom, B. R., and Bennett, B., *Science*, 153, 80 (1966).
⁷ Hughes, D., Caspary, E. A., and Field, E. J., *Z. Immunitäts. Allergie* (in the press).
⁸ David, J. R., Al Askari, S., Lawrence, S., and Thomas, L., *J. Immunol.*, 93, 264 (1964).
⁹ Wilson, D. B., and Billingham, R. E., *Advances in Immunology* (edit. by Dixon, F. J., and Kunkel, H. G.), 7, 248 (Academic Press, New York and London, 1967).

Thymic Origin of Lymphocytes in Developing Peyer's Patches of Newborn Mice

THE question of whether the gut-associated lymphoid tissue in mammals is a "primary" lymphoid organ, as proposed for the bursa of Fabricius in birds^{1–3}, or a secondary organ whose immunological development is at least partly thymus-dependent, has so far not been resolved by kinetic studies. This report provides direct evidence for the thymic origin of most lymphocytes in developing Peyer's patches of newborn mice, that is when antigenic stimulation from the intestinal flora sets in.

One day old Charles River albino mice of both sexes were anaesthetized with ether. With the aid of a stereomicroscope the thymus was exposed, and tritiated thymidine (specific activity 1.9 Ci/mmol, diluted to contain 20 μ Ci/ml.) was injected subcapsularly into the frontal parts of both thymic lobes (1 μ l./thymus) with a glass microcapillary approximately 25 μ m in diameter. A small amount of Evans blue dye was added to the thymidine solution to visualize intrathymic injection. Groups of five animals each were killed under anaesthesia 1 h, 2 h and 1, 2 and 3 days after injection. Thymus, Peyer's patches and spleen were processed for 57 days of histological autoradiography. An average of 800 thymic lymphocytes and 800 Peyer's patch lymphocytes were evaluated from each mouse. Based on grain counts on splenic erythroblasts and intestinal crypt cells, lymphocytes, or their precursors, with three or more grains over the nucleus were considered to have been labelled in the thymic region. Autoradiographic studies with tritiated thymidine have shown that in neonatal mice thymic cells migrate from the outer cortical zone to the cortico-medullary junction and then disappear

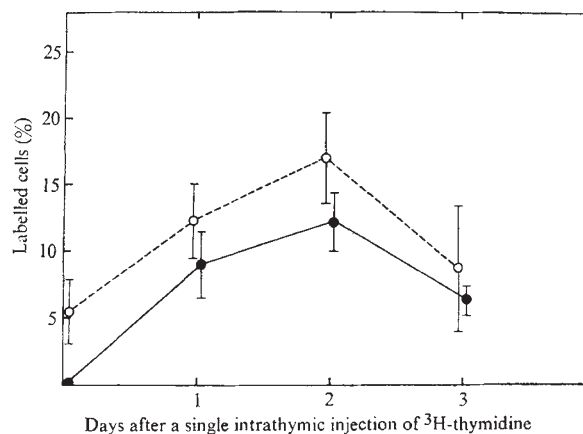


Fig. 1 Labelling indices of thymic cortical lymphocytes (○ --- ○) and Peyer's patch lymphocytes (● — ●) as a function of time after a single intrathymic injection of tritiated thymidine. Lymphoid cells of varying sizes with three or more grains over the nucleus were considered to be labelled. Individual points represent the mean and standard deviation obtained from five mice.